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EFFECT OF DIETARY ELECTROLYTE BALANCE AND ARGININE TO LYSINE RATIOS ON PERFORMANCE, NUTRIENT DIGESTIBILITY, EGG QUALITY AND HATCHING TRAITS OF LAYING HENS UNDER CYCLIC HEAT STRESS

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ABSTRACT

This research sought to study the relationship between dietary electrolyte balance (DEB) and the arginine-to-lysine ratio (Arg/Lys) concerning the sustainability of both productivity and reproductive efficiency in laying hens exposed to cyclic heat stress (CHS). A completely randomized design was used with 245 laying hens and 35 cocks randomly assigned to seven distinct experimental treatments between week 32 and 44 of age. The initial treatment was maintained under thermoneutral conditions with a temperature interval of 22–24 °C, accompanied by a relative humidity (RH) level fluctuating between 45% and 55%. The birds in this group were provided with a basal diet consisting of 180 mEq of DEB and a ratio of 1.25 for arginine to lysine (Arg/Lys), serving as a positive control with a DEB-to-Arg/Lys ratio of 144 (thermoneutral group). The remaining treatments were kept under CHS conditions for three consecutive days a week from Sunday to Tuesday at 38 °C and 55-65 % RH from 11.00 am to 15.00 pm. The birds were fed diets with various concentrations of DEB and an Arg/Lys ratio, organized into the following treatments: 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, and 320 mEq and 1. 37, respectively. The CHS treatment administered a dosage of 250 mEq of DEB and a ratio of 1.37 for Arg/Lys (resulting in a DEB/Lys ratio of 182.5), which significantly enhanced egg production percentage (EP%). The digestibility of protein and dry matter (DM) percentages were significantly improved by increasing Arg supplementation from 1.25 to 1.37 in 180 DEB. The diet 1.37 Arg/Lys and 250 DEB could be used to elevate CHS adverse effects, improve the apparent protein digestibility. This suggests that the ideal DEB: Arg/Lys ratio for laying hens exposed to CHS is about 182.5.

Keywords: thermal stress, animal health and production, animal nutrition, amino acid metabolism, egg evaluation.

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INTRODUCTION

Climate change is placing increasing pressure on global ecosystems, significantly impacting animal production. The poultry industry, especially laying hens, is highly vulnerable to rising environmental temperatures, which impair both productive and reproductive performance (Vandana *et al*., 2021). The ideal environmental conditions for optimal egg production are around 20 °C (North and Bell, 1990). However, heat stress (HS) is typically observed when temperatures exceed 27 °C and become severe above 30 °C (Bollengier-Lee *et al*., 1999). High environmental temperatures disrupt normal physiological and metabolic processes in poultry, leading to reduced feed intake, impaired nutrient utilization, and decreased egg production and quality (Oluwagbenga and Fraley, 2023). Given the economic burden associated with cooling poultry facilities, alternative strategies to mitigate heat stress in laying hens are essential. Among these, dietary manipulation has emerged as a practical and cost-effective approach (Attia *et al*., 2018).

One of the main physiological responses to heat stress is the alteration of amino acid metabolism, which

affects protein synthesis and energy balance. Amino acids play a critical role in various metabolic functions and their requirements may change under hyperthermic conditions (Temim *et al*., 2000). Arginine (Arg) and lysine (Lys) are two essential amino acids in poultry nutrition, which influence growth, immune function and overall metabolic efficiency. The balance between Arg and Lys (Arg/Lys ratio) has been identified as a crucial determinant of poultry performance, especially under stressful conditions such as high ambient temperatures (Saeed *et al*., 2018a; b). Previous research suggests that arginine supplementation can mitigate the detrimental effects of heat stress by enhancing immune responses, improving egg quality, and optimizing metabolic homeostasis in laying hens (Attia *et al*., 2016; 2024).

In addition to amino acid balance, dietary electrolyte balance (DEB) is another critical factor that influences poultry performance under heat stress. DEB plays a critical role in maintaining acid-base homeostasis, osmoregulation, and nutrient absorption (Philip *et al*., 2022). Modification of DEB levels in poultry diets has been shown to influence feed efficiency, egg production and overall resilience to heat stress (Gezen *et al*., 2005). However, the interaction between DEB and the Arg/Lys ratio in relation to heat stress adaptation has not been thoroughly studied. A better understanding of how these nutritional components interact under cyclic heat stress (CHS) conditions could provide valuable information to improve poultry management strategies. The aim of this study is to fill this knowledge gap by evaluating how different DEB levels and Arg:Lys ratios influence the sustainability of productive and reproductive performance in heat-stressed hens.

MATERIALS AND METHODS

Animals and experimental design: The current study was conducted at the El-Sabahia Poultry Research Station, belonging to the Animal Production Research Institute and affiliated with the Agricultural Research Centre of the Ministry of Agriculture. It is located within the Alexandria Governorate. The Ministry of Agriculture ethically approved the experimental procedures (protocol no. 01-10-003-37, record no.1563). The local Mandara strain, derived from a cross between ♂Alex and ♀Dokki-4 (Abd-El-Gawad *et al*., 1981), was utilized in this study. The experimental subjects comprised 245 hens exhibiting a mean body weight of 1536 g (±46.7), and 35 cocks, with an average weight of 1673 g (±58.3). They were allocated into seven treatment groups and housed in 35 floor pens, each 1x1.5 m size with a density of 0.214 m² per bird. The pens were equipped with wheat straw. Each treatment composed of five replicates, with each replicate comprising of seven hens and one cock (Attia *et al*., 2024). It was held for 12 weeks from week 32 to 44 of age. Seven treatments arranged in a design that employs complete randomization served as the experimental protocol.

Environmental conditions and experimental treatments: The hens were reared in the same facility, which was divided into two separate sections, allowing independent environmental control, light-proof house with a 16:8 light-dark cycle. The environmental separation between sections was ensured through independent climate control systems, insulated partitions, and continuous monitoring of temperature and humidity using calibrated sensors. In the first sector, hens were kept under ideal temperature environments from 22 to 24°C with RH levels between 45 and 55%. The subjects received a diet containing mainly corn and soybean meal (Gezen *et al*., 2005), called the basal diet, which had a 180 mEq dietary electrolyte balance and an Arg/Lys ratio of 1.25. This was considered the positive control group or thermoneutral group, with a ratio of 144 DEB: Arg/Lys. The remaining six treatments were maintained in the second section of the facility under Controlled Humidity and Temperature (CHS) conditions. High temperatures (38 °C ± 1) and relative humidity (55-65%) were administered over three consecutive days each week, specifically from Sunday to Tuesday, and from 11:00 AM to 3:00 PM. This experimental design aimed to replicate the intermittent heat stress conditions that reflect the actual climatic patterns observed in various geographical areas. Extreme heat tends to occur in waves rather than being constant throughout the summer.

The CHS groups were provided with a foundational diet distinguished by different proportions of DEB and the ratio of Arg/Lys. In this study, Group 1 administered a dietary electrolyte balance (DEB) of 180 mEq, characterized by an Arg/Lys ratio of 1.25, and served as the negative control (NC) with a DEB/Arg ratio of 144. Group 2 had a DEB of 250 mEq but the same Arg/Lys ratio of 1.25, giving them a DEB/Arg ratio of 200. Group 3 had a DEB of 320 mEq with an Arg/Lys ratio of 1.25, giving a DEB/Arg ratio of 256, whereas Group 4 had a DEB of 180 mEq but with an Arg/Lys ratio of 1.37, giving a DEB/Arg ratio of 131.4. For Group 5, the dietary inclusion level was 250 mEq of DEB, in association with an Arg/Lys ratio of 1.37, yielding a ratio of DEB/Arg of 182.5. The last group, Group 6, consisted of animals fed 320 mEq of DEB, combined with an Arg/Lys ratio of 1.37 and thus providing a ratio of DEB/Arg of 233.6. Table 1 depicts the formulation of experimental diets, as well as the levels of DEB, Agr/Lys ratios, and DEB/Agr:Lys ratio.

Breeding conditions and production parameters: The animals had unrestricted access to food and water. A daily photo period of 16 hours of light and 8 hours of darkness was implemented. The mortality rate, feed intake, live body weight, egg production percentage, and egg weight were recorded weekly for each replicate

group. In addition, calculations were performed for body weight (BW) change, egg mass (EM), and feed conversion ratio (FCR).

Digestibility analysis: The apparent digestibility of dry matter, organic matter (OM), crude protein (CP), ether extract (EE), and crude fiber (CF) was estimated in four cocks of each group (four replicates of one male) using the total collection method described by Attia (2020a) after chronic exposure to the cyclic heat stress regimen, ensuring that the birds had adequately undergone heat-stressed conditions sufficient to accurately measure the effects of dietary interventions at 48 weeks of age. The fecal CP was estimated after the separation of fecal nitrogen from urine nitrogen according to Colvin *et al*. (1966). Nutrient analyses were conducted following the AOAC (2007) procedures, specifically method 934.01 for DM, 942.05 for OM, 954.01 for CP, 920.39 for EE, and 978.10 for CF. The apparent digestibility of dry matter (DM), ether extract (EE), organic matter (OM), CF, and CP was computed by dividing the daily retained amount (g/d) by the daily intake (g/d).

Chemical and amino acid analysis: For the analysis of arginine and lysine concentrations, freeze-dried samples were processed using a 1-mm mesh screen and arranged for immediate amino acid assessment. Hydrolysis of the samples was carried out 24 hours using 6N hydrochloric acid at 110°C, which enabled amino acid quantification via a High-Speed Amino Acid Analyzer (Hitachi LA8080 Amino SAAYA). Nitrogen content was analyzed with a Leco nitrogen analyzer, while pump (1) facilitated the use of buffer solutions and RG, with ammonia filtration conducted at temperatures ranging from 20 to 80 °C. The TDE3 reactor operated at temperatures between 125 and 140 °C, and the detector was calibrated for wavelengths ranging from 440 to 570 nm. The washing solution and ninhydrin reagent were dispensed using a pump (2). Each sample was analyzed in triplicate.

Egg quality and fertility: At 44 weeks of age, five eggs per replicate group (totaling 25 eggs per group) were randomly collected on the same day as laying to assess egg quality. The hatching percentage for each treatment was calculated at 44 weeks based on 19 eggs per replicate (95 eggs per treatment). The fertility and hatchability rates were calculated by expressing the proportion of fertile eggs as a percentage of the total number of eggs subjected to incubation. Pipes that hatched were weighed to the nearest gram on the day of hatching, and the relative weight was determined by calculating the ratio of the chick's weight to the egg weight (EW) and then multiplying the result by 100.

Environmental monitoring and blood biochemical analysis: During the experimental period, indoor temperature and relative humidity (RH) were consistently observed, and the temperature-humidity index (THI) was computed following the methodology outlined by Berman (2016). The Weather Safety Index zones were identified based on THI, as described by Thom (1959), LCI (1970), and Gross and Siegel (1983). Upon conclusion of the experimental phase, blood samples (5 ml) were obtained from the wing vein of five hens using two types of blood collection tubes with and without heparin. The blood samples underwent centrifugation at 3,000 rpm for a duration of 20 minutes to facilitate the separation of plasma and serum. Following this process, the serum and plasma were preserved at − -20 °C until they were subjected to subsequent analyses. The quantification of serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, globulin, albumin, immunoglobulins IgG, IgM, and IgA was performed utilizing commercial diagnostic kits procured from Egypt Spectrum Diagnostic, as indicated by the studies of Attia *et al*. (2017, 2020b).

Statistical analysis: The dataset was assessed for normal distribution through the application of the Shapiro-Wilk test (1965) and subsequently analyzed statistically using SAS software (2009). For this analysis, a one-way ANOVA model was utilized as follows:

$Yij=\mu+\tau i+\epsilon ij$

where

Yij is the value of the response variable for the j^{th} trial of the i^{th} factor level,

μ is a constant of mean of trait,

τi represents the treatment effect,

ϵij represents the random experiment error.

The unit of experimentation employed in this investigation was replicated. Significant differences among the variables were assessed through the Tukey test (SAS, 2009).

RESULTS AND DISCUSSION

Environmental Conditions and Heat Stress: The mean indoor ambient temperature and relative humidity recorded in the HS sector indicated that the layers were exposed to significantly elevated ambient temperatures during the CHS period (Table 2). The THI index, calculated according to Berman (2016), was 91.5, confirming that the environmental conditions imposed severe heat stress on the layers throughout the experimental period. According to the Weather Safety Index (Gross and Siegel, 1983), the observed mean ambient temperature (37.6 ± 0.47 °C) exceeded the thermoneutral range for poultry, which is defined as 12–24 °C in temperate climates (Plyaschenko and Sidorov, 1987) and 20.9–28.5 °C in tropical regions (Prinzinger *et al*., 1991). The significant deviation from these optimal temperature ranges supports the conclusion that birds in the CHS sector were exposed to prolonged heat stress

conditions. Furthermore, our results are consistent with previous research by Abdalla *et al*. (2018), who demonstrated that poultry experience significant heat stress when THI exceeds 29 °C. Prolonged exposure to high THI values likely affected the physiological responses of layers, potentially affecting their productivity and metabolic balance, as discussed in the following sections.

Body Weight and Mortality Rate: The results indicate that body weight (BW) at the end of the experimental period did not show significant differences between groups (Table 3). Similarly, health status (HS) did not exert a significant effect on final BW or weight changes in the experimental treatments. These results suggest that local layer breeds show a strong capacity to adapt to cyclic heat stress (CHS), potentially due to physiological acclimation mechanisms. According to Felver-Gant *et al*. (2014), laying hens can adapt to high temperatures, maintaining their metabolic efficiency under stressful environmental conditions. However, the mortality rate was significantly higher in the CHS-exposed group compared to the thermoneutral one (P = 0.01) (Table 3). This is in line with the results of Attia *et al*. (2016), who reported a similar increase in mortality rates among heat-stressed layers. The increased risk of mortality under CHS conditions is likely due to prolonged exposure to elevated temperature, which leads to panting behavior and increased respiratory rate (Ayo *et al*., 2010). These physiological responses can induce dehydration, resulting in heatstroke-related mortality. This evidence highlights the critical impact of HS on the survival of breeding hens, reinforcing the need for targeted nutritional and environmental management strategies to mitigate the effects of heat stress.

Egg Production and Feed Intake: The current experiment revealed that cyclic heat stress exposure resulted in a significant decline in EP% of 13.8% during the entire experimental period from week 33 to 44 of age compared to the thermoneutral group (Table 3). These results are consistent with the findings of Christopher *et al*. (1995). The reduction in EP% under HS conditions could be attributed to the decreased feed intake, lower digestibility of feed, and reduced energy and protein utilization (Mahmoud *et al*., 1996). These observations are consistent with the study conducted by Attia *et al*. (2016). Furthermore, the changes in EP% in the heat-exposed group compared to the control before HS were 27.13%, 22.34%, and 42.48% during the early, middle, and late phases of the study, respectively (Aswathi *et al*., 2019).

In contrast, diets containing 180 and 250 mEq DEB with an Arg/Lys ratio of 1.37 (corresponding to 131.4 and 182.5 DEB, respectively) resulted in a significant increase in EP% compared to the CHS-treated group. The results indicate that the group fed 250 mEq DEB and an Arg/Lys ratio of 1.37 (equivalent to 182.5 DEB) showed an increase in EP% of 47.82% compared to the NC group (144 DEB/Arg/Lys) under CHS conditions and of 33.01% compared to the thermoneutral group (144 DEB/Arg/Lys). A similar response was observed when comparing the DEB/Arg/Lys ratio of 131.4 with that of 144 under thermoneutral and CHS conditions. These data suggest that the response to the DEB/Arg/Lys ratio varies with thermal conditions, with the optimum value being 144 in thermoneutral conditions and 182.5 in HS conditions. In this context, EP% increased with NaHCO3 levels ranging from 0% to 1.5% (Ghorbani and Fayazi, 2009). However, the laying percentage in hens fed a diet containing 170 mEq/kg DEB showed a significant increase (P<0.01) compared to those fed 250 mEq/kg (Chiericato *et al*., 2005). In contrast, several studies have shown that laying performance was not significantly affected by diets with DEB ranging from 176 to 242 mEq/kg or from 0 to 360 mEq/kg (Nizamettin *et al*., 2005; Gezen *et al*., 2005; Nobakht *et al*., 2007). The benefits of arginine supplementation are supported by multiple studies. Youssef *et al*. (2015) and Duan *et al*. (2015) showed that hens fed a diet containing 1.36% digestible arginine achieved optimal egg production rates. Sahin *et al*. (2018) also demonstrated that laying hens fed a standard diet enriched with 500 or 1000 mg of an arginine-silicate-inositol compound per kg for 90 days showed a significant increase in EP% compared to the control group (P<0.001). As reported in Table 3, hens subjected to cold heat stress (NC) produced 7.36% lower egg weight (EW) and 20.2% lower egg mass (EM) compared to hens raised in a thermoneutral environment between 33 and 44 weeks of age. These results are consistent with those of Attia *et al*. (2016). The data indicates that dietary adjustment in laying hens exposed to CHS for the Arg/Lys ratio or DEB significantly improved EW and EM compared to the NC group. Hens fed 250 mEq DEB and an Arg/Lys ratio of 1.37 (equivalent to 182.5 DEB) showed the highest energy metabolism (EM), comparable to that observed with 180 mEq DEB at the same Arg/Lys ratio of 1.37 (131.4 DEB) (Table 3). This suggests that the best DEB/Arg/Lys ratio for EP%, EW and EM in laying hens exposed to CHS is approximately 182.5 or 131.4 (180 or 250 DEB with Arg/Lys ratio of 1.37). In addition, the present research reveals that the heat stress (CHS) group experienced a significant 2.84% reduction in protein digestibility compared to the thermoneutral group (Table 4). However, CHS subgroups fed variable concentrations of dietary electrolytes (DEB) and specific arginine to lysine (Arg) ratios showed improved protein digestibility, reaching levels statistically comparable to those of the thermoneutral group. Results indicated that most egg quality traits were not affected by chronic heat stress (CHS) and dietary energy balance (DEB) fortification in relation to the arginine/lysine (Arg) ratio,

except for eggshell weight percentage and yolk index (Table 5). In particular, the shell percentage in the CHS group underwent a significant reduction of 8.8% compared to that observed in the thermoneutral group receiving the same DEB/Arg ratio. In addition, the yolk flow index decreased significantly in the groups ranging from 6.13% to 9.09% compared to the thermoneutral group, while the different DEB/Arg/Lys ratio did not influence the yolk index (Table 5).

Fertility and Hatchability: The results indicate that fertility and hatchability rates significantly decreased in hens subjected to chronic heat stress (CHS). Compared to the thermoneutral control group, fertility decreased from 91.3% to 89.4% ($P < 0.05$), hatchability of total eggs from 89.2% to 76.1% ($P < 0.01$), and hatchability of fertile eggs from 97.6% to 85.0% ($P < 0.001$) (Table 6). These results are consistent with previous research by Attia *et al*. (2018), who reported a significant reduction in fertility and hatchability due to heat stress exposure to breeding hens. However, dietary supplementation with 180 mEq of dietary electrolyte balance (DEB) and an Arg/Lys ratio of 1.37 (equivalent to 131.4 DEB/Arg ratio) resulted in a significant 9.72% increase in fertility ($P = 0.037$) compared to the CHS group that received an Arg/Lys ratio of 1.25 (DEB/Arg ratio of 144). Fertility rates in this group were statistically comparable to those of the thermoneutral group, indicating that regulation of DEB and Arg/Lys ratios can mitigate heat stress-induced fertility declines (Sharideh *et al*., 2016; Youssef *et al*., 2015). Similarly, hatchability of total and fertile eggs was significantly lower in hens exposed to CHS compared to thermoneutral conditions ($P < 0.001$). However, hens supplemented with 180 mEq DEB and an Arg/Lys ratio of 1.37 showed complete recovery of hatchability, except those in the CHS group that received a DEB/Arg ratio of 144. These results suggest that optimal DEB and Arg/Lys ratios can effectively restore reproductive performance under heat stress conditions (Chiericato *et al*., 2005; Daghir & Jones, 2008). Embryo mortality was significantly elevated in the CHS-exposed group that received a DEB/Arg ratio of 144, while hens supplemented with 180 mEq DEB and an Arg/Lys ratio of 1.37 showed a reduced embryo mortality rate of 4.65% ($P = 0.022$) (Table 6). This rate was statistically comparable to that of thermoneutral control, reinforcing the protective effect of DEB and arginine supplementation against HS-induced embryonic loss (Ao *et al*., 2020). In addition, chick weight and relative weight were significantly lower in hens exposed to CHS compared to the thermoneutral group ($P < 0.05$) (Table 6). However, hens supplemented with 250 mEq/kg DEB and an Arg/Lys ratio of 1.37 (DEB/Arg ratio of 182.5) showed the highest relative chick weight (%), like other supplemented groups. These results are in line with Youssef *et al*. (2015), who demonstrated that dietary arginine improves fertility and hatchability while simultaneously optimizing chick development.

The observed fertility and hatchability heat under CHS conditions may be linked to reduced sperm viability and fertilization capacity during heat stress. Studies have shown that high temperatures impair spermatogenesis, reduce sperm motility, and impair sperm penetration ability (Daghir & Jones, 2008; Sharideh *et al*., 2016). Furthermore, arginine supplementation was found to improve sperm penetration by 10%, which was positively correlated with increased fertility rates ($P \leq 0.05$). In addition, L-arginine is involved in chick embryo development, and increased dietary arginine levels have been associated with higher fertility and hatchability rates. For example, Youssef *et al*. (2015) reported that increasing L-arginine from 0.700% to 0.728% in Fayoumi and Golden Montazah breeds significantly improved reproductive performance.

Metabolic Response: Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) showed a significant increase in the CHS-exposed group compared to the thermoneutral group ($P < 0.05$) (Table 7). However, no significant changes were observed between different DEB/Arg ratios, indicating that adjustments in dietary electrolyte balance (DEB) and Arg/Lys ratio did not affect liver enzyme activity. These results are consistent with those of Attia *et al*. (2018), who reported similar enzymatic responses under heat stress conditions. Furthermore, the AST/ALT ratio remained unaffected by CHS exposure and changes in DEB/Arg/Lys ratios ($P = 0.136$) (Table 7), suggesting that liver function was not impaired despite increased enzyme levels. Renal function markers, including urea, creatinine, and creatinine-to-urea ratio, did not show significant differences between the different dietary treatments ($P > 0.05$) (Table 7). These results indicate that heat stress and dietary modifications did not have a measurable impact on renal function, corroborating previous findings (Chiericato *et al*., 2005). However, plasma glucose levels were significantly reduced in hens exposed to CHS that received a DEB/Arg ratio of 144 compared to the thermoneutral group ($P < 0.01$) (Table 8). Increasing arginine levels from 1.25 to 1.37 in the 180 DEB group resulted in a significant increase in blood glucose ($P < 0.001$), consistent with previous studies by Attia *et al*. (2016, 2017). Similarly, layers exposed to CHS and supplemented with 250 or 320 DEB maintaining an Arg/Lys ratio of 1.25 (equivalent to 200 and 256 DEB for Arg ratio) showed significantly higher glucose concentrations than the CHS control group ($P < 0.001$) (Table 8). These results agree with Pirsaraei *et al*. (2018), who reported that higher levels of DEB can improve glucose homeostasis in poultry subjected to heat stress. Plasma concentrations of total protein, albumin, α-, β- and γ-globulin, as well as

the albumin-globulin ratio (Alb/Glob), showed no significant differences in different dietary treatments (P > 0.05) (Table 8). These results suggest a lack of correlation between the DEB/Arg ratio and the metabolic protein profile, reinforcing previous results reported by Chiericato *et al*. (2005). The absence of significant changes in protein metabolism markers suggests that heat stress primarily affects glucose regulation rather than protein turnover, probably due to increased energy demands and metabolic shifts toward glucose-dependent pathways under heat stress conditions

Table 1. Ingredient Composition and Chemical Constituents (g/kg) of the Experimental Diet.

Ingredients composition, %	Relationship between arginine/lysine ratio and dietary electrolyte balance					
Yellow corn	630	630	628	630	630	628
Soybean meal 44%	260	260	260	260	260	260
Vit+Min Premix[1]	3	3	3	3	3	3
$NaHCO_3$	0	1.9	2	0	1.9	2
KCO_3	0.23	3	8.47	0.23	3	8.5
KCl	0	2.2	2.4	0	2.1	2.3
NaCl	3.9	1.95	1.7	3.80	1.95	1.5
Dicalcium phosphate	19.4	19.4	17.2	19.4	19.4	17.5
Limestone	82.47	77.55	76.33	81.59	76.67	75.3
DL-Methionine	1	1	0.9	1	1	0.92
Arginine	0	0	0	0.98	0.98	0.98
Total	1000	1000	1000	1000	1000	1000
Calculated composition,						
ME, kcal/Kg	2706	2706	2699	2706	2706	2699
Calcium, g/kg	32.7	31.1	30.2	32.4	30.8	29.9
Av. Phos, g/kg	4.90	4.90	4.50	4.90	4.90	4.50
Argnine 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
Arg/Ly	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)[2]	180	250	320	180	250	320
ER[3]	3.6	4.3	5.4	3.6	4.3	5.4
Dietary electrolyte balance /arginine/lysine ratio	144	200	256	131.4	182.5	233.6
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
Chemical analysis,						
CP, g/kg	161.3	164.3	165.1	163.3	162.7	163.1
Lysine, g/kg	10.32	10.41	10.47	11.38	11.51	11.47
Arginine, g/kg	8.46	8.45	8.51	8.47	8.49	8.53
Ether extract, g/kg	25.0	24.9	26.0	25.8	24.5	26.0
Crude fibre, g/kg	29.0	28.0	27.8	27.4	26.8	28.9
Ash, g/kg	57.8	52.3	88.5	62.6	58.1	67.0

[1]Vit+Min mixture provides per kilogram of diet: vitamin A, 12000 IU, vitamin E, 10 IU, menadione, 3 mg, Vit. D3, 2200 ICU, riboflavin, 10 mg, Ca pantothenate, 10 mg, nicotinic acid, 20 mg, choline chloride, 500 mg, vitamin B12, 10 µg, vitamin B6, 1.5 mg, vitamin B1, 2.2 mg, folic acid, 1 mg, biotin, 50 µg. Trace mineral (milligrams per kilogram of diet): Mn, 55, Zn, 50, Fe, 30, Cu, 10, Se, 0.10, Anti oxidant, 3 mg.
[2]Dietary electrolyte balance (DEB)= (Na++K+–Cl-), [3]-Electrolyte ratio (ER)= [(K++Cl-)/Na+].

Table 2. The recorded values of temperature (Tdb, °C) and relative humidity (RH, %) observed during experimental phases.

Month	Before HS			During HS		
	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±1.71	49.3 ± 0.84	64.9 ± 3.18	37.6 ± 0.47	39.4 ± 3.22	91.5 ± 1.62

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

Table 3. Impact of dietary electrolyte balance and the arginine-to-lysine ratio on the final body weight, changes in body weight, egg production efficiency, and survival rates of Mandara laying hens subjected to cyclical heat stress conditions*.

DEB + (Arg /lys ratio)		DEB/Arg/Lys ratio	Bodyweight (BW), g			EP, %	EW, g/d	EM, g/d	FI, g/h/d	FCR, kg/kg	Mortality, %
			Initial BW	Final BW	BW Change	33-44 wk	33-44 wk	33-44 wk	33-40 wk	33-44 wk	33-44 wk
180 +1.25 (Thremonetural group)		144	1549	1669	120	50.9 ^{b}	55.9 ^{a}	28.4 ^{c}	121 ^{a}	4.22 ^{b}	0.0^{b}
	180 +1.25 (NC)	144	1556	1663	107	43.9 ^{c}	51.7 ^{b}	22.7 ^{d}	120 ^{ab}	5.31 ^{a}	2.5^{a}
Cyclic heat stress	250 +1.25	200	1597	1709	113	56.2 ^{b}	52.2 ^{b}	29.3 ^{bc}	119 ^{ab}	4.08 ^{b}	0.0^{b}
	320 +1.25	256	1541	1709	113	57.9 ^{b}	53.3 ^{b}	30.9 ^{b}	118 ^{ab}	3.85 ^{b}	0.0 ^{b}
	180 +1.37	131.4	1507	1654	125	65.2 ^{a}	53.5 ^{b}	34.8 ^{a}	117 ^{b}	3.37 ^{c}	0.0 ^{b}
	250 +1.37	182.5	1520	1632	118	67.7 ^{a}	52.8 ^{b}	35.7 ^{a}	119 ^{ab}	3.32 ^{c}	0.0 ^{b}
	320 +1.37	233.6	1497	1614	118	56.4 ^{b}	53.0 ^{b}	30.0 ^{b}	117 ^{b}	3.89 ^{b}	0.0 ^{b}
	SEM		46.74	46.17	12.12	0.384	0.204	0.898	0.402	0.042	0.526
	P Value		0.161	0.084	0.465	0.001	0.001	0.001	0.024	0.001	0.01

$^{a, b, c}$ means bearing various letters in the same column are markedly various (P<0.05). SEM: standard error of means; P value: probability level; DEB=dietary electrolyte balance; Arg/Lys= arginine/lysine ratio; EP%= egg production percentage; FI g/day= feed intake (g/day); FCR= feed conversion ratio (g feed/ g egg); EW= egg weight (g); EM= egg mass (g); *n=5 replicates of 7 hens for each treatment.

Table 4. Effects of dietary electrolyte balance and the arginine-to-lysine ratio on the nutrient digestibility traits in laying hens experiencing cyclic heat stress conditions*.

DEB + (Arg /lys ratio)		DEB/Arg/Lys ratio	Organic Matter %	Dry matter%	protein%	Ether extract%	Fiber%
180 +1.25 (thermoneutral group)		144	58.11	82.56^{c}	81.84^{a}	77.28	27.67
	180 +1.25 (NC)	144	57.87	82.62^{bc}	79.11^{b}	77.97	26.52
	250 +1.25	200	59.89	83.42^{ab}	81.35^{a}	77.70	26.23
Cyclic heat stress	320 +1.25	256	59.72	83.22^{abc}	80.43^{ab}	78.10	26.17
	180 +1.37	131.4	59.38	83.62^{a}	81.35^{a}	78.00	26.21
	250 +1.37	182.5	60.11	83.52^{a}	81.04^{ab}	78.16	26.20
	320 +1.37	233.6	54.68	83.42^{a}	80.59^{ab}	78.14	26.74
	SEM		0.393	0.067	0.226	0.668	0.731
	P Value		0.5825	0.0001	0.0204	0.6647	0.9996

$^{a, b, c}$ means bearing various letters in the same column are markedly various (P<0.05). SEM: standard error of means; P value: probability level; DEB=dietary electrolyte balance; Arg/Lys= arginine/lysine ratio. *n=4 replicates per treatment.

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Table 5. Effects of dietary electrolyte balance and the arginine-to-lysine ratio on specific external and internal quality traits of eggs in laying hens exposed to cyclical heat stress conditions*.

DEB + (Arg /lys ratio)		DEB/Arg/ Lys ratio	External and internal egg quality traits								
			Shape index, %	Shell, %	Sh. Thickness, µm	SWU SA[1], mg/cm[2]	Yolk, %	Albumen, %	Yolk index	Yolk color	Haugh unit score
180 +1.25 (Thermoneutral group)		144	75.3	10.2[b]	341	108	38.1	51.7	47.3[a]	6.7	85.7
	180 +1.25 (NC)	144	74.7	9.30[c]	316	108	37.7	53.1	44.0[b]	6.5	83.2
	250 +1.25	200	75.2	10.6[b]	337	108	38.5	51.0	44.4[b]	6.1	86.8
Cyclic heat stress	320 +1.25	256	76.9	11.3[a]	333	108	37.5	50.2	43.1[b]	6.7	88.1
	180 +1.37	131.4	76.2	11.2[a]	332	107	39.1	49.7	43.0[b]	7.0	85.1
	250 +1.37	182.5	74.4	11.1[a]	333	109	38.9	50.0	43.4[b]	6.2	86.9
	320 +1.37	233.6	75.7	11.1[a]	338	109	38.1	50.8	43.4[b]	6.7	87.4
	SEM		0.375	0.039	0.0267	0.312	0.361	0.383	1.49	0.096	0.867
	P Value		0.462	0.001	0.084	0.693	0.816	0.086	0.001	0.084	0.699

[a, b, c] means bearing various letter in the same column are markedly various (P<0.05); SEM: standard error of means; P value: probability level; DEB=dietary electrolyte balance; Arg/Lys=arginine/lysine ratio; Sh, W.= Shell Weight, %; Sh. Thick =Shell thickness; SWUSA=: shell weight per unit of surface area; Alb.W.= Albumen weight. *n=5 eggs per replicate for each treatment.

Table 6. Influence of dietary electrolyte balance and the arginine-to-lysine ratio on hatchability attributes in laying hens under cyclic heat stress conditions*.

DEB + (Arg /lys ratio)		DEB/Arg/ Lys ratio	Fertility, %	Hatchability of total eggs, %	Hatchability of fertile eggs, %	Dead, %	Chick weight, (g)	Chick weight,%
180 +1.25 (Thremonetural group)		144	91.3[ab]	89.2[a]	97.6[a]	8.7[ab]	36.7[abc]	68.6 [d]
	180 +1.25 (NC)	144	89.4[b]	76.1[b]	85.0[b]	10.6[a]	36.0[c]	64.2 [e]
	250 +1.25	200	89.1[b]	84.8 [ab]	95.1[a]	10.4 [a]	36.5[bc]	70.2 [b]
Cyclic heat stress	320 +1.25	256	92.5[ab]	88.7 [a]	96.0[a]	7.5[ab]	37.2[ab]	69.7 [bc]
	180 +1.37	131.4	95.4[a]	90.0[a]	94.4[a]	4.70[b]	36.6[abc]	69.9 [bc]
	250 +1.37	182.5	93.3[ab]	88.5[a]	94.8[a]	6.7[ab]	37.7[a]	71.5 [a]
	320 +1.37	233.6	93.5[ab]	88.5[a]	94.6[a]	6.6[ab]	37.1[abc]	69.2[cd]
	SEM		0.464	0.974	0.741	0.477	0.114	0.375
	P Value		0.005	0.003	0.002	0.008	0.003	0.007

[a, b, c] means having various letters in the same column are markedly various (P<0.05). SEM: standard error of means; P value: probability level; DEB=dietary electrolyte balance; Arg/Lys= arginine/lysine ratio. *n=19 eggs per replicate for each treatment.

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Table 7. Effect of dietary electrolyte balance and the arginine-to-lysine ratio on hepatic and renal performance in laying hens exposed to conditions of thermal stress*.

DEB + (Arg /lys ratio)		DEB/Arg/Lys ratio	AST, U/L	ALT, U/L	AST/ALT	ALP, U/L	Urea (mg/dl)	create (mg/dl)	Creat/ Urea
180 +1.25 (Thremonetural group)		**144**	51.8 ^{b}	60.4^{c}	0.85	102.6 ^{b}	2.57	1.22	0.480
	180 +1.25 (NC)	**144**	55.4^{a}	64.2^{ab}	0.87	119.0^{a}	2.50	1.16	0.464
	250 +1.25	**200**	55.6^{a}	67.0^{a}	0.83	109.0^{ab}	2.43	1.22	0.502
Cyclic	**320 +1.25**	**256**	56.8^{a}	64.4^{ab}	0.88	107.4^{b}	2.37	1.32	0.556
heat stress	**180 +1.37**	**131.4**	57.0 ^{a}	65.4^{ab}	0.87	112.4 ^{ab}	2.33	1.16	0.496
	250 +1.37	**182.5**	55.2^{a}	63.0^{bc}	0.88	107.4^{b}	2.50	1.34	0.534
	320 +1.37	**233.6**	58.4^{a}	65.0^{ab}	0.90	107.6^{b}	2.43	1.34	0.546
	SEM		0.339	0.379	0.007	1.63	0.159	0.029	0.012
	P Value		0.019	0.0004	0.151	0.001	0.611	0.182	0.148

$^{a, b, c}$ means bearing various letters in the same column are markedly various (P<0.05); SEM: standard error of means; P value: probability level; ALT: Alanine amino transferase; AST: Aspartate amino transferase; Creat: creatinine. ALP: alkaline phosphatase; DEB=dietary electrolyte balance; Arg/Lys= arginine/lysine ratio; *n=5 blood samples per treatment; *n=5 replicates for each treatment.

Table 8. Effects of dietary electrolyte balance and the arginine-to-lysine ratio on the concentrations of total glucose, total protein, and immunoglobulin in laying hens subjected to heat stress*.

DEB + (Arg /lys ratio)		DEB/Arg/ Lys ratio	Glucose (mg/dl)	Total protein (g/dl)	Albumen (g/dl)	Globulin (g/dl)	α – globulin (g/dl)	β –globulin (g/dl)	γ– globulin (g/dl)	Albumen/ Globulin ratio
180 +1.25 (Thermoneutral group)		144	176^{a}	6.26	3.32	2.96	1.78	1.12	0.200	1.12
	180 +1.25 (NC)	144	171^{b}	6.46	3.40	3.06	1.90	1.14	0.167	1.11
	250 +1.25	200	176^{a}	6.36	3.44	2.94	1.84	1.20	0.100	1.17
Cyclic heat	320 +1.25	256	175^{a}	6.36	3.36	3.04	1.82	1.06	0.367	1.22
stress	180 +1.37	131.4	175^{a}	6.22	3.20	3.00	1.90	1.14	0.167	1.07
	250 +1.37	182.5	174^{ab}	6.20	3.38	2.86	2.00	1.22	0.133	1.19
	320 +1.37	233.6	174^{ab}	6.20	3.32	2.84	2.03	1.16	0.133	1.18
	SEM		0.334	0.291	0.35	0.435	0.029	0.021	0.106	0.026
	P Value		0.001	0.056	0.431	0.687	0.053	0.301	0.659	0.751

$^{a, b, c}$ means bearing various letters in the same column are markedly various (P<0.05); SEM: standard error of means; P value: probability level; DEB=dietary electrolyte balance; Arg/Lys= arginine/lysine ratio; *n=5 replicates for each treatment.

Conclusions: Supplementation of a diet with an electrolyte balance of 250 mEq and an Arginine/Lysine ratio of DEB significantly attenuated the negative effects of cyclical heat stress in laying hens, improving egg production and nutrient digestibility. This study highlights the importance of balancing electrolyte balance and amino acid profile to optimize egg productivity and quality under adverse environmental conditions. However, the research was conducted in a controlled experimental setting, which may not fully reflect commercial farming conditions. Furthermore, the analysis focused on short-term effects without assessing long-term metabolic or physiological implications. Further studies are needed to validate these findings in different breeds and more variable climatic conditions.

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Ethics approval: The protocol for the current study was approved during a session of the Scientific and Ethics Committee at the Animal Production Research Institute (Protocol No. 01-10-003-37; Record No. 1563).

Author Contributions: conceptualization was undertaken by YAA and AAA; methodology was developed by YAA, AAA, AEA, AShE, and RMZ; software utilized was contributed by YAA, FB, and AAA; data collection involved AAA and RMZ; investigation was conducted by YAA, NFA, FB, AAA, AEA, AShE, MAB, and RMZ; resources were provided by AAA, RMZ, RAA, and FB; writing of the original draft was prepared by YAA, AAA, and RMZ; writing-review and editing were performed by YAA, NFA, FB, AAA, AEA, AShE, RAA, and RMZ; project administration and supervision were managed by YAA; and funding acquisition was secured by RAA and YAA. All authors have reviewed and approved the final version of the manuscript for publication.

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