Influence of dietary electrolyte balance and arginine: lysine ratio on performance of developed layer chickens reared under heat stress condition

A thesis

Submitted to the Graduate School

Faculty of Agriculture, Damanhour University

In Partial Fulfillment of the

Requirements for the Degree

Of

Doctor of Philosophy

In

Poultry Production Damanhour University

By

Reda Mohammed Zaki Ibrahim

B.SC., Animal and Poultry Production (2007),

M.SC., Poultry Production (2013),

Faculty of Agriculture (Damanhour),

Damanhour University

2021

TABLE OF CONTENTS	
Title	Page
1. Introduction	1
2. Review of literature.	3
2.1. Production performance of laying hens under heat stress.	3
2.1.1. Production performance.	3
2.1.1.1. Body weight and body weight change.	3
2.1.1.2. Viability of birds.	4
2.1.1.3. Egg Production.	4
2.1.1.4. Egg weight and egg mass.	5
2.1.1.5. Feed consumption and feed conversion.	6
2.1.1.6. Digestibility of nutrients.	6
2.1.1.7. Egg quality traits.	7
2.1.1.8. Fertility and hatchability.	8
2.1.2. Physiological traits of laying hens under heat stress.	8
2.1.2.1. Immune response.	8
2.1.2.2. Hematological traits	9
2.1.2.3. Blood biochemical constituents.	9
2.1.2.3.1. Plasma total protein, albumin and globulin.	9
2.1.2.3.2. Plasma lipid profile.	9
2.1.2.3.3. Liver and kidney function.	10
2.1.2.3.4. Plasma total glucose concentration.	10
2.1.2.3.5. Plasma ions concentrations.	10
2.1.2.4. Oxidative biomarker and antioxidant status.	11
2.1.2.5. Hormones concentrations.	11
2.1.2.6. Body temperature and respiration rate.	12
2.1.2.7. Carcass Traits	13
2.2. Production performance of laying hens and the effect of DEB under heat stress.	14

2.2.1. Role of Na+, K+ and Cl- in the body.	14
2.2.2. Productive performance.	16
2.2.2.1. Body weight and body weight change.	16
2.2.2.2. Viability of birds.	17
2.2.2.3. Egg Production.	17
2.2.2.4. Egg weight and egg mass.	18
2.2.2.5. Feed consumption and feed conversion.	19
2.2.2.6. Digestibility of nutrients.	19
2.2.2.7. Egg quality traits.	19
2.2.2.8. Fertility and hatchability.	21
2.2.3 Physiological and biochemical traits of laying hens under heat stress.	21
2.2.3.1. Hematological traits.	22
2.2.3.2. Blood biochemical constituents	22
2.2.3.3. Plasma total protein, albumin and globulin	22
2.2.3.4. Plasma total glucose concentration.	22
2.2.3.5. Plasma ions concentrations.	23
2.2.3.6. Serum hormone concentrations, Oxidative biomarker and antioxidant status.	23
2.2.4. Body temperature and respiration rate.	23
2.2.5. Carcass traits.	24
2.3. Production performance of laying hens and the effect of arginine or arginine/lysine ratio (Arg/Lys) supplementation under heat stress.	25
2.3.1. Arginine.	25
2.3.2. Arginine biochemistry.	25
2.3.3. Productive performance.	26
2.3.3.1. Body weight and body weight change.	27
2.3 3.2. Egg production.	27
2.3 3.3. Egg weight and egg mass.	27
2.3 3.4. Feed consumption and feed conversion.	28
2.3 3.5. Digestibility of nutrients.	29
2.3 3.6. Egg quality traits.	29

2.3 3.7. Fertility and hatchability.	29
2.3.4. Carcass traits.	30
2.3.5. Physiological traits of laying hens under heat stress.	31
2.3.5.1. Immune response.	34
2.3.5.2. Hematological traits.	34
2.3.5.3. Blood biochemical constituents.	34
2.3. 5.3.1. Plasma total protein, albumin, and globulin.	34
2.3.5.3.2. Plasma total glucose concentration.	34
2.3.5.3.3. Lipped prevail.	35
2.3.5.3.4. Kidney function.	35
2.3.5.3.5. Liver function.	36
2.3.5.3.6. Plasma ions concentrations.	36
2.3.5.3.7. Hormone concentrations.	36
2.3.5.3.8. Oxidative biomarker and antioxidant status.	36
2.4. The interaction effect of DEB and arginine or arginine/lysine ratio (Arg/Lys) on production performance of laying hens under HS.	38
2.4.1. Body weight and body weight gain.	38
2.4.2. Viability of birds.	38
2.4.3. Egg Production.	38
2.4.4. Egg weight and egg mass.	39
2.4.5. Feed consumption and feed conversion.	39
2.4.6. Digestibility of nutrients.	40
2.4.7. Physiological traits of laying hens under HS.	40
2.4.7.1. Immune response.	40
2.4.7.2. Blood biochemical constituents.	40
3. 3. Materials and Methods.	41
3.1. Description the light proof house.	41
3.1.1. Temperature.	41
3.1.2. Relative humidity.	41
3.1.3. Ventilation.	41

3.1.4. Light source.	41
3.2. Experimental birds and design	42
3.3. Data collections.	44
3.3.1. Body weight.	44
3.3.2. Body weight gain.	44
3.3.3. Feed intake.	44
3.3.4. Feed conversion ratio.	44
3.3.5. Mortality rate.	44
3.3.6. Egg production traits.	44
3.3.6.1. Egg production (%).	44
3.3.6.2. Egg weight (g).	44
3.3.6.3. Egg mass	45
3.3.7. Apparent digestibility of nutrients.	45
3.3.8. Egg quality traits.	45
3.3.8.1. External egg quality.	45
3.3.8.1.1. Egg shape index.	45
3.3.8.1.2. Shell percent.	46
3.3.8.1.3. Shell thickness.	46
3.3.8.1.4. Shell weight per unit of surface area (mg/cm ²).	46
3.3.8.2. Internal egg quality.	46
3.3.8.2.1. Haugh units.	46
3.3.8.2.2. Yolk index.	47
3.3.8.2.3. Albumen percent.	47
3.3.8.2.4. Yolk percent.	47
3.3.8.2.5. Yolk color.	47
3.3.9. Hatching characteristics.	47
3.3.9.1. Fertility and hatchability.	47
3.3.9.2. Absolute and relative weight of chicks at day old of hatch.	48
3.3.10. Blood biochemical characteristics.	48
3.3.10.1. Blood sample collection.	48

3.3.10.2. Hematological parameters.	48
3.3.10.2.1. Packed cell volume (PCV %).	48
3.3.10.2.2. Hemoglobin concentration (Hgb g/dl).	48
3.3.10.2.3. Blood pH.	48
3.3.10.2.4. Red blood cell's (RBC's) and white blood cell's (WBC's) Counts.	48
3.3.10.2.5. Mean corpuscular volume (MCV).	49
3.3.10.2.6. Mean corpuscular hemoglobin (MCH).	49
3.3.10.2.7. Mean corpuscular hemoglobin concentration (MCHC).	49
3.3.10.2.8. Differential leucocytic count.	49
3.3.10.3. Blood biochemical constituents.	49
3.3.10.4. Hormonal assay.	50
3.3.11. Evaluation of immune responses.	50
3.3.11.1. Determination of HI titers against NDV.	50
3.3.11.2. Determination of phagocytic activity and phagocytic Index.	51
3.3.11.3. Lymphocyte transformation test (LTT).	51
3.3.11.4. Determination of serum immunoglobulin's.	52
3.3.12. Body and reproductive organs measurements.	52
3.3.13 Cloaca temperature (Body temperature)	52
3.3.14. Respiration rate.	52
3.3.15. Economic evaluation.	52
3.3.16. The Temperature-Humidity Index (THI)	53
3.3.17. Statistical analysis:	53
4. Results	54
4.1. Temperature degrees (°C) and relative humidity (%)	56
4.2. Performance of laying hens as affected by heat stress, dietary manipulation of DEB and arginine/lysine ratio (Arg/Lys).	57
4.2.1. Body weight and body weight change.	57
4.2.2. Survival rate.	57
4.2.3. Egg production rate.	57
4.24.Feed intake.	59

4.2.5. Feed conversion ratio	59
4.2.6. Egg weight.	61
4.2.7. Egg mass.	61
4. 3. Egg quality traits.	63
4.4. Reproductive traits and relative weight of day-old chicks.	65
4.5. Dressed carcass weight, inner and reproductive organs.	68
4.6. Blood hematology and biochemical constituents.	71
4.6.1. Red blood cells characteristics.	71
4.6.2. White blood cells and its fractions.	71
4.6.3. Blood biochemical constituents.	74
4.6.3.1 Plasma glucose, total protein, protein fractions and lipid profile constituents and catalase and malondialdehyde activity.	74
4.6.3.2. Indices of liver and kidney functions.	77
4.6.3.3. Plasma calcium and phosphors concentrations and Serum estrogen, progesterone, tri-iodothyronin, thyroxin.	80
4.6.4. Blood total antioxidant capacity, some immunological traits	80
4.6.5. Antibody titration against New-Castle disease virus (HINDV, Haem agglutination inhibition, HI)	83
4.7. Cloaca temperature and respiration rate.	83
4-8. Economical evaluation.	86
5. Discussion	87
5.1. Effect of chronic heat stress.	87
5.1.1 Temperature degrees (°C) and relative humidity (%)	87
5.2. Effect of nutritional manipulation:	87
5.2.1 Productive traits	87
5.2.2. Egg quality and reproductive performance:	92
5.2.3. Biochemical constituents:	95
5.2.4 Liver and kidney function:	97
5.2.5. Immune response:	98
5.2.6. Oxidative biomarker and antioxidant status:	98
5.2.7. Hormones concentrations:	99
5.2.8. plasma ions concentrations	100

6. Summary and Conclusion.	102
7. References.	106
8. Arabic Summary.	1

LIST OF TABLES

No	Title	Page
1	Ingredient and chemical composition of the experimental diet for laying hens.	43
2	The temperature (°C) and relative humidity (RH, %) during the experimental periods.	56
3	Effect of dietary electrolyte balance and arginine/lysine ratio on final and change body weight (BW), egg production percentage, and survival rate of Mandara laying hens under heat stress condition.	58
4	Effect of dietary electrolyte balance and arginine/lysine ratio on feed intake (g/day) and feed conversion ratio (g feed/ g egg) of Mandara laying hens under heat stress condition.	60
5	Effect of dietary electrolyte balance and arginine/lysine ratio on egg weight (g) and egg mass of Mandara laying hens under heat stress condition.	62
6	Effect of dietary electrolyte balance and arginine/lysine ratio on some external and internal egg quality traits of Mandara laying hens under heat stress conditions.	64
7	Effect of dietary electrolyte balance and arginine/lysine ratio on hatchability traits of Mandara laying hens under heat stress.	66
8	Effect of dietary electrolyte balance and arginine/lysine ratio on nutrient digestibility traits of Mandara laying hens under heat stress	77
9	Effect of dietary electrolyte balance and arginine/lysine ratio on some carcass characteristic of Mandara laying hens under heat stress condition.	69
10	Effect of dietary electrolyte balance and arginine/lysine ratio on some carcass characteristic of Mandara laying hens under heat stress condition.	70
11	Effect of dietary electrolyte balance and arginine/lysine ratio on hematological parameters of Mandara laying hens under heat stress.	72
12	Effect of dietary electrolyte balance and arginine/lysine ratio on White blood cells and differential leukocyte of Mandara laying hens under heat stress	73
13	Effect of dietary electrolyte balance and arginine/lysine ratio on glucose, total protein, and protein fractions of Mandara laying hens under heat stress.	75
14	Effect of dietary electrolyte balance and arginine/lysine ratio on lipid profile s, catalase, and malondialdehyde of Mandara laying hens under heat stress.	76
15	Effect of dietary electrolyte balance and arginine/lysine ratio on liver and kidney function of Mandara laying hens under heat stress.	78
16	Effect of dietary electrolyte balance and arginine/lysine ratio on blood calcium and phosphorus concentrations and some hormones activity of Mandara laying hens under heat stress	79

17	Effect of dietary electrolyte balance and arginine/lysine ratio on some immune parameters of Mandara laying hens under heat stress	81
18	Effect of dietary electrolyte balance and arginine/lysine ratio on HI titers of Newcastle disease of Mandara laying hens under heat stress	82
19	Effect of dietary electrolyte balance and arginine/lysine ratio on body temperature and respiration rate of Mandara laying hens subjected to heat stress at a different time of measuring (before, during, and after heat exposure).	84
20	The correlation coefficients between body temperature (BT) and respiration rate (RR) of Mandara laying hens subjected to heat stress (before, during, and after heat exposure).	85
21	Effect of dietary electrolyte balance and arginine/lysine ratio on economic efficiency (EE) and relative economic efficiency (REE) at the end of the experiment.	86

LIST OF FIGURES

No	Title	Page
1	Structure of Arginine	25
2	Recent advances in arginine metabolism: roles and regulation of the arginases	26
3	Temperature-Humidity Index (THI) chart	56

6. SUMMARY and CONCLUSION

The present study was conducted from January to March 2018 to investigate the effect of dietary electrolyte balance and arginine to lysine ratio on productive, reproductive performance, egg quality, immune responses, and physiological and blood biochemical constituents of Mandara laying hens exposed to chronic heat stress. The experiment lasted for 12 wks from 32 to 44 weeks of age.

A total of 245 hens and 35 cocks of Mandarah developed local strain (32 Wks age) were divided into seven groups and housed in 35-floor pens furnished with wheat straw. Each treatment was represented by 5 replicates (7 hens + 1 cock, each). Hens were housed in an environmentally controlled light-proof house (close system).

The first treatment was kept in the first sector under thermoneutral conditions (22:24 °C) and relative humidity (RH) 45:55 %. Whereas the other six treatments were kept in the second sector under chronic heat stress conditions (38 °C \pm 1, 55-65 % RH) for three successive days a week from 10.00 am until 15.00 pm.

Experimental design:

- **1-The first treatment**: birds were fed the basal diet (corn-soybean meal diet) with dietary electrolyte balance (DEB) equal 180 mEq, and 1.25 arginine/lysine ratio and used as a positive control (control +, PC).
- **2- The second treatment**: birds were fed the basal diet with DEB equal 180 mEq, and 1.25 Arg/Lys and used as a negative control (control -, NC).
- **3- The third treatment:** birds were fed the basal diet with 250 mEq DEB and 1.25 Arg/Lys.
- **4- The fourth treatment:** birds were fed the basal diet with 320 mEq DEB and 1.25 Arg/Lys.
- **5- The fifth treatment:** birds were fed the basal diet with 180 mEq DEB and 1.37 Arg/Lys.
- **6-The sixth treatment:** birds were fed the basal diet with 250 mEq DEB, and 1.37 Arg/Lys.
- **7- The seventh treatment:** birds were fed the basal diet with 320 mEq DEB, and 1.37 Arg/Lys.

The obtained results could be summarized as follows:

- **1-**The final body weight of breeding hens at 44 wks of age in different treatment groups was insignificantly affected by HS and other dietary manipulation.
- **2-** The Laying rate for layer groups exposed to HS (NC) was significantly decreased compared with the control group. The Laying rate decrease was 12.1, 12.9, 16.3, and 13.8% during 33-36, 37 40, 41 44, and 33-44 wks of age, respectively.
- **3-**The laying rate of layers exposed to HS, and fed 250 mEq DEB with 1.37 Arg/Lys recorded the highest laying rate during 33 -36, 41-44, and 33- 44 wks of age by

61.9, 65.1 and 54.2%, respectively than the NC group, and by 54.0, 38.2, and 33.0%, respectively compared with the PC group.

- **4-** The amount of FI was decreased for all experimental groups under HS compare with PC group and the lowest amount was recorded for the group fed monblueted diet equal DEP 320 mEq with 1.37Arg/Lys.
- **5-** Layers exposed to HS (NC) significantly declined FCR than the other groups. The decline in FCR was 22.5, 26.4 and 25.8 % during 33 -36, 37 40 and 41 44 wks of age, respectively, and 25.89% for the whole experimental period (33- 44 wks of age).
- 6- DEB equal to 250 mEq, and Arg/Lys equal 1.37 recorded the significant improvement on FCR during 33 -36, 41-44, and 33-44 wks of age by 37.79, 21.74, 40.23, and 32.93 %, respectively more than the NC group. Also, the same group significantly improved FCR during the same period by 21.2, 43.5 and 37.5%, respectively compared with the PC group.
- 7- The EW for the NC layer groups (HC) was significantly decreased compared with those in the PC group for all experimental periods.
- **8-** The layer groups exposed to HS and fed different Arg/Lys ratios or BED were significantly improved EW compared to the EW for the NC group.
- **9-** Layer groups exposed to HS (NC) significantly declined EM compared with the other experimental groups during different periods.
- **10-** Shell weight % for the NC group was significantly decreased by 8.8% compared with that recorded for PC group, also its recorded the significantly lowest percentage compared with the different experimental groups.
- **11-**Hens on diets had 320 mEq of DEB with 1.25 Arg/Lys, 180 mEq of DEB with 1.37Arg/Lys, and 250 mEq of DEB with 1.37 Arg/Lys recorded the highest eggshell weight compared with the other groups.
- 12- Fertility and hatchability (of total or fertile eggs) were significantly decreased of chickens exposed to HS (NC) from 87.36, 82.91, 97.3 to 85.3, and 97.3 85.3, 79.9, and 91.5% respectively compared with the PC group.
- 13- Laying hen on 180 mEq of DEB with 1.37 Arg/Lys significantly improved fertility, and both hatchability traits, percentages compared with the NC group from 85.3, 79.9, and 91.5 % to 92.2, 90.5, and 98.1 %, respectively.
- 14- Feeding 180 DEB with 1.37 Arg/Lys recorded the significantly lowest embryonic mortality (4.88 %) than the other experimental groups.
- **15-** The relative weight of baby chick (%) hatched from the group fed manipulated DEB diet equal 250 mEq/kg, andArg/Lys 1.37 was the highest than that of the other groups.
- 16- The digestibility percentage of protein significantly differed among experimental groups and the groups under HS and fed monblueted diet with different levels of DEB and Arg/Lys were statistically equal with the PC group and the lowest value was recorded for the NC group.
- 17- Layer groups exposed to HS (NC) significantly decreased spleen, abdominal fat, total ovarian follicle numbers, large yellow follicle numbers, small white follicle

numbers (SWF), and oviduct weight compared to the PC group. At the same time, different DEB with Arg/Lys significantly improved those treats.

- **18-**Layer exposed to HS (NC) had significantly decreased RBCs characteristics (RBCs count, PCV, Hgb, MCV, MCH, and MCHC) compared with PC group, while blood pH was significantly increased.
- **19-** Layers fed different DEB with Arg/Lys significantly enhanced RBCs count, PCV, Hgb, MCV, MCH, and MCHC values compared with those recorded for the NC group.
- **20-** CH had a significant adverse effect on WBCs, and its fractions (lymphocyte, monocyte, heterophil, neutrophil percentages) and increased on heterophil/lymphocyte ratio (H/L ratio).
- **21-** Layers fed different DEB with Arg/Lys resulted in diminishing the negative effect of CH on WBCs, lymphocyte, heterophil, and neturophile, percentages compared with the NC group.
- 22- Layer exposed to HS (NC) had significantly lower glucose than the PC.
- 23- Layer exposed to HS had significantly increased plasma total lipid, total cholesterol, low-density lipoprotein (LDL), triglyceride constituents, and malondialdehyde (MDA) activity compared to PC and all the other experimental groups.
- **24** The layer under HS, and fed on 250 and 320 DEB with 1.37 Arg/Lys recorded the lowest concentration of low-density lipoprotein (LDL) compared with the other experimental groups.

25- The activity of alkaline phosphatase activity (ALP) was significantly increased for the NC group (under HS) compared with the PC group, while the other experimental groups were almost statistically equals.

- **26**-Layers exposed to HS (NC) had significantly decreased plasma calcium (Ca) and phosphorus (P) concentrations compared with the PC and the other experimental groups.
- **27** Triiodothyronine (T3) activity was not differed among all experimental groups, while T4 activity for layer exposed to HS (NC group) recorded the significantly highest activity compared with PC group.
- **28-** Layers exposed to CH (NC group) significantly decreased TAC, LTT and BA compared with PC group, and Haemagglutination inhibition of the Newcastle disease virus (HINDV) at 7 d post-vaccination compared with the PC, and the other experimental groups.
- **29** Haemagglutination inhibition of Newcastle virus was significantly incensed at 14, and 21 d compared with the titer value at 7 d of post-vaccination.
- **30-** Laying hens exposed to HS (during and after HS time) significantly increased cloaca temperature for all experimental heat stress groups compared with the PC group.
- **31-** The highest economic efficiency (EE), and relative economic efficiency (REE) were recorded for the group fed dietary manipulation diet 250 DEB with 1.37 Arg/Lys than the other experimental groups.

In conclusion,

It can be concluded that Mandara laying hens fed monblueted diet equal DEP 250 mEq with 1.37Arg/Lys could be applied to elevate the adverse effect of HS and improve the productive, reproductive, egg quality traits, blood haematological and biochemical traits, antioxidants, and immunity and represented the highest EE.