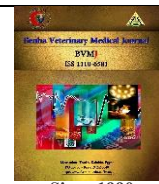




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The impact of biosecurity and heat stress on the poultry performance under field condition

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ABSTRACT

Heat stress (HS) is one of the most important environmental stressors challenging poultry production worldwide. This study aimed to measure the relationship between the biosecurity measure which applied in poultry farms and the effect of heat stress severity. We collected of 120 blood and 24 tissue samples to show the effect of heat stress on blood parameters and some histopathological changes. There were a direct relationship between biosecurity level and heat stress on egg production and FCR. The egg production was the highest in layer farm B with biosecurity (90%) as well as the FCR was the highest in farm A with biosecurity (62.5%). In addition to the heat stress effect on blood in poultry. Serum glucose was the highest in layer farm A and broiler farm B, thyroid hormone triiodothyronine (T3) was the highest in layer farm A and broiler farm A, thyroxine (T4) was the highest in layer farm B and broiler farm A. Furthermore, the level of cortisol was the highest in layer farm A and broiler farm A. There were some histopathological changes in liver and intestine. Finally, we concluded that efficient heat stress control through strong biosecurity plan relieves the HS effect and maintains high FCR and egg production.

1. INTRODUCTION

Climate change and HS affected on chickens that occur when the internal temperature rises above the thermoneutral zone that is range of temperatures where an organism does not require additional energy to regulate its body temperature. For chickens, the thermoneutral zone is between 18°C and 24°C and once the temperature rises above 27°C, the birds must reduce their heat burden. Climate change is predicted to raise global temperatures in the twenty-first century, which will have a negative impact on poultry production (Schmidt and Lamont, 2024).

Heat stress has many series effect in poultry as decrease feed intake and egg production such as laying hens exposed to heat stress showed significantly lower body weight, feed intake, egg production rate, egg weight, and their eggs also had lower quality while broiler chicks affect through slows their growth, reduces their feed intake (Heidari and Toghyani, 2018).

Biosecurity application and good management are the best control for the serious effect of HS that requires careful management of poultry housing as proper ventilation and temperature control in environmentally controlled housing in order to minimize heat stress (Saeed et al., 2019).

Glucose concentration in blood one of the predominant changes occurred by HS as synthesized in the liver via gluconeogenesis rather than glycolysis. The liver also plays a critical role in maintaining glucose homeostasis during prolonged starvation to meet the energy demands of the broilers. Generally, glucose metabolism decreases during heat stress for maintaining body temperature by restricting feed intake (Zhang et al., 2019).

Poultry are impacted by high temperatures due to raise corticosterone levels and activating the hypothalamic-

pituitary-adrenal (HPA) axis, they regulate development, metabolism, T3 and T4 (Quinteiro-Filho et al., 2012).

Heat stress affected on the immune response in animals that influenced on sympathetic-adrenal medullar (SAM) and hypothalamic-pituitary-adrenal (HPA) axes. The presence of receptors for a variety of neuroendocrine products, including cortisol (Butts and Sternberg, 2008).

The intestinal tract is highly susceptible to heat stress. The production of poultry depends on the intestinal tract's effective operation because it significantly impacts the birds' overall health and productivity. The intestinal microbiota will be significantly impacted by heat stress. Although it is becoming more evident that heat stress affects the composition and functionality of the gut microbiota, the exact mechanisms causing these effects are still being investigated and are not fully understood (Kers et al., 2018).

Liver damage is one of series effect of HS due to formation of reactive oxygen species (ROS). ROS lead to lipid peroxidation in muscles, protein, liver damage and change in morphology in liver cells (Kumar et al., 2012). This study aimed to detect the relationship between the biosecurity measure which applied in poultry farm and the effect of heat stress severity. In addition to, determine the effect of HS by measuring some blood parameters concentration and histopathological changes in intestine and liver of poultry.

2. MATERIAL AND METHODS

2.1. Poultry farms:

The present study was carried out on four poultry farms (two broiler and two layer) at Qalyubia governorates.

Broiler farm A located in Qalyubia with closed deep litter system, Pollution sources is moderate, quarantine of sick

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birds was present, water and feed hygiene are good, control of pets and knowledge about biosecurity is high. The temperature control system by good ventilation system, air hoods and air conditioning.

Broiler Farm B located in Qalyubia with closed deep litter system, Pollution sources are moderate, presence of quarantine of sick bird's water and feed hygiene are good, control of pets and knowledge about biosecurity is low. the temperature control system by good ventilation system and air hoods only.

Layer farm A located in Qalyubia with open deep litter system, Pollution sources is moderate, presence of quarantine of sick birds, water and feed hygiene are good, control of pets and knowledge about biosecurity is good. The temperature control by natural ventilation and water nibbles to sprinkle water.

Layer Farm B located in Qalyubia governorate, the system of housing is closed slatted floor system, Pollution sources are moderate, presence of quarantine of sick birds, water and feed hygiene are good, control of pets and knowledge about biosecurity is good. The control of temperature through good ventilation system, air hoods and air conditioning. The selection of the farms was based on variation in farm hygiene, housing system, variation in temperature control, humidity control and applied biosecurity measures.

2.2 .Hygienic scoring of poultry farms.

Biosecurity scoring system which applied in each farm such as wheel dip- foot bath, inner farm construction, pollution sources, type and state of ventilation system, water hygiene, outer farm construction, feed hygiene, litter hygiene and pest control. The final total score of farm biosecurity was the sum of the different scores according to (Dewulf et al., 2019).

Calculation = $\frac{\text{Sum scores of total applied biosecurity measures}}{\text{Total full application of biosecurity measures (score 2)}} \times 100$

2.3. Collection of data:

The data about temperature, humidity, egg production in layer farms were collected per each day and FCR was calculated during the summer season according to Jahejo et al., (2016).

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Live weight}}$$

2.4. Sampling:

The temperature and humidity were recorded daily; the samples were collected during the highest temperature and humidity. A total of 120 blood samples were collected from four poultry farms along three visits with five samples per each visit (Schmittgen, 2001). The collection of samples was approved with Institutional Approval Number (BUFVTM 07-12-24).

2.4.1. Blood sampling:

The Samples were collected by using sterile tubes without anticoagulant from the wing vein for live chickens and the jugular vein for chickens that had just died, The blood samples were centrifuged at 3,000 RPM for 10 minute, and the obtained serum was stored at -20 immediately according to Liang et al., (2024).

2.4.2. Tissue sampling:

Intestine and liver of broilers and layers were examined, small tissue specimens were taken from necropsied bird and fixed in 10% neutral buffered formalin. After proper fixation, these specimens were dehydrated through ascending grading of ethyl alcohols, cleared in xylol and

embedded in paraffin wax. Four-µm-thick serial sections were cut and stained with hematoxylin and eosin (H&E) according to (Bancroft et al., 1996), and examined using a light microscope equipped with an ocular micrometer (Nikon Eclipse E600, Japan).

2.5 . Blood analysis

2.5.1 .Determination of serum glucose concentration.

Serum glucose concentration was determined according to Lott and Turner, (1975) using a commercial kit supplied by biodiagnostic, Giza, Egypt.

$$\text{Serum Glucose Concentration (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times 100$$

2.5.2. Determination of serum T3 concentration.

T3 concentration was examined according to (Larsen, 1972) Using a commercial kit supplied by Abnova, Catalog Number KA0198.

2.5.3. Determination of serum T4 concentration.

T4 concentration was applied according to (Tietz and Berger, 1976) Using a commercial kit supplied by Abnova, Catalog Number KA4013, This test is based on competition enzyme immunoassay principle.

2.5.4. Determination of serum cortisol concentration.

Serum cortisol concentration was measured using Rabbit Cortisol (COR) Elisa kit (Cat. No. MBS752096) according to (Munro and Lasley, 1988).

2.6. Statistical analysis:

The statistical analysis was carried out using two-way ANOVA using SPSS, ver. 27 (IBM Corp. Released 2013) according to (Steel and Torrie, 1960). Multiple comparisons were carried out applying Duncun test the significance level was set at < 0.05.

3. RESULTS

3.1 .Biosecurity scoring:

The biosecurity score was the highest in layer farm B (90%) with good ventilation system, air hoods and air conditioning. followed by layer farm A (67. 5%) with natural ventilation, broiler farm A (62.5%) with good ventilation system, air hoods and air conditioning and broiler farm B was the lowest (60%) with good ventilation system and air hoods only. (Figure 1).

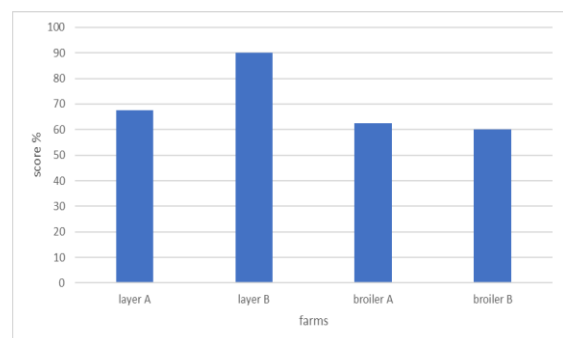


Figure (1): The biosecurity score (%) of different poultry farms.

3.2 Egg production calculated in layer farm.

The mean of egg production was the highest in layer farm B (39803.25 eggs) with the highest biosecurity score (90%). In contrast, it was (16534.65 eggs) in layer farm A with biosecurity score (67. 5%) (Figure 2).

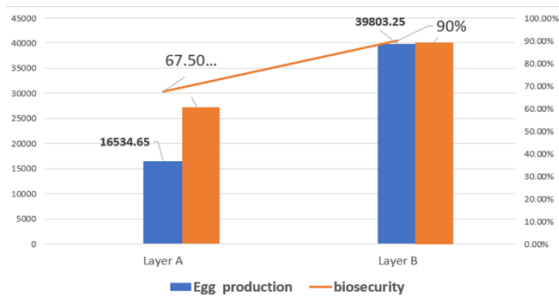


Figure (2): The egg production in different layer farms in relation to the biosecurity.

3.3 .Feed conversion rate calculated in broiler farm

The FCR was calculated in broiler farms under the period of study. It was the highest (1,008) in broiler farm A which had (62.5%) biosecurity score while it was the lowest (0.814) in broiler farm B with biosecurity score (60%) (Figure 3).

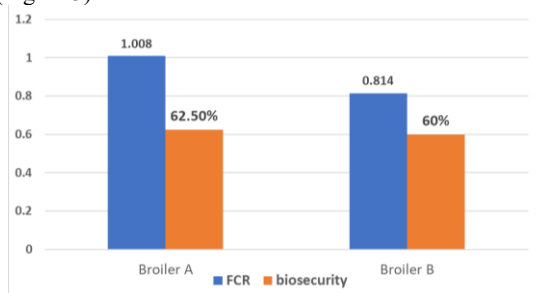


Figure (3): The FCR of different broiler farms in relation to biosecurity.

3.4 .Determination of serum glucose concentration.

(Table1) showed that there was a significant difference between serum glucose concentration and different poultry farms. The level of serum glucose was the highest in layer

Table (1): Serum biochemical analysis in heat stress between farms.

| Parameter | Type | Farm A | | | Farm B | | |
|-----------|---------|----------------------------|----------------------------|-------|----------------------------|---------------------------|-------|
| | | 1 st | 2 nd | mean | 1 st | 2 nd | mean |
| Glucose | Layer | 202.6±7.15 ^{bC} | 232.63±10.39 ^{aB} | 217 | 182.20±5.70 ^{bD} | 211.09±6.35 ^{bC} | 196.5 |
| | Broiler | 275.4±9.97 ^{aA} | 167.82±4.59 ^{bE} | 221 | 215.40±6.47 ^{aBC} | 229.07±9.69 ^{aB} | 222 |
| T3 | Layer | 68.16±3.52 ^{bB} | 91.75±1.95 ^{bA} | 79.5 | 65.58±3.22 ^{bB} | 56.40±2.51 ^{bC} | 60.5 |
| | Broiler | 206.79±10.32 ^{aB} | 327.07±4.02 ^{aA} | 266.5 | 147.79±6.39 ^{aC} | 136.50±2.04 ^{aD} | 141.5 |
| T4 | Layer | 0.69±0.10 ^{bC} | 0.69±0.00 ^{aC} | 0.69 | 1.00±0.10 ^{bAB} | 0.75±0.02 ^{bBC} | 0.8 |
| | Broiler | 1.48±0.25 ^{aA} | 0.87±0.04 ^{aC} | 1.175 | 1.25±0.19 ^{aAB} | 1.07±0.05 ^{aBC} | 1.16 |
| Cortisol | Layer | 0.46±0.08 ^{bC} | 0.94±0.04 ^{bA} | 0.7 | 0.59±0.09 ^{bBC} | 0.19±0.02 ^{aD} | 0.39 |
| | Broiler | 2.24±0.28 ^{aA} | 1.33±0.11 ^{aC} | 1.785 | 1.96±0.13 ^{aB} | 0.23±0.05 ^{aE} | 1.09 |

a, b and c: There is no significant difference ($P>0.05$) between any two means, within the same column have the same superscript letter.

A, B and C: There is no significant difference ($P>0.05$) between any two means, within the same row have the same superscript letter.

3.8.2 .Intestinal histopathology:

As shown in Fig. 4C, the examined intestines collected from layer farm exposed to high temperature showed mild to moderate shortening of intestinal villi with slight epithelial degeneration. The mucosa demonstrated mild epithelial desquamation. The glands within the lamina propria showed slight distortion, with mild vacuolar degeneration in the glandular cells. There was also a mild inflammatory cell infiltration. The muscular layer exhibited slight disorganization and separation of smooth muscle fibers with mild interstitial edema and occasional infiltration of inflammatory cells, indicating the early effects of heat stress. As shown in Fig. 4D, the intestine collected from broiler farm showed demonstrated severe pathological alterations, including significant villus atrophy, marked epithelial degeneration, and desquamation with severe glandular necrosis, and disorganized muscular layers with pronounced edema and inflammatory infiltration.

farm A (217) with low biosecurity score, in contrast, it was the lowest in layer farm B (196.5) with high biosecurity score.

3.5 .Determination of serum T3 concentration.

The T3 had significant differences between different poultry farms, the level of serum T3 was the highest in broiler farm A (266.5), in contrast, it was the lowest in layer farm B (60.5) (Table 1).

3.6 .Determination of serum T4 in concentration.

(Table 1 showed that there were significant differences between poultry farms. The level of T4 was the highest in broiler farm A (1.175), in contrast, it was the lowest in layer farm A.(0.69)

3.7 .Determination of serum cortisol concentration.

(Table 1) the cortisol had significant differences between different poultry farms. The level of serum cortisol was the highest in broiler farm A (1.785), in contrast, it was lowest in layer farm B.(0.39) .

3.8 .Histopathological findings in liver and intestine of broiler and layer chickens.

3.8.1. Liver histopathology:

Layer farm had the liver tissues displayed mild dilatation and congestion in the central veins and sinusoids, accompanied by moderate degeneration in hepatocytes (Fig. 4A). Occasional hepatocellular necrosis was also noted, with mild inflammatory cell infiltration

Broiler farm had liver histopathology revealed marked hepatocellular damage, with severe sinusoidal dilatation, hydropic degeneration, and multifocal coagulative necrosis infiltrated with mononuclear inflammatory cells (Fig. 4B) .

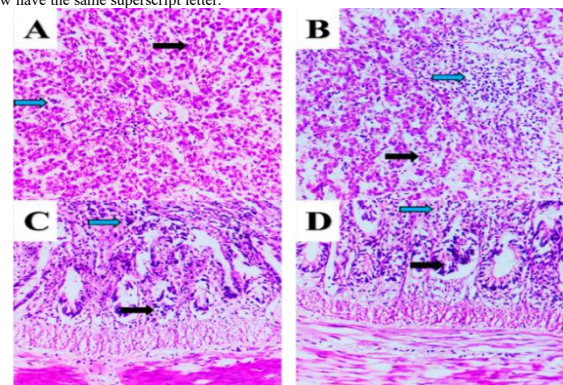


Figure (4): Histopathological analysis of liver and intestinal tissues at different temperatures showing progressive pathological changes. (A) liver of layer chickens showing mild sinusoidal dilatation, hepatocellular degeneration (B) liver of broiler, showing sinusoidal dilatation, vacuolar degeneration, coagulative necrosis, and prominent inflammatory infiltration, (C) intestine of layer, showing mild villus shortening, slight epithelial degeneration, and mild slight disorganization and separation of smooth muscle fibers with mild interstitial edema (D) intestine of broiler, showing significant villus atrophy, epithelial desquamation, severe glandular necrosis, and disorganized muscular layers with pronounced edema and inflammatory infiltration. Blue arrows: inflammatory cell infiltration, black arrows degenerative and necrotic changes. H&E stain X200

4. DISCUSSION

Heat Stress was occurred when poultry are unable to withstand high temperatures, they produce more metabolic heat than they can expel, which has a significant negative impact on their performance, health, and general well-being. Heat stress during broiler growth has been linked to unfavourable meat quality. Heat stress has been demonstrated to adversely impact the quantity and quality of eggs produced by laying chickens (Tsiouris et al., 2018). Numerous health-management techniques are included in biosecurity, which aims not only to stop infections from entering farms (external biosecurity) and from spreading within a flock (internal biosecurity) but also, to stop the bad impact of heat stress (Damiaans et al., 2018).

The biosecurity score of layer farm B was the highest due to applied basic hygienic measure as good ventilation, proper stocking density to control HS, good feed and good water in two layer farms. In contrast, broiler farm B was the lowest biosecurity score. This discrepancy can be attributed to farm have lower biosecurity measures (Greening et al., 2020).

Egg production was the highest in layer farm B with the highest biosecurity score. This attributed to farm have good monitoring of temperature and humidity, good management to birds and good stocking density so the farm less exposure to heat stress effect, while layer farm A was the lowest in egg production and biosecurity score so more exposure to heat stress effect that caused low in feed intake, increase water intake, which decreases the amount of nutrients of Ca, Mg, and P that are available for egg formation, and increased respiratory alkalosis, which reduces blood CO₂ and HCO₃ and elevates blood PH so decreases of egg shell quality, it reduces carbonic anhydrase levels in the shell gland and kidneys and reduces calcium (Kim et al., 2024). FCR was the highest in broiler farm A which had high biosecurity score. Farms with low biosecurity level with low ventilation system led to increase the temperature and humidity inside the poultry house that led to heat stress. Heat stress broilers limits the broiler feed intake due to metabolic effort. HS alters nutrient use, which in turn reduces hunger, which in turn slows growth and reduces feed conversion efficiency (Teyssier et al., 2022).

The serum glucose was varied in different poultry farm. It was the highest in layer farm A with low biosecurity score that contain bad ventilation, temperature exceed the thermoneutral zone above 27°C that led to presence of high heat stress, so increase glucose due to increased level of circulating glucocorticoids which stimulates gluconeogenesis causing high blood glucose levels (Igboke, 2018).

The T3 serum concentration was the highest in broiler farm A with moderate biosecurity score, moderate ventilation so moderate heat stress. The heat stress effects on the thyroid gland action. Their distinct physiological functions are influenced by their separate impacts, as T3's function in cellular metabolism. The coordinated regulation of metabolic and developmental processes, which are essential for the optimal growth and overall health of chickens, is achieved through the combined activities of T3. The balance of thyroid hormones (T3), crucial for regulating body temperature and metabolism, is disrupted under heat stress may increase level of T3 (Mack et al., 2013).

The level of T4 was the lowest in layer farm A with low biosecurity score, bad ventilation system so led to high heat stress. The thyroid gland's size of broiler and layer were decreased by high ambient temperatures which was

responsible for drop in T4 plasma concentration (Gonzalez-Rivas et al., 2020).

The cortisol serum concentration was the highest in broiler farm A with moderate biosecurity score and moderate heat stress. The level of cortisol increase due to effect of high temperature on internal body and blood to outcome high temperature (Shini et al., 2008). Short exposure to an environmental stressor induces corticosterone production (Ataollahi et al., 2020).

Our current study showed some morphological change, the intestinal tract is extremely sensitive to heat stress. Layer farm showed that there was mild to moderate shortening of intestinal villi. The mucosa demonstrated mild pathological changes, including a reduction in villus height and mild epithelial desquamation. The glands within the lamina propria showed slight distortion and in broiler farm showed that significant villus atrophy, marked epithelial degeneration, and desquamation. The integrity of the intestinal barrier is compromised by heat stress leading to an increase in intestinal permeability that also agree with (Slawinska et al., 2019), while liver of layer farm showed that the liver tissues displayed mild dilatation and congestion in the central veins and sinusoids, accompanied by moderate degeneration in hepatocytes and in broiler farm showed marked hepatocellular damage, with severe sinusoidal dilatation, diffuse hydropic degeneration, and extensive areas of coagulative necrosis. Hepatocellular degeneration and occasional necrosis were observed at higher temperature with mild inflammatory infiltration ROS occurred lipid peroxidation that caused liver damage that agree with (Kumar et al., 2012).

5. CONCLUSIONS

Heat stress is affection not infection that can't treat but may be control by many biosecurity practices as good control of temperature, good ventilation, proper stocking density, good feed and water quality. The effect of heat stress may be one of the most proper and frequent reason of sudden drop of egg production in layer and decrease FCR in broiler not only that but also high mortality rate with some serum biochemical and histopathology changes.

6. REFERENCES

1. Ataollahi, M., Nejad, J. G., Song, J.-I., Kim, J.-S. and Park, K.-H., 2020, Effects of feather processing methods on quantity of extracted corticosterone in broiler chickens. *Journal of Animal Science and Technology*, 62,6, 884 -892.
2. Bancroft, J.D., Stevens, A. and Turner, D.R., 1996. *Theory and practice of histological techniques*. 4th Ed. Churchill Livingstone, Edinburgh, London, Melbourne, New York, 4, 184-193
3. Butts, C. L. and Sternberg, E. M., 2008, Neuroendocrine factors alter host defense by modulating immune function. *Cellular immunology*, 252,1-2, 7-15.
4. Damiaans, B., Sarrazin, S., Heremans, E. and Dewulf, J., 2018, Perception, motivators and obstacles of biosecurity in cattle production. *Vlaams Diergeneeskundig Tijdschrift*, 87,3. 150-163.
5. Dewulf, J., Postma, M., Immerseel, F. v., Vanbeselae, B. and Luyckx, K., 2019, How to measure biosecurity and the hygiene status of farms. *Book How to measure biosecurity and the hygiene status of farms*. CABI Wallingford UK.
6. Gonzalez-Rivas, P. A., Chauhan, S. S., Ha, M., Fegan, N., Dunshea, F. R. and Warner, R. D., 2020, Effects of heat stress on animal physiology, metabolism, and meat quality: A review. *Meat science*, 162,108025.
7. Greening, S., Mulqueen, K., Rawdon, T., French, N. and Gates, M., 2020, Estimating the level of disease risk and

- biosecurity on commercial poultry farms in New Zealand. *New Zealand veterinary journal*, 68,5, 261-271.
8. Heidari, S. and Toghyani, M., 2018, Effect of stocking density and methionine levels on growth performance and immunity of broiler chicks. *Iranian Journal of Applied Animal Science*, 8,3, 483-489.
 9. Igbokwe, N., 2018a, Effects of environmental heat stress on reproduction and its management in chickens. *Nigerian Veterinary Journal*, 39,2, 101-114.
 10. Jahejo, A. R., Rajput, N., Rajput, N. M., Leghari, I. H., Kaleri, R. R., Mangi, R. A., Sheikh, M. K. and Pirzado, M. Z., 2016, Effects of heat stress on the performance of Hubbard broiler chicken. *Cells, Animal and Therapeutics*, 2,1, 1-5.
 11. Kers, J. G., Velkers, F. C., Fischer, E. A., Hermes, G. D., Stegeman, J. A. and Smidt, H., 2018, Host and environmental factors affecting the intestinal microbiota in chickens. *Frontiers in microbiology*, 16, 9,235. doi: 10.3389/fmicb.2018.00235
 12. Kim, H.-R., Ryu, C., Lee, S.-D., Cho, J.-H. and Kang, H., 2024, Effects of heat stress on the laying performance, egg quality, and physiological response of laying hens. *Animals*, 14,7, 1076. doi: 10.3390/ani14071076
 13. Kumar, B., Manuja, A. and Aich, P., 2012, Stress and its impact on farm animals. *Frontiers in Bioscience-Elite*, 4,5, 1759-1767.
 14. Larsen, P., 1972, Triiodothyronine: review of recent studies of its physiology and pathophysiology in man. *Metabolism*, 21,11, 1073-1092.
 15. Liang, Q., Huan, S., Lin, Y., Su, Z., Yao, X., Li, C., Ji, Z. and Zhang, X., 2024, Screening of heat stress-related biomarkers in chicken serum through label-free quantitative proteomics. *Poultry Science*, 103,2, 103340.
 16. Lott, J. A. and Turner, K., 1975, Evaluation of Trinders glucose oxidase method for measuring glucose in serum and urine. *Clinical chemistry*, 21,12, 1754-1760.
 17. Mack, L., Felver-Gant, J., Dennis, R. and Cheng, H., 2013, Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry science*, 92,2, 285-294.
 18. Munro, C. J. and Lasley, B. L., 1988, Non-radiometric methods for immunoassay of steroid hormones. *Progress in clinical and biological research*, 285:289-329.
 19. Quinteiro-Filho, W. M., Gomes, A., Pinheiro, M. L., Ribeiro, A., Ferraz-de-Paula, V., Astolfi-Ferreira, C. S., Ferreira, A. J. P. and Palermo-Neto, J., 2012, Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella Enteritidis*. *Avian Pathology*, 41,5, 421-427.
 20. Saeed, M., Abbas, G., Alagawany, M., Kamboh, A. A., Abd El-Hack, M. E., Khafaga, A. F. and Chao, S., 2019, Heat stress management in poultry farms: A comprehensive overview. *Journal of thermal biology*, 84,414-425.
 21. Schmidt, C. J. and Lamont, S. J., 2024, Integrated Genomic Approaches to Characterize and Mitigate Heat Stress in Poultry. *Annual Review of Animal Biosciences*, 13, 1:121-141.
 22. Schmittgen, T. D., 2001, Real-time quantitative PCR. *National Library Of The Medicine*. 25,4:402-8.
 23. Shini, S., Kaiser, P., Shini, A. and Bryden, W. L., 2008, Differential alterations in ultrastructural morphology of chicken heterophils and lymphocytes induced by corticosterone and lipopolysaccharide. *Veterinary immunology and immunopathology*, 122,1-2, 83-93.
 24. Slawinska, A., Mendes, S., Dunislawska, A., Siwek, M., Zampiga, M., Sirri, F., Meluzzi, A., Tavaniello, S. and Maiorano, G., 2019, Avian model to mitigate gut-derived immune response and oxidative stress during heat. *Biosystems*, 178,10-15.
 25. Steel, R. G. D. and Torrie, J. H., 1960, Principles and procedures of statistics.
 26. Teyssier, J.-R., Brugaletta, G., Sirri, F., Dridi, S. and Rochell, S. J., 2022, A review of heat stress in chickens. Part II: Insights into protein and energy utilization and feeding. *Frontiers in Physiology*, 13,943612.
 27. Tietz, N. W. and Berger, S., 1976; *Fundamentals of clinical chemistry*. 2d ed. Saunders. 27, 1263
 28. Tsiouris, V., Georgopoulou, I., Batzios, C., Pappaioannou, N., Ducatelle, R. and Fortomaris, P., 2018, Heat stress as a predisposing factor for necrotic enteritis in broiler chicks. *Avian Pathology*, 47,6, 616-624.
 29. Zhang, X., Zhang, X., Sun, N., Wang, S., Wang, X. and Jiang, Z., 2019, High production of levulinic acid from cellulosic feedstocks being catalyzed by temperature-responsive transition metal substituted heteropolycarboxylic acids. *Renewable Energy*, 141,802-813.