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Physiological and Immunological Responses to Episodic Heat Stress in Broiler Chicks Reared in Uncontrolled Ventilation

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Abstract: Broiler chickens were reared from one to twenty two days old in uncontrolled ventilation, then they were exposed to daily cyclic constant 41°C for 4 hours in completely isolated subunit, the exposure was repeated daily until 31 day old. Blood sampling were taken at 1 hour before and immediately after heat exposure at 1st, 5th and 10th day of exposure. Different plasma physiological and immune parameters were measured. It could be concluded that heat stress has speed, immediate and extreme negative influences on assessed parameters which resulted in to say that metabolites glucose, cholesterol and malondialdehyde (MDA) in addition to immunoglobulin (IgM and IgG) as well as ascorbic acid may be considered useful as good quantitative bio-markers for rapid heat stress effects. Post Hoc statistics' test revealed that all parameters level were inhibited except glucose and MDA which increased significantly at the level 0.05 post every exposure to heat, but chickens could restore their normal concentrations at next pre-exposure of latest stages when compared with basal level. This model of response may indicate the ability of this type (breed) of broiler to adapt to the high heat temperature. Statistics' Person test revealed that most assessed indices were correlated with other significantly at the level 0.01 except (IgM and IgG) which were inhibited greatly by heat stress without any significant correlation with the metabolites, it may indicated that this relation was indirect. Therefore necessary to say that further physiological mediators such as leptin, growth hormone, corticosteroids and cytokines-with wider period-were recommended for future studies to clarify this relationship to present an integral insight to manage poultry physiological and immunological functions under heat stress.

Key words: Poultry · Heat stress · Immunoglobulin · Ascorbic acid · Glucose · Cholesterol · MDA

INTRODUCTION

Physiological stress is one of many concerns facing the modern broiler producer. It is one of the most important factors adversely affecting overall poultry production in the tropics. The domestic fowl is a homoeothermic which can live comfortably only in a very relatively narrow zone of thermo-neutrality ranged from 18-22°C within which the heat from normal maintenance and productive functions of the animal in non-stressful situations offsets the heat loss to the environment without requiring an increase in rate of metabolic heat production [1]. When the thermo-neutral zone exceeds the upper critical temperature, animals must employ evaporative heat loss mechanisms such as sweating and panting, the animal is then considered to be heat stressed [2]. Elevation of environmental temperatures has deleterious effects on poultry, reducing rate of growth, basal metabolic rate, immunity and mortality [3-5]. Research has yet to reveal mechanisms that would allow the producer to efficiently minimize the detrimental impacts of physiological stress on broiler performance [6]. Stresses leads to protein and lipid catabolism in turn elevating plasma cholesterol concentration snd corticosterone which inhibits antibody production [7]. Furthermore, heat stress is known to decrease T-helper 2 cytokines [8], which are important for antibody production [9]. The oxidative stress induced by heat stress was reflected in the increase of protein oxidation (increased protein carbonyls) and lipid peroxidation increased Malondialdehyde (MDA) [10,11]. Chronic heat exposure decreases metabolic oxidation capacity in skeletal muscle of broiler chickens [12]. Sahin *et al.* [13] showed that exposure to high ambient temperature induced decreases hepatic superoxide dismutase, catalase and glutathione peroxidase activities but increases in

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hepatic malondialdehyde concentrations suggesting that supplemental resveratrol reduces oxidative stress in heat-stressed quails through modulating the hepatic heat shock proteins and nuclear transcription factors. Kutlu and Forbes [14] reported that exposure to high ambient temperature may result in hypercholesterolemia. In addition, marked elevation of temperature increases blood glucose and cholesterol concentrations [15]. Al-Azraqi [16] showed that metabolites such as glucose, cholesterol and triglycerides concentrations have been increased in heat-stressed pigeons. Similar findings of heat stress effects on concentrations of glucose [17, 18], cholesterol [19] and triglycerides [20] have been reported in birds. Soleimani and Zulkifli [21] found that heat exposure significantly decreased serum levels of glucose, showed lower LDH concentration, stage of heat treatment had no significant effect on LDH activity, the effect of heat stress on serum cholesterol in chickens has been inconsistent. Physiological responses to noisy also documented in broilers included increased levels of corticosterone, glucose, triglycerides and cholesterol [22]. The heat stress affects consequently the rate of the metabolic pathways [23] and the concentration of the anabolic and catabolic products, such as the molecules of growth hormone [24], cholesterol [17], corticoid hormones and thyme hormones [25, 26]. Vitamin C helped to improve the carcass traits of chicken broilers reared at a high ambient temperature of 32°C [27], to improve the broiler performance [28], as well as a decrease in serum corticosterone and MDA concentrations. A combination of vitamin C (250 mg/kg of diet) and chromium (400 mg Cr/kg of diet) may offer a potential protective management practice in preventing heat stress-related depression in performance of broiler chickens, because this vitamin could decrease corticosterone level in the blood circulation [29].The most clearly established functional role for vitamin C involves collagen biosynthesis and corticosteroids in the adrenal glands may involve ascorbic acid related hydroxylation steps [30]. The presence of antioxidant could partially inhibit adversely oxidative protein denaturizing and would improve nutrients digestibility and feed efficiency [31]. It was found that the heat stress not only had an increase in the H/L ratio, indicating the birds were under increased stress, but also a decrease in antibody titer. Heat stress reduces immune response [32]. In addition, antibody production was significantly inhibited and mortality was higher in hens in the heat stress compared to the cyclic and control groups [33]. El-Ghamdi [34] concluded that heat stress has fast, direct and sever effects on ascorbic acid, IgG and IgM of plasma concentration on the same day of heat exposure. Released corticosterone causes impaired immune system function and regression of the lymphoid tissues [6]. In poultry production, it is very important to improve immunity so as to prevent infectious diseases, minimizing immunosuppression and its impact is an important strategy for success in the broiler industry [35]. Significant deviation from normal biochemical values as well as hormonal disturbances is the outcome of stress in birds [36].Because Dammam region in Saudi Arabia-characterized by hot weather.

The Objectives: Was to assess the effects of episodic heat stress on some physiological functions in broilers metabolites (glucose, cholesterol, MAD), immunological responses (IgM, IgG) and ascorbic acid concentrations in broiler under heat stress in uncontrolled ventilation.1-To determine the ability and quality of these indices as benefit biomarkers for express response to heat stress in broiler.2-To find the correlation between physiological metabolites and immunity because most of works neglected evaluation of the impact of interaction of studied parameters under heat stress. Results of this study will contribute futurity in improvement of physiological functions and minimize immunosuppression of broiler reared in open system in hot regions.

MATERIALS AND METHODS

One day-old broiler chicks (Cobb 500) were obtained from Dammam Modern Poultry Company, Saudi Arabia and housed under Uncontrolled ventilation (Open system) in experimental unit area 20 m^2 [37], which was a conventional open-sided house with temperatures (minimum, 31.9±1.61°C, maximum 34.8±1.142°C) at mean 33.8 ± 1.02 which exceeded the temperature recommended by Sainsbury [38]. The relative humidity Rh% was between (52±3.19% and 59.8 ± 4.54%), it was not exceed Rh% recommended by Maroufyan *et al.* [35] and Aengwanich and Simaraks [39].Two fans were run through the day with maximum speed. Lighting 23L: 1D.Wood shaving litter was used at 3-5 cm thickness and renew weekly. All disinfection and preparation procedures were done in the unit before chicks receiving, feed and cold water were provided *ad libitium* with traditional prophylactic. Chicks were allowed to adapt to new environment unit for three weeks with Stocking density: (10 birds/m^2) [40]. Formalin 0.1% as spray was used to reduce ammonia concentration to normal (1 part/million), when they reached 22 day-old, heat stress experimental was started.

Exposure to Heat Stress: Chicks were reared at uncontrolled ventilation from 1 to 21 day, then and to impose a stress response. Thirty birds at 22 days old [41, 42] were exposed daily to cyclic constant 41°C for 4 hrs from 01.00-04.00 pm (4h heat stress episodes) as decided by Mashaly *et al*. [33], in completely isolated subunit in the experimental unit where the electric heater was 2000 watts placed at 50 cm height of floor. This exposure was repeated daily till 31days old [34].

Blood Sampling and Plasma Estimations: Individual blood samples were taken at one hour before and immediately after treatment with heat stress at 1st, 5th and 10th day of exposure (22,26 and 31 days old) and collected from wing vein of the same birds' group in tubes treated with anticoagulant, after centrifugation at 3000 r.p.m./15 min. Separated plasma was kept in-18°C until the following assays.

Antibodies and Ascorbic Acid: Immunoglobulin estimation (IgG and IgM) was done immunochemically using tripantigen plates [43], kits reference (CU50045SD, Serotec, Oxford, UK). Immunoglobulins were measured by nephlometry using Beckman Array Analyzer (Beckman, Instruments Inc., California, USA).Total ascorbic acid concentrations were estimated spectrophotometrically by method of Maickel [44]. All assays included manufactures calibration standards for comparison with chicken samples. Analysis for total glucose and cholesterol, MDA were conducted on an automated spectrophotometer (Ultraspec, 300, Cobas-Mira, Roche Diagnostic System, CH4070, Basel, Sw 1t2er land) using stand and diagnostic kits.

Statistical Analysis: Data were analyzed using SPSS software ver. 17, ANOVA was applied to compare the means of various treatment groups, then Post Hoc test were done to obtain LSD. Person correlation test was carried out. The results were expressed as mean ± standard error of mean. Statistical significance was considered at the level 0.05 and 0.01.

RESULTS AND DISCUSSION

Stress physiological responses were measured by blood biochemistry [14, 45]. It was suggested that blood biochemistry such as serum concentrations of immunoglobins, ascorbic acid, glucose, malondialdehyde (MDA) and cholesterol may be considered useful and convenient measurements for stress physiological response.

Effect of Episodic Exposure to Heat Stress on Plasma Concentration of Glucose, MDA in Chicks: Data presented in Table 1 indicated that at pre-exposure to heat in stage 2 and 3, levels of glucose and MDA did not affected by heat stress when compared with Basal level, but their levels increased significantly ($p< 0.05$) at every post exposure to heat stress, the maximum values of them were (15.13±0.27) and (2.40±0.037) µ mole/L, respectively when compared with their pretreatment and basal levels. In this study, heat stress was assessed by glucose and MDA that has been shown to be increased significantly. The elevated blood glucose level was respond to heat in post exposure stages and was consistent with earlier studies reported by Zulkifli *et al.* [17], Kataria *et al.* [18], Sahin *et al.* [19] and Rashidi *et al.* [46]. Recent evidence have been reported a significant increase of glucose and MDA in birds as response for heat stress [16] and of glucose for noise[22], this increase could be explained by Habeeb [47] who found that plasma insulin concentration decreases significantly with high temperature. Decreased plasma insulin concentration due to the large decrease in potassium retention under heat stress has also been reported in cow [48, 49]. Elevation in blood glucose level also may be attributed to increase in glucocorticoids secretion which play a major role in glucose metabolism [50]. After broilers were exposed to high ambient temperature, their body temperature increased more than the normal body temperature [51] and the corticosterone which stored in adrenal cortex was released into the blood circulation to improve increase metabolism which consequently exert catabolic effects [49, 52, 53]. Richard [54] found that MDA and glucose has been indicated to be good quantitative measures of stress. On the contrary, in poultry [55] and in sheep [56] heat stress resulted in hypoglycemia, they may be attributed that to a decrease in concentration of thyroxine. The oxidative stress induced by heat or exercise stress was reflected in the increase lipid peroxidation via (increased MDA) [10, 11,57] who reported that, high ambient temperature has been shown to increase the free radicals and other Reactive Oxygen Species (ROS) production in body fluids and tissues. Although, low levels of ROS are essential for many biochemical processes, their accumulation may be due to over-production or a decreased antioxidant defense, leads to damage of biological macromolecules and disruption of normal cell metabolism. Previous studies found that heat stress [19], exercise [10] and ischemia [58] can result in increased protein oxidation and lipid peroxidation. Changes in glucose and lipid could be explained by AL-Azraqi [16] who found Parallel increase in the concentrations of leptin

and metabolites and oxidative markers in pigeon and found that leptin seems to partially modulate the oxidative stress in pigeons. Leptin directly affects glucose and lipid metabolism in isolated adipocytes, myotubes and skeletal muscles [59, 60]. Moreover, in pregnant obese women, increased fat oxidation has been correlated with increasing leptin concentration [61]. In current study MDA and glucose returned to its normal levels at pre exposure to heat in two latest stages, it may seem to results of Azad *et al.* [12] who concluded that chronic heat stress did not induce oxidative damage to a major extent.

Effect of Episodic Exposure to Heat Stress on Plasma Concentration of Cholesterol in Chicks: Regarding to cholesterol, data in Table 1 showed that heat stress also markedly at ($p<0.05$) decreased cholesterol at every post-treatment, it reached minimum value (4.30±0.040) µmole/L at stage 2 when compared with basal level (6.71± 0.134) µmole/L. Alnaimy *et al.* [62] indicated that the phenomenon could be attributed to an increase in total body water or a decrease in acetate concentration, which is the primary precursor for the synthesis of cholesterol, also Yalçin *et al.* [41] found that broiler (65 weeks) which were exposed to high daily cyclic heat 38.5°C treatment reduced triglycerides. In contrast of current results, some researchers found that the effect of heat stress on plasma cholesterol in chickens has been inconsistent, Kutlu and Forbes [14] reported that exposure to high ambient temperature may result in hypercholesterolemia, But Barbour *et al.* [63] documented that the mean cholesterol level showed was no significant difference among the different treatments of heat acclimatized birds, however they agree with this study in their tow groups (heat non acclimatized birds and fasted birds which had a significant drop in cholesterol level compared to the control). Based on the results of Soleimani and Zulkifli [21] stage of heat treatment had not a significant influence on serum cholesterol concentration, Plasma metabolites of cholesterol and glucose concentrations decreased significantly by short (2 days) period of exposure to solar radiation but prolonged exposure (4 days) increase these parameters in both Balady and Damascus goat breeds [64]. Chloupek *et al.* [65] did not find any changes in plasma triglycerides and glucose concentrations in broilers exposed to noise at 80 and 100 dB for 10 min, however Bedáňová *et al.* [22] also described a significant increase in the cholesterol level in broilers following 10 min of noise exposure at 100 dB, which is in contradiction with our findings, but they found a significant increase in plasma triglycerides and glucose concentrations during the first 12 min in broilers exposed to 100 dB noise in experiment

Effect of Episodic Exposure to Heat Stress on Plasma Concentration of Ascorbic Acid in Chicks: Data in Table 1 revealed that ascorbic acid levels in chicks plasma decreased significantly by exposure to heat in most stages when compared with basal level at 22 day pre-exposure (57.10± 1.04), but showed highly significance decrease at post-treatment when compared with its level at pre-treatment of every stage, ascorbic acid

Table 1: Effect of heat stress on physiological and immunological parameters in plasma of 22 day old broilers rearing in open system

	Stages					
	Stage 22 day		Stage 26 day		Stage 31 day	
Parameters	Pre-exposure to heat	Post-exposure to heat	Pre-exposure to heat	Post-exposure to heat	Pre-exposure to heat	Post-exposure to heat
Glucose (µM/l)	11.0± 0.1	13.5± 0.4 [a,b]	10.9± 0.1	15.1± 0.3 [a,b]	10.6± 0.1	15.1±0.03 [a,b]
MDA (µM/l)	0.82 ±0.01	2.10±0.04 [a,b]	0.81±0.01	2.4±0.04 [a,b]	0.9 ±0.0	2.2±0.9 [a,b]
Cholesterol (µ M/l)	6.7± 0.1	5.0±0.03 [a,b]	6.5±0.1	4.4±0.03 [a,b]	6.5±0.1	4.3±0.04 [a,b]
Ascorbic acid (mg/dl)	57.1±1.0	32.±0.3 [a,b]	56.1±0.1	29.1±0.3 [a,b]	55.1±0.1 [a]	27.2±0.4 [a,b]
IgG (g/l)	1325. ±5.16	1100.±1.9 [a,b]	1340.±7.2 [a]	980.±2.3 [a,b]	1350.±5.8 [a]	950.±2.3 [a,b]
IgM (g/l)	132. ± 0.6	105.±0.4 [a,b]	121.±0.6 [a]	90.±0.4 [a,b]	120.±0.7 [a]	85.±0.4 [a,b]

Data indicate mean ±SE

[a]. there was a significant difference with basel level in the same row at ($p = 0.05$).

[b]. there was a significant difference in every stage between before and after exposure to heat in the same row at ($p = 0.05$).

Stage 1: Starting level (Basal level). At 1st day after heat stress exposure.

Stage 2: At 5th day before daily heat stress exposure. At 5th day after daily heat stress exposure.

Stage 3: At 10th day before daily heat stress exposure. At 10th day after daily heat stress exposure.

MDA = Malondialdehyde = a lipid peroxidase indicator.

level decrease gradually among stages and found that average minimum value was (27.20± 0.375) mg/L in last one. These results were agreed with Richards [66] who reported that this vitamin is used in the poultry diet because of their anti-stress effects, Sahin *et al.* [55] found that the negative effect of heat stress was the decreased concentration of vitamins C in the serum and liver, El-Ghamdi [34] reported similar results of ascorbic acid in plasma chickens exposure to heat stress reared in closed system. Stress increased mobilization of vitamin from tissue and their excretion [30, 49, 67, 68]. Vitamin C is involved in a number of biochemical processes and is necessary for various biosyntheses (colagene, carnitine, 1, 25 dihydroxyvitamin D, adrenaline etc.) as well as for the regulation of diverse reactions (secretion of corticosterone, regulation of body temperature, activation of the immune system) [2]. The results of Wang *et al.* [69] suggested that dietary vitamin C supplements of 400mg/kg feed provide optimal effects on growth performance, antioxidant status and parameters of humoral immunity of layer ducklings.

There was a significant correlation at the 0.01 level (1 and 2-tailed) between most of estimated parameters: negative significant correlation between ascorbic acid with glucose and MDA, while positive between ascorbic acid and cholesterol. Also there was a significant correlation reflection between MDA with glucose and cholesterol. And then a negative one between glucose and cholesterol. These significant correlations make sure the importance of vitamin C assay and its correlation with other physiological parameters here, Gursu *et al.* [70] found reported that vitamin C has proved to reduce the serum cholesterol level and to alleviate the effects of heat stress in broiler quails. Ascorbic acid has been widely used to reduce the stress in chickens, because of decreasing corticosterone level in the blood circulation [29, 71]. Similarly Gross [72] reported that ascorbic acid could improve immune response in birds under stress and disease condition. Current correlations between assessed parameters could be clarified also by some previous reports which documented that the heat stress affects the water and ionic balance in broilers [73] and thus affecting the homeostasis in the body that relies on metabolic pathways that occur in the extra and intra-cellular aqueous environment of the chicken cells [23] as well as and the concentration of the anabolic and catabolic products, such as the molecules of growth hormone [24] and cholesterol [17].

Effect of Episodic Exposure to Heat Stress on Plasma Concentration of Antibodies in Chicks: Data in Table 1 showed that at 2nd and 3ed pre-exposure to heat, IgG did not show any reduction in its level but IgM showed a significant decrease in all stage when compared with basal level. Two immunoglobulins revealed highly significant decrease post every exposure to heat treatment gradually (1100.00 ± 1.89, 980.00 ± 2.30, 950.00 ± 2.30, g/L) for IgG and (105.00 ± 0.36, 90.00 ± 0.43, 85.00 ± 0.433 g/L) for IgM, respectively. There is highly significant reduction in IgG and IgM levels post treatment when compared with their levels pre every stage and the minimum values appeared at the latest stage. Similar behavior appeared for ascorbic acid level during the experiment which may indicated that there was a commutative effect of heat on ascorbic acid, IgG and IgM, levels. Antibodies in all stages were probably affected to some degree by high temperature, this result is in agreement with those obtained by Zulkifi *et al.* [74] and Ogle *et al.* [75] who showed that heat stress caused a reduction in antibody synthesis and production in young chicken, this reduction could be indirectly due to an increase in inflammatory cytokines under stress, which may stimulates the hypothalamic production of corticotrophin releasing factor [76], corticosterone inhibits antibody production [7]. Jain [77] reported that corticosteroid caused lympholysis in blood and lymphoid tissue, increased shift of lymphocytes from blood to other body compartments, or both in mammals, moreover, T cell in blood and tissues are most sensitive to the lympholytic effect. Heat stress is known to decrease T-helper 2 cytokines, which are important for antibody production [9, 8]. Lower concentration of antibodies (IgM, IgG) compared with basal level may be attributed to the negative effects of heat stress, these results were promoted by Bobeck and Cook [78] who found that the antibody was nearly denatured by five minutes (79% lost); their results showed a downward slope in the loss of antibodies as the time in heat increases. Also current results were similar to study of EL-Ghamdi [34] who noticed that heat exposure to 41°C for 4 hours episodes had fast, direct and intensive effect on the reduction of IgM and IgG concentrations. Previous studies showed that heat-induced immune-suppression may depend on many factors as breed of bird [79] or the length and intensity of the heat exposure [80]. On the contrary Heller *et al.* [81] found significantly increased antibody titers to SRBC following heat exposure; however Donker *et al.* [82] found that heat exposure did not reduce antibody

production to SRBC. Mashaly *et al.* [33] attributed the difference in these findings to age and type of bird used or due to the methodology that was applied.

Some results of assessed parameters here revealed swift and immediate effect which were similar to results of El-Ghamdi [34] who suggested that antibodies concentration (IgG, IgM) and ascorbic acid in plasma are good indicators for fast, direct and sever action of heat stress (41°C) more than H/L ratio on the same day of heat exposure. Also Table 1 showed that the birds have ability to restore their level of most parameters to normal at next pre-treatment stages 26 and 31 day old which indicated that stress heat effects was temporary, this result is in agreement with those obtained by Heller *et al.* [81] who found that the measurements of lymphocyte counts and plasma ascorbic acid were back to normal, Our experiment were carried out in Dammam city in KSA which located in hot region. It is generally considered that native or indigenous breeds of chickens in the tropical countries are better able to withstand high ambient temperatures [83] and with Bedáňová *et al.* [22] noticed a subsequent decrease of triglycerides and glucose levels can also be seen approximately from time 12 min and a gradual return to the pre stress value at 28 min of noise and reported that type of response may indicate the ability of broilers to adapt to an increased level of noise at 100 dB intensity after the first 12 min of exposure. Matteo *et al.* [84] studied the heat stress in two poultry genetic types and found that Ross birds could better adapt to heat stress respect Naked neck ones making metabolic changes in their antioxidant mechanisms. It is worth noting, that Barbour *et al.* [63] reported that measures can be used to indicate a stress response and the measurements can be done without harming the animal, future investigation should include a wider period of heat acclimatization, which could result in differences of certain important physiological parameter(s) such as the level of GH.

CONCLUSION

- Exposure chicks reared in uncontrolled ventilation to heat stress as described could disturb all estimated parameters, increase glucose, MDA and decrease IgG, IgM, vitamin C and cholesterol.
- Generally all parameters changed significantly post every exposure to heat but chicks could restore their normal levels at next pre-exposure stages at 26 and 31 day old, this type of response may indicate the ability of this broilers type to episodes daily for 10 days exposure to heat stress (41°C).
- Post Hoc test (LSD) revealed that there were a clear significant gradual decrease in concentrations of ascorbic acid, IgG and IgM among three stages post exposure to heat, at the 0.05 level, which indicated that there was a cumulative effect of heat stress on these three indices
- Current study concluded that heat stress has swift, immediate and extreme influence on levels of ascorbic acid, IgG and IgM and metabolites indices (glucose, cholesterol and MDA) in plasma concentration on the same day of heat stress.
- Results showed that indices used here, were blood biochemistry: glucose, MDA, cholesterol, ascorbic acid and immunoglobulin (IgG and IgM) may be considered useful as good quantitative biomarkers during ten days of heat physiological stress
- It is worth noting that this work searched a relationship between the different measures used in the study, Person test showed that all assessed parameters except (IgM and IgG) were correlated significantly at the 0.01 level, this may be attributed to their strong physiological relation metabolically, if any of them disturbed by heat stress the body homeostasis would be affected.
- Although this study found intensive negative effects of heat stress on all parameters including IgM and IgG, but statistical Person test revealed no significant correlation between these immunoglobulin and other physiological assays, this may be indicate that there was indirect link between them.
- Other mediators such as leptin, corticosteroids, growth hormone and cytokines must be suggested to future study-during wider period-for the relationships between antibodies and metabolic indices to produce an integral insight to manage poultry physiology and immunity under heat stress.

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