



The Protective Role of *N*-Acetyl Cysteine (Nac) on Broilers Exposed to Chronic Heat Stress

Faten Zahran¹, Samia S. Hagag^{2*}, Osama E. Ramadan³, Hoda ML. Abdallah²,
Mohamed S. Gharib⁴

¹Chemistry Department, Faculty of Science, Zagazig University, Egypt.

²Biochemistry Department, Animal Health Research Institute, Agriculture research centre, Egypt.

³Pharmacology Department, Animal Health Research Institute, Agriculture research centre, Egypt.

⁴Chemistry Department, Faculty of Science, Port-Said University, Port-Said, Egypt.

* Corresponding author: Samia_Hagag@yahoo.com

ABSTRACT

In hot areas, heat stress (HS) is one of the most prominent stressors in the broiler industry. To provide comprehensive information about the effect of *N*-acetyl cysteine (NAC) on hematology, biochemistry, and thyroid hormones in broilers under thermoneutral (TN) (22 ± 4 °C) and heat stress (HS) (34 ± 2 °C), The current study was carried out. A total of 80 one-day-old Cobb broiler chicks were used, with 20 birds in each group receiving either a control (basal diet) or a NAC (100 mg/Kg diet). Chronic HS at 42 days resulted in a considerable decrease in HGB, PCV, RBCs, total protein, Albumin, serum T3 and T4, while greatly increasing H/L, triglycerides, cholesterol, LDL-cholesterol, uric acid, AST, ALT, ALP, TBIL, DBIL, and creatinine. When compared to HSC broilers at 42 d old, NAC supplementation greatly increased PCV content with a substantial decrease in total leukocytic count, heterophils, and H/L ratio with an increase in lymphocytes and monocytes. When compared to HSC population, chickens given NAC had a significant decrease in serum AST, ALP, ALT and triglyceride levels, as well as a significant increase in serum T3 concentration.

Keywords:

Heat stress, Thermoneutral, Broiler chickens, NAC, *N*-acetyl cysteine.

1. INTRODUCTION

Broilers are genetically selected for rapid growth and high feed productivity, according to the poultry industry. Because of their rapid metabolic rates and lack of sweat glands, they are extremely sensitive to high ambient temperatures [1] that can lead to high mortality [2]. Chronic HS is characterised by a cycle of extreme heat followed by a safe temperature for the remainder of the day [3]. Oxidative stress caused by HS has been identified as a primary factor in the pathogenesis of so many diseases, the reduction of production

results, and the oxidative deterioration of poultry meat [4]. Broilers are raised in open-house systems in tropical and subtropical countries, as opposed to environmentally regulated housing systems in hot regions. However, due to the high cost of cooling poultry homes, dietary manipulations are becoming more popular as potential approaches to alleviating the detrimental effects of elevated air temperatures in poultry [5].

Elevated temperature increases serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and creatinine [6], as well as cholesterol, triglycerides, and low-density lipoproteins (LDL-C) in broiler chickens [7]. Chronic heat stress induces a variety of hormonal responses in warm-blooded animals, including impairment of endocrine functions. Thyroid hormones are of particular interest, especially triiodothyronine (T3) and thyroxine (T4). At high ambient temperatures, serum concentrations of T3 and T4 and immune system suppression have been observed [8].

N-acetylcysteine (NAC) is a powder of white crystalline shape that has a faint odour. It is a naturally occurring sulfur-containing compound produced in living organisms from the amino acid cysteine. It is a precursor to L-cysteine and can also be quickly metabolised to produce GSH, which protects cells and cellular components from oxidative stress due to its strong antioxidant impact [9]. N-acetylcysteine contains a thiol, which acts as both a precursor for glutathione synthesis and an enhancer of the cytosolic enzymes involved in glutathione restoration, allowing it to directly protect cells from oxidative damage by interacting with reactive oxygen species [10]. NAC has shown to directly react with ROS including such hydroxyl radicals, hypochlorous acid, and hydrogen peroxide and ascorbate [11].

2. MATERIALS AND METHODS

I. MATERIALS

I.1 Diets: NAC is a white sterile crystalline powder with a very mild odour that was acquired from Medical Union Pharmaceuticals Company, Abu-Sultan, Ismailia, Egypt. NAC was included in the basal diets at 100 mg/Kg diet at 21 years of age [12], and the basal diets met the NRC guidelines [13].

I.2 Birds in Experiment Design: On the tenth of Ramadan in Al-Sharkia governorate, Egypt, eighty healthy one-day old Cobb broiler chickens were bought from El-Kahera Poultry Company and checked during the summer season (August). They were housed in floor pens bedded with fresh wood shavings litter of 8 cm depth and given 10 birds per m² in Egypt's Animal Health Research Institute's experimental poultry provided unit and fed with the basal diet until 42 d of age. All chickens were raised at 32°C from day one to day twenty, which was then reduced by 2°C per week.

Table 1: The broiler diet structure (for 100 kg feed)

	Starter (0-21 days of age)	Finisher (22-42 days)
Components		
Corn	48.20	58.70
Wheat	8.00	7.50
Soybean meal (40%)	28.50	20.50
Protein concentration (50%)	10.00	10.00
Vegetable oil	4.00	2.50
Salt	1.00	0.50
Vit + Min mix*	0.30	0.30
Total	100.00%	100.00%
Composition calculated**		
ME (kcal/kg)	3079.00	3102.60
Crude protein	22.06	19.37
Lys.	1.21	1.03
Meth + Cys.	0.82	0.75
Ca (%)	1.2	0.95
P (%)	0.44	0.42

*Per kg of diet, the vitamin and mineral combination provided.

** calculations determined according to NRC [12].

II. METHODS

2.1 Experimental design

At day 21, all chickens were divided into four equal groups of twenty chickens each: Class I: Thermoneutral state (TN), with daily ambient temperature of ($22\pm 4^{\circ}\text{C}$) and humidity levels of (55 ± 3), divided into two groups: thermoneutral control group (TNC) supplemented with broiler diets only but thermoneutral N-acetyl cysteine group (TNN) supplemented with NAC (100 mg/Kg diet). Class II: Heat stress condition (HS), with an ambient temperature of ($34\pm 2^{\circ}\text{C}$) for 8h/d. and a relative humidity of 65% split into two groups: heat stress control (HSC) group received only a basal diet and heat stressed N-acetyl cysteine (HSN) group received NAC dose of 100 mg/Kg diet.

2.2 Haematological parameters and blood leucocyte profiles

On the 42nd day, five birds were chosen for each treatment and venous blood was collected from the wing vein into EDTA anticoagulant treated vials. Haematological indices presented in table (2) were measured using the Sysmex XT 2000 IV Automated Haematology Analyzer (Japan), as stated by [14]. For microscopic examination of the WBC with Wright-Giemsa complex staining, one 100 WBCs were counted per sample using the Neubauer haemocytometer according to the procedure of [15] then H/L values were calculated.

2.3 Serum biochemical parameters

Feed was removed from all birds for 12 hours prior to blood sample collection. At 42 days, 4 mL of blood was collected from the wing vein and allowed to clot before being centrifuged at 3000 rpm for 15 minutes to separate serum, which was then stored in Eppendorf tubes (1.5 mL) stored at -20°C for estimation of total protein, albumin, AST, ALT, ALP, total and direct bilirubin, uric acid, creatinine, Total cholesterol (TC), Triacylglycerol (TAG), High-density lipoprotein (HDL-C), and LDL-C were measured using colorimetric techniques and the process outlined by Spinreact Co., Spain [16]. All of these assays were carried out spectrophotometrically on a Turner 690 Chemistry Analyzer in the chemistry department of AHRI in Egypt. The serum globulin concentration was determined by the difference between the concentration of total protein and albumin.

2.4 Serum thyroid hormones analysis

T3 and T4 hormone levels are measured using Biochrom Asys Expert Plus Microplate Readers from Biochrom Ltd., Cambridge, UK, using enzyme-linked immunosorbent assay (ELISA) kits approved for chicken analysis (MyBioSource, Inc. San Diego, California, USA) [17].

2.5 Statistical Analysis

The obtained collected data were analysed using the statistical computer programme SPSS 16.00 Software and a one-way ANOVA procedure (SPSS Inc., Chicago, IL, USA) [18].

3. RESULTS

3.1. Effect of HS and NAC on Haematology

When compared to the TNC population, chronic heat stress (HSC group) resulted in a substantial decrease in RBC, PCV, and monocyte counts. In addition, there was a substantial ($P<0.05$) increase in total leukocytic count, heterophils, and H/L ratio with a significant decrease in lymphocytes, monocytes, and eosinophils when compared to control broilers subjected to room temperature. NAC supplementation greatly increased PCV parameters with a substantial ($P<0.05$) decrease in total leukocytic count, heterophils, and H/L ratio

with an enhancement in lymphocytes and monocytes when compared to HSC broilers at 42 years old (**Table 2**).

3.2. Effect of HS and NAC on serum metabolites measurements

When compared to the TNC group, HSC broilers had a significant decrease ($P<0.05$) in total protein and albumin levels while having a significant increase ($P<0.05$) AST, ALT, ALP, T. bilirubin, D. bilirubin, Uric acid, and creatinine levels. Extreme ambient temperature resulted in a substantial elevation in serum total cholesterol, triglyceride, and LDL-C data at the end of the experiment. On the 42nd day of chicken life, broilers experimentally supplemented with NAC in an HS environment had a substantial ($P<0.05$) decrease in serum AST, ALT, ALP and triglycerides on the 42nd day of age when compared to the HSC group (**Table 3**).

3.3 Effect of HS and NAC on thyroid hormones

With respect to results of thyroid hormones, heat stress resulted in a substantial decrease in serum T3 and T4 hormone concentrations as compared with TNC group. When compared to HSC broilers, the addition of NAC increased serum T3 concentration markedly ($P<0.05$).

Table 2: Effect of *N*-acetyl cysteine (NAC) on haematological indices of broilers at 42 d of age under thermoneutral and heat stress conditions ($34\pm 2^\circ\text{C}$) (Mean \pm SE).

Parameters ^{1,2}	Treatments ²			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNN (0.5%) (n=5)	HSC (n=5)	HSN (0.5%) (n=5)
PCV %	31 \pm 1.39 ^a	31.6 \pm 2.2 ^a	24.8 \pm 0.71 ^b	31 \pm 0.93 ^a
Hb (g/dl)	2.1 \pm 0.38 ^a	11.494 \pm 0.22 ^a	9.00 \pm 0.23 ^b	10.17 \pm 0.22 ^b
RBCs ($\times 10^6/\text{mm}^3$)	3.73 \pm 0.27 ^{ab}	3.884 \pm 0.18 ^a	2.454 \pm 0.05 ^c	3.082 \pm 0.29 ^{bc}
MCV (fl)	85.45 \pm 8.62 ^a	82.68 \pm 8.19 ^a	101.33 \pm 4.22 ^a	103.97 \pm 9.27 ^a
MCH (pg)	32.96 \pm 1.92 ^a	29.91 \pm 1.76 ^a	36.722 \pm 1.08 ^a	34.35 \pm 3.69 ^a
MCHC (g/dL)	39.38 \pm 2.32 ^a	37.02 \pm 2.38 ^a	36.44 \pm 1.54 ^a	32.90 \pm 1.01 ^a
Platelets	5.55 \pm 0.43 ^a	5.57 \pm 0.37 ^a	4.876 \pm 0.18 ^a	4.998 \pm 0.29 ^a
WBCs ($\times 10^3/\text{mm}^3$)	4.46 \pm 0.15 ^{bcd}	4.344 \pm 0.16 ^{cd}	5.768 \pm 0.24 ^a	5.068 \pm 0.33 ^b
Monocyte (%)	2.00 \pm 0.10 ^b	1.61 \pm 0.16 ^{bc}	1.41 \pm 0.03 ^c	1.82 \pm 0.15 ^b
Eosinophils (%)	2.10 \pm 0.45 ^{ab}	2.20 \pm 0.21 ^a	1.10 \pm 0.15 ^{cd}	1.40 \pm 0.2 ^{bcd}
Basophils (%)	0.30 \pm 0.06 ^c	0.20 \pm 0.07 ^c	1.60 \pm 0.52 ^a	1.06 \pm 0.16 ^{ab}
Lymphocyte (%)	73.1 \pm 2.49 ^a	73.75 \pm 1.52 ^a	67.06 \pm 1.8 ^b	70.34 \pm 2.09 ^{ab}
Heterophils (%)	22.5 \pm 1.37 ^b	22.24 \pm 2.07 ^b	28.83 \pm 0.67 ^a	25.38 \pm 1.66 ^{ab}
H/L ratio	0.31 \pm 0.01 ^{bc}	0.30 \pm 0.03 ^{bc}	0.43 \pm 0.02 ^a	0.36 \pm 0.02 ^b

¹Abbreviations: PCV; Haematocrit; Hb: Haemoglobin; RBCs: Erythrocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; WBCs: Leukocytes; H/L ratio: Heterophils / Lymphocyte ratio.²TNC: Thermoneutral control, TNN: Thermoneutral *N*-acetyl cysteine, HSN: Heat stress *N*-acetyl cysteine, HSC: Heat stress control.

^{a-d} Means with different superscript letters within the same column are significantly different at $P<0.05$. SEM stands for Standard Error of Mean Values.

4. DISCUSSION

The haematological profiling is an important indicator of the physiological state of the body [19]. The current study discovered that broilers in HSC had significantly lower RBC, Hb, and PCV levels, which is

consistent with [20], who discovered that broilers in response to HS (341°C for 9 h/d) had a significant decrease in HCT, Hb concentrations, and RBC count after 42 days. These findings were consistent with [21], who indicated that under heat stress, birds had significantly lower levels of Hb, PCV, and RBC compared to the stress-free group. This was attributed to the effect of oxidative stress, which improved red

Table 3: Effect of *N*-acetyl cysteine (NAC) on serum metabolites of broilers at 42 d of age under thermoneutral and heat stress conditions (34±2°C) (Mean ±SE).

Parameters ^{1,2}	Treatments ²			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNN (0.5%) (n=5)	HSC (n=5)	HSN (0.5%) (n=5)
Total protein (g/dL)	3.95±0.22 ^b	4.01±0.18 ^b	2.71±0.13 ^c	3.07±0.12 ^c
Albumin (g/dL)	2.24±0.22 ^b	2.25±0.07 ^b	1.212±0.06 ^c	1.504±0.05 ^c
Globulin (g/dL)	1.704±0.03 ^{abc}	1.79±0.21 ^{abc}	1.5±0.11 ^c	1.56±0.11 ^{bc}
AST (U/L)	237.2±2.22 ^d	233.80±2.03 ^d	287.88±3.85 ^a	252.66±4.81 ^{bc}
ALT (U/L)	8.67±0.34 ^b	7.918±0.41 ^b	11.55±1.14 ^a	9.274±0.22 ^b
ALP (IU/dL)	148.34±100.1 ^c	143.92±41.37 ^c	246.66±40.32 ^a	215.86±64.95 ^b
TBIL (mg/dL)	0.946±0.05 ^b	0.924±0.05 ^b	1.6686±0.09 ^a	1.616±0.04 ^a
DBIL (mg/dL)	0.158±0.01 ^b	0.1438±0.0 ^b	0.326±0.04 ^a	0.2902±0.03 ^a
T. Cholesterol (mg/dL)	124.61 ±1.57 ^{bc}	121.10 ±1.22 ^c	150.12 ±2.71 ^a	143.60±1.21 ^a
Triglyceride (mg/dL)	96.02 ± 1.72 ^d	94.03 ±1.52 ^d	137.8 ±2.96 ^a	121.6±2.27 ^b
HDL-Cholesterol (mg/dL)	56.8 ±1.77 ^{abc}	61.34 ±1.87 ^{ab}	48.20 ±2.4 ^d	50.8±1.93 ^{cd}
LDL-Cholesterol (mg/dL)	48.62 ±1.67 ^b	41.23 ±2.73 ^b	74.24 ±3.72 ^a	68.48 ±2.96 ^a
Uric acid (mg/dL)	5.51 ±0.28 ^b	5.12 ±0.32 ^b	8.558±0.48 ^a	7.76±0.32 ^a
Creatinine (mg/dL)	0.35 ±0.04 ^{bc}	0.34±0.03 ^c	0.538±0.01 ^a	0.47±0.03 ^a

¹Abbreviations: ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TBIL: total bilirubin; DBIL: direct bilirubin; U/L=unit of enzyme activity/L.

²TNC: Thermoneutral control, TNN: Thermoneutral *N*-acetyl cysteine, HSN: Heat stress *N*-acetyl cysteine, HSC: Heat stress control.

^{a-d} Means with different superscript letters within the same column are significantly different at P<0.05. SEM stands for Standard Error of Mean Values.

Table 4: Effect of *N*-acetyl cysteine (NAC) on serum thyroid hormones of broilers at 42 d of age under thermoneutral and heat stress conditions (34±2°C) (Mean ± SE).

Parameters ^{1,2}	Treatments ²			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNN (0.5%) (n=5)	HSC (n=5)	HSN (0.5%) (n=5)
T3 (ng/ml)	154.66 ±3.38 ^{ab}	161.18 ±2.16 ^b	139.36 ±1.5 ^d	152.38 ±4.65 ^{bc}
T4 (ng/ml)	1.90 ±0.08 ^a	1.98 ±0.16 ^a	1.03 ±0.07 ^c	1.31 ±0.02 ^{bc}

¹Abbreviations: T3: Triiodothyronine; T4: Thyroxine.

²TNC: Thermoneutral control, TNN: Thermoneutral *N*-acetyl cysteine, HSN: Heat stress *N*-acetyl cysteine, HSC: Heat stress control.

^{a-d} Means with different superscript letters within the same column are significantly different at P<0.05. SEM stands for Standard Error of Mean Values.

blood susceptibility to oxidation, impaired the production of erythropoietin, and resulted in decreased Hb and PCV concentrations with decline in RBCs count of HS birds. In vivo endotoxin can directly damage erythrocytes as heat stress is prolonged [22]. On day 42, data for WBC profiles showed a higher lymphocyte

number with a lower heterophil number in the thermoneutral control group compared to the heat stressed control group [20].

The current findings agreed with the findings of [23], who reported that NAC 200 mg orally prescribed to patients resulted in a substantial increase in haematocrit level. Stress can affect haematological counts by inducing ACTH and producing epinephrine and norepinephrine, resulting in heterophils, the first line of defence with an effective chemotactic response against florogens [24]. It was suggested that increased WBC counts in the HS population may be due to a stress-related rise in cortisol concentration, which is responsible for increased leukocyte production, whereas NAC supplementation (0.01%) at week no. 4 resulted in a decrease in WBC counts when during HS environments, so it improved many haematological parameters [21].

It was found that broilers fed NAC in diet had lower CORT concentrations than broilers given only the basal diet because NAC relieves HS and thus lowers the WBC counts of broilers under HS partly by lowering the CORT level [25]. It is still unknown how NAC affects hypothalamic-pituitary-adrenal response and thus inhibits CORT elevation [26]. As a result, increased succinyl-CoA production was almost certainly followed by increased haemoglobin synthesis. In stressful conditions, glucocorticoids are released, causing an increase in the growth of heterophils from the bone marrow and, as a result, an increase in the number of heterophils in circulation [27]. The current study discovered that the H and H/L scores of broilers subjected to HS were significantly higher, with a significant decrease in monocytes, than that in the TNC community as a result of serum corticosterone elevation that was in accordance with [21, 28].

Heat stress can cause changes in some of the body's biochemical indexes, particularly the enzyme content, and can be assessed using indicators including clinical behaviour and blood biochemical parameters that can indicate tissue damage [29]. Clearly, different tropical hot temperatures affected biochemical parameters in broilers [30]. The current study discovered that HS significantly reduces serum total protein and albumin concentrations, which is consistent with [27], who showed that mean serum protein in broilers reared in HS zones was significantly affected by temperature area. Oxidative stress increases the production of ROS, which causes desaturation of biomolecules such as nucleic acid, protein, and enzymes. Increased oxidative stress caused by HS can be linked to a decrease in serum protein which is due in part to a decrease in protein intake and a shortage of essential amino acids. Decreased plasma protein level can also result in diminished protein digestibility at HS [31]. The 38°C programme for 10 hours resulted in a significant reduction in total protein and albumin levels at HS compared to control levels [6]. Currently, neither diet nor temperature had an effect on serum total protein levels, implying that protein metabolism was unaffected, which is consistent with the findings of [32].

Serum ALT levels are clinically part of a diagnostic liver function test [33, 34]. Hot stress causes changes in the production of serum enzymes [35]. The current study found a significant increase in AST, ALT, and ALP in the HSC group compared to the TNC group, which is consistent with [36], who found that heat stress-induced chicken liver injury caused an extraordinarily significant increase in the activity of AST and ALT liver damage enzymes. The biochemical parameters increase could theoretically be related to higher cellular production (ROS). Free radicals have an impact on blood serum metabolites [37]. Increased serum ALT levels may be due to a decrease in cell-level antioxidant enzymes [38].

The current study discovered that supplementing NAC in the heat stressed broiler diet significantly reduced the activities of blood enzymes like ALT, AST, and ALP, resulting in improved digestion and increased nutrient metabolism, which was consistent with [32]. There is a lack of similar work in poultry. NAC, but in the other hand, has been shown in mammals to improve kidney function by regulating ammonia and nitrogen metabolism [39]. NAC is an L-cysteine precursor which is used in the synthesis of reduced glutathione (GSH). A significant amount of evidence proved that the NAC possessed both direct and indirect antioxidant properties [40].

Interference with the normal functions of the liver affects the rate of bilirubin conjugation and excretion. As a result, increased bilirubin levels are used to determine liver function and bile excretion status [41]. According to [6], the other most recent findings demonstrate a significant increase in TBIL and DBIL. Furthermore, dietary NAC has been shown to decrease liver injury in a porcine model by improving antioxidative capacity and energy metabolism [42]. Similarly, NAC reduces aflatoxin toxicity in broilers by enhancing antioxidant capacity and energy metabolism [43]. As a result, it was hypothesised that NAC could benefit hot-stressed hepatic and cardiac function of hot-stressed broilers by modulating the energy and antioxidant status.

Elevated plasma corticosterone levels may raise serum uric acid levels. In poultry, uric acid is a powerful free radical scavenger, and rising levels of ACTH have been linked to higher uric acid concentrations. This may be due to increased plasma corticosterone levels caused by heat stress, which raises blood uric acid levels [44]. The lower uric acid levels in birds fed phytogetic supplements may be due to their ability to suppress ACTH and uric acid production. When compared to the TNC group, the chronic heat stressed HSC group broilers had a substantial increase in creatinine [44]. The current study found that when compared to broilers in the thermoneutral group, chronic heat stress (HSC group) resulted in a significant increase in total cholesterol, triglyceride, and LDL-C. (TNC). It is worth noting that dietary NAC supplementation resulted in a significant ($P < 0.05$) reduction in cholesterol and triglycerides, as well as a non-significant decrease in LDL-C content and an increase in HDL-C (**Table 3**).

The current findings are consistent with previous study indicated that, on day 42 in elevated air temperature chronic heat stress (HSC group), in comparison to the TNC group, there were an increase in cholesterol, triglycerides, and LDL-C which were probably due to an increase in corticosterone concentrations caused by hypothalamic-pituitary-adrenal stimulation [45]. Thyroid hormones are also affected by high temperatures. When broilers were subjected to HS, the average of T3 and T4 response declined dramatically when compared to the control group, according to the current study, which was consistent with the results [8]. According to current research, the effect of chronic heat stress on broiler T3 and T4 has been significantly reduced when compared to TNC, whereas dietary supplementation with NAC has increased T3 significantly ($p > 0.05$) when compared to the HSC group (**Table 4**). These results were consistent with the findings of [25], who claimed that supplementation of NAC (1 g/kg diet) in broiler diet gave an elevation of both serum T3 and T4 of heat-stressed broiler chickens ($36 \pm 1^\circ\text{C}$ for 10 h/d) for 27 days, which were not important, and that this could be due to the chicken getting exposed to high temperatures for an extended period of time, diluting the treatment effect of NAC.

5. CONCLUSION:

The experimental feed supplement showed a positive dominance in oxidative stress treatment based on the administered dose of NAC, which was reflected in the improvements of measured parameters and suggested that NAC has an antagonistic effect during heat stress.

List of abbreviations

ACTH: Adrenocorticotrophic hormone

ROS: Reactive oxygen species

NRC: National Research Council

EDTA: Ethylene diamine tetra acetic acid

REFERENCES

- [1] LARA, J. AND ROSTAGNO, M. Impact of heat stress on poultry production. *Animals*, 3(2): 356–69, 2013.
- [2] GÜNAL, M. The effects of early-age thermal manipulation and daily short-term fasting on performance and body temperatures in broiler exposed to heat stress, *Journal of Animal Physiology and Animal Nutrition*, 97, 854–860, 2013.

- [3] AKBARIAN, A., MICHIELS, J., DEGROOTE, J., MAJDEDDIN, M., GOLIAN, A., AND DE SMET, S. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology*, 7(1), 37, 2016.
- [4] SALAMI, S.A., MAJOKA, M.A., SAHA, S., GARBER, A. AND GABARROU, J.F. Efficacy of dietary antioxidants on broiler oxidative stress, performance and meat quality: science and market, *Avian Biology Research*, 8, 65-78, 2015.
- [5] KHAN, R. U., NAZ, S., NIKOUSEFAT, Z., SELVAGGI, M., LAUDADIO, V., & TUFARELLI, V. Effect of ascorbic acid in heat-stressed poultry. *World's Poultry Science Journal*, 68(3), 477-490, 2012.
- [6] HUANG, S., YANG, H., REHMAN, M. U., AND TONG, Z. Acute heat stress in broiler chickens and its impact on serum biochemical and electrolyte parameters. *Indian Journal of Animal Research*, 52(5), 683-686, 2018.
- [7] CHAND, N., NAZ, S., REHMAN, Z., AND KHAN, R. U. Blood biochemical profile of four fast-growing broiler strains under high ambient temperature. *Applied Biological Chemistry*, 61(3), 273-279, 2018.
- [8] MACK, L. A., FELVER-GANT, J. N., DENNIS, R. L., AND CHENG, H. W. Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry science*, 92(2), 285-294, 2013.
- [9] CLOUGH, S.R. Cysteine, N-Acetyl-L. *Encyclopedia of toxicology*, 2.ed. Westford: [s.n.], p. 716-718, 2005.
- [10] BANACLOCHA, M. M. Therapeutic potential of N-acetylcysteine in age-related mitochondrial neurodegenerative diseases. *Medical hypotheses*, 56(4), 472-477, 2001.
- [11] VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M. T., MAZUR, M., & TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, 39(1), 44-84, 2007
- [12] KOPKE, R.; BIELEFELD, E.; LIU, J.; ZHENG, J.; JACKSON, R. AND HENDERSON, D. N-acetylcysteine (NAC) and Acetyl-L-Carnitine (ALCAR) show different effects in protecting the cochlea from noise in chinchilla . *Assoc. Res. Otolaryngol. Abs.* 27 (686), 231, 2004.
- [13] NRC, NATIONAL RESEARCH COUNCIL. *Nutrient Requirements of Poultry*. 9th revised ed. National Academy Press, Washington, DC, USA, 1994.
- [14] CAMPO, J. L., AND DAVILA, S. G. Estimation of heritability for heterophil: lymphocyte ratio in chickens by restricted maximum likelihood. *Effects of age, sex, and crossing. Poultry science*, 81(10), 1448-1453, 2002.
- [15] CRAY, C. AND ZAIAS, J. Laboratory procedures. *Veterinary Clinical Exotic Animal*, 7: 487-51, 2004.
- [16] YOUNG DS. *Effects of drugs on Clinical Lab. Tests*, 4th ed AACC Press, 1995.
- [17] TIETZ N. W. *Clinical Guide to Laboratory Tests*. 3rd ed. Philadelphia, Pa: WB Saunders Co, 612, 1995.
- [18] IBM CORP. Released. *IBM SPSS Statistics for Windows*, Version 23 Armonk, NY: IBM Corp, 2013.
- [19] KHAN, T. A., & ZAFAR, F. Haematological study in response to varying doses of estrogen in broiler chicken. *International Journal of Poultry Science*, 4(10), 748-751, 2005.
- [20] XU, Y., LAI, X., LI, Z., ZHANG, X., AND LUO, Q. Effect of chronic heat stress on some physiological and immunological parameters in different breed of broilers. *Poultry science*, 97(11), 4073-4082, 2018.
- [21] SWATHI, B., GUPTA, P. S. P., AND NAGALAKSHMI, D. Effect of tulsi (*Ocimum sanctum*) and turmeric (*Curcuma longa*) on broiler performance and blood constituents during heat stress in broilers. *Int. J. Pharm. Bio Sci*, 3, 446-453., 2012.

- [22] BOUAZIZ, H., CROUTE, F., BOUDAWARA, T., SOLEILHAVOUP, J. P., & ZEGHAL, N. Oxidative stress induced by fluoride in adult mice and their suckling pups. *Experimental and Toxicologic Pathology*, 58(5), 339-349, 2007.
- [23] HSU, S. P., CHIANG, C. K., YANG, S. Y., & CHIEN, C. T. N-acetylcysteine for the management of anemia and oxidative stress in hemodialysis patients. *Nephron Clinical Practice*, 116(3), c207-c, 2010.
- [24] KUMARI, P., GUPTA, M. K., RANJAN, R., SINGH, K. K., AND YADAVA, R. Curcuma longa as feed additive in broiler birds and its patho-physiological effects. *Indian J. Exp. Biol.* 45, 272–277, 2007.
- [25] YI, D., HOU, Y., TAN, L., LIAO, M., XIE, J., WANG, L., ... & GONG, J. N-acetylcysteine improves the growth performance and intestinal function in the heat-stressed broilers. *Animal Feed Science and Technology*, 220: 83-92, 2016.
- [26] OMID, K., AMIRALI, S., & AHMAD, K. N-Acetyl cysteine improves performance, reproduction, antioxidant status, immunity and maternal antibody transmission in breeder Japanese quail under heat stress condition. *Livestock Science*, 217, 55-64, 2018.
- [27] HUWAIDA E.E. MALIK, ALI O.H.A., MOHAMED, E.A.A. AND YOUSIF, I.A. Effect of season and dietary protein level on immune response of three exotic broiler strains in Sudan. *Online J. Anim. Feed Res.*, 3(1): 31-35, 2013.
- [28] RUELL, P. A., SIMAR, D., PÉRIARD, J. D., BEST, S., CAILLAUD, C., AND THOMPSON, M. W. Plasma and lymphocyte Hsp72 responses to exercise in athletes with prior exertional heat illness. *Amino Acids*, 46(6), 1491-1499, 2014.
- [29] ZHANG, J. F., HU, Z. P., LU, C. H., YANG, M. X., ZHANG, L. L., AND WANG, T. Dietary curcumin supplementation protects against heat-stress-impaired growth performance of broilers possibly through a mitochondrial pathway. *Journal of Animal Science*, 93(4), 1656-1665, 2015.
- [30] AMRUTKAR, C., KIM, Y. S., AND TRAYNOR, P. Detecting mobile malicious webpages in real time. *IEEE Transactions on Mobile Computing*, 16(8), 2184-2197, 2016.
- [31] MAJID A, QURESHI MS, KHAN RU. In vivo adverse effects of alpha-tocopherol on the semen quality of male bucks. *J Anim Physiol Anim Nutr* 99:841–846, 2015.
- [32] LI, C., PENG, M., LIAO, M., GUO, S., HOU, Y., DING, B., ... & YI, D. EFFECTS of N-acetylcysteine on the energy status and antioxidant capacity in heart and liver of cold-stressed broilers. *Asian-Australasian journal of animal sciences*, 33(9): 1444, 2020.
- [33] ALHIDARY IA, ABDELRAHMAN MM, KHAN RU. Comparative effects of direct-fed microbial alone or with a traces mineral supplement on the productive performance, blood metabolites and antioxidant status of grazing Awassi lambs. *Environ Sci Pollut Res* 23:25218–25223, 2016.
- [34] ABUDABOS, A. M., ALYEMNI, A. H., DAFALLA, Y. M., AND KHAN, R. U. The effect of phytogenic feed additives to substitute in-feed antibiotics on growth traits and blood biochemical parameters in broiler chicks challenged with *Salmonella typhimurium*. *Environmental Science and Pollution Research*, 23(23), 24151-24157, 2016.
- [35] ABUDABOS AM, ALYEMNI AH, DAFALLA YM, KHAN RU. Effect of organic acid blend and *Bacillus subtilis* alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to *Salmonella typhimurium* challenge during the starter phase. *J Appl Anim Res* 45:538–542, 2017.
- [36] YE, W.Q., DU, B.W., LI, D.H. AND LI, N.B. Establishment of experimental animal models of kirin chicken(chicken feather volume)liver injury induced by heat stress. *China Animal Husbandry and Veterinary Medicine*. 42(3):741-744, 2015.
- [37] ALHIDARY IA, RAHMAN Z, KHAN RU, TAHIR M. Anti-aflatoxin activities of milk thistle (*Silybum marianum*) in broiler. *World's Poult Sci J* 73:559–566, 2017.
- [38] ABUDABOS AM, ALYEMNI AH, DAFALLA YM, KHAN RU. The effect of phytogenics on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to *Clostridium perfringens* challenge. *J Appl Anim Res* 46:691–695, 2018.

- [39] ONK D, ÖZÇELİK F, ONK OA, GÜNAY M, AYAZOĞLU TA, ÜNVER E. Assessment of renal and hepatic tissue-protective effects of N-acetylcysteine via ammonia metabolism: a prospective randomized study. *Med Sci Monit.* 24:1540-6, 2018.
- [40] DEKHUIJZEN PN. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. *Eur Respir J.*, 23:629-36, 2004.
- [41] OCHE, O., SANI, I., CHIAKA, N.G., SAMUEL, N.U. AND SAMUEL, A. Pancreatic islet regeneration and some liver biochemical parameters of leaf extracts of *Vitex doniana*, in normal and streptozotocin-induced diabetic albino rats. *Asian Pac. J. Trop. Med.* 4(2):124-130, 2014.
- [42] WANG, D., HUANG, H., ZHOU, L., LI, W., ZHOU, H., HOU, G., AND HU, L. Effects of dietary supplementation with turmeric rhizome extract on growth performance, carcass characteristics, antioxidant capability, and meat quality of Wenchang broiler chickens. *Italian Journal of Animal Science*, 14(3), 3870, 2015.
- [43] PUVAČA, N., ČABARKAPA, I., BURSIĆ, V., PETROVIĆ, A., AND AĆIMOVIĆ, M. Antimicrobial, antioxidant and acaricidal properties of tea tree (*Melaleuca alternifolia*). *Journal of Agronomy*, 15, 2018.
- [44] KANANI, P. B., DANESHYAR, M., AND NAJAFI, R. Effects of cinnamon (*Cinnamomum zeylanicum*) and turmeric (*Curcuma longa*) powders on performance, enzyme activity, and blood parameters of broiler chickens under heat stress. *Poultry Science Journal*, 4(1), 47-53, 2016.
- [45] BISHOP-WILLIAMS, K. E., BERKE, O., PEARL, D. L., HAND, K., AND KELTON, D. F. Heat stress related dairy cow mortality during heat waves and control periods in rural Southern Ontario from 2010–2012. *BMC veterinary research*, 11(1), 291, 2015.