Physiological Responses of Ross 308 Broilers to Aspirin Supplementation and Feed Deprivation Under Cyclic Heat Stress

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Abstract

Heat Stress (HS) poses a significant constraint on broiler production, compromising physiological performance, immune function, and antioxidant capacity. This study investigated the combined effects of aspirin supplementation and feed deprivation as an integrated strategy to mitigate the adverse physiological impacts of cyclic heat Stress in Ross 308 broilers. A total of 240 one-day-old chicks were randomly allocated to four treatments: T1 (control, ad libitum feed and water), T2 (feed deprivation 11:00–17:00 h), T3 (aspirin 100 mg L□¹ in drinking water 11:00–17:00 h), and T4 (combined feed deprivation 11:00–17:00 h followed by aspirin 17:00–23:00 h). At 35 days of age, hematological and serum biochemical parameters were assessed. Results indicated that the combined treatment (T4) significantly reduced the heterophil-to-lymphocyte (H/L) ratio ($P \leq 0.01$), elevated red blood cell count and hemoglobin concentration ($P \leq 0.01$), and improved packed cell volume ($P \leq 0.05$) compared with the control. Serum total protein and globulin concentrations increased significantly ($P \leq 0.05$), whereas glucose and uric acid levels decreased ($P \leq 0.01$) in all treated groups. Antioxidant markers, including superoxide dismutase (SOD) activity and total antioxidant capacity, were significantly enhanced in T3 and T4 ($P \leq 0.05$). Body temperature was markedly reduced at peak heat hours (1400 h and 2200 h) in T2, T3, and T4 ($P \leq 0.01$). These findings demonstrate that the synergistic application of feed deprivation and aspirin supplementation effectively alleviates cyclic heat stress in broilers by attenuating inflammatory responses, enhancing hematological function, and improving antioxidant status.

Keywords: Aspirin; Feed deprivation; Ross 308 broilers; Thermoregulation; Poultry welfare

Introduction

Broiler chickens represent a vital source for global food security owing to their high production efficiency, characterized by a short growth cycle and an excellent feed conversion ratio (FCR). Concurrently, the poultry industry plays a strategic role in driving economic growth and generating employment opportunities [1]. Despite this significance, seasonal elevations in ambient temperature impose severe challenges on poultry producers, resulting in substantial economic losses driven by reduced bird productivity, increased disease susceptibility, and elevated mortality rates [2]. Heat Stress (HS) remains a primary bottleneck in poultry production, severely impairing feed intake, nutrient utilization efficiency, meat quality, reproductive performance, and intestinal integrity [3].

To mitigate the adverse effects of HS, various interventions have been explored, including the administration of aspirin

ISSN 2072-3857

(acetylsalicylic acid). Renowned for its anti-inflammatory, antipyretic, and analgesic properties [4], aspirin serves as an effective agent in mitigating oxidative damage induced by environmental stress in poultry flocks [2]. Furthermore, Aspirin may indirectly reduce oxidative stress through its anti-inflammatory actions [5,6] by alleviating the detrimental physiological disruptions triggered by thermal stress [7].

In tandem with chemical interventions, feed deprivation during peak thermal hours constitutes a crucial managerial and nutritional strategy to alleviate heat load and enhance physiological performance [8]. This practice effectively reduces metabolic heat production associated with digestion and nutrient absorption, thereby diminishing the cumulative thermal load on the bird's body and ultimately leading to a marked reduction in mortality rates [9]. Consequently, the present study aimed to evaluate the combined application of aspirin supplementation and feed deprivation at different periods as an integrated approach to mitigate the negative physiological impacts of cyclic heat stress on Ross 308 broiler chickens.

Materials and Methods:

Experimental Site and Birds:

The experiment was conducted at the Poultry Research Unit, Department of Animal Production, College of Agriculture, University of Kufa, Iraq, from 4 September to 9 October 2025. The study evaluated the effects of aspirin supplementation through drinking water and feed deprivation on the physiological responses of broiler chickens exposed to heat stress conditions.

A total of 240 one-day-old, unsexed Ross 308 broiler chicks with an average initial body weight of 45 ± 2 g were obtained from Al-Anwar Hatchery (Al-Muradiya, Babylon Province, Iraq). Upon arrival, chicks were individually weighed and randomly allocated to four experimental treatments. Each treatment consisted of 60 birds distributed among three replicate pens, with 20 birds per replicate. Birds were reared on a floor system using coarse wood shavings as litter material at a depth of approximately 5 cm. Standard management and vaccination programs recommended for Ross 308 broilers were followed throughout the experimental period.

Feeding Program

Birds were fed a three-phase feeding program formulated to meet or exceed the nutritional requirements of broiler chickens. The starter diet was provided from 1 to 10 days of age and contained 3015 kcal ME kg□¹ and 23.11% crude protein (CP). The grower diet was offered from 11 to 24 days of age and supplied 3081 kcal ME kg□¹ and 21.51% CP. The finisher diet was fed from 25 to 35 days of age and contained 3210 kcal ME kg□¹ and 19.58% CP. Feed and water were provided ad libitum unless otherwise specified by the treatment protocol.

Experimental Design and Treatments

The experiment was arranged in a completely randomized design (CRD) comprising four treatments:

- T1 (Control): Birds had unrestricted access to feed and drinking water throughout the experimental period.
- T2 (Feed deprivation): Feed was withdrawn daily for 6 h (11:00–17:00 h), while water remained available.
- T3 (Aspirin supplementation): Aspirin was administered through drinking water at a concentration of 100 mg L□¹ for 6 h daily (11:00–17:00 h).
- T4 (Combined treatment): Birds were subjected to feed deprivation for 6 h (11:00–17:00 h), followed immediately

ISSN 2072-3857

by aspirin supplementation in drinking water at 100 mg L□¹ for a subsequent 6 h period (17:00–23:00 h).

All treatments were maintained throughout the experimental period, and the physiological responses of broilers were assessed under heat stress conditions to determine the effectiveness of aspirin supplementation, feed deprivation, and their combined application in mitigating heat-induced physiological disturbances.

At the end of the fifth week, blood samples were collected from two male birds per treatment. Hematological parameters were analyzed, including red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), hemoglobin concentration (Hb), platelet count (Thrombocytes), and erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), using a Genix Vet 60 hematology analyzer.

Several blood biochemical parameters were also evaluated, (total protein, albumin and cholesterol) concentrations were determined according to the methods described by Tietz [10]. Globulin concentration was calculated according to the equation described by Al-Omari [11]. Blood glucose concentration was determined following the method of Coles [12], while uric acid concentration was estimated according to the method of Fossati and Prencipe [13].

Statistical Analysis

Experimental data were statistically analyzed using a Completely Randomized Design (CRD) to evaluate the effects of the different treatments on the studied traits. Significant differences among treatment means were determined using Duncan's Multiple Range Test at significance levels of ($P \leq 0.05$) and ($P \leq 0.01$). Statistical analyses were performed using the SAS software package (2012), according to the following mathematical model:

$Yij = \mu + Ti + eij$

Where:

Yij = observed value.

μ = overall mean.

Ti = effect of treatment.

eij = experimental error.

Results and Discussion

The experimental treatments exerted significant effects on several hematological parameters in heat-stressed broiler chickens, as presented in Table 1. Total white blood cell (WBC) count was significantly elevated in the feed deprivation group (T2) relative to the untreated control (T1) ($P \leq 0.05$), while groups T3 and T4 did not differ significantly from either T1 or T2.

Heterophil percentages and heterophil-to-lymphocyte (H/L) ratios were significantly reduced in all treatment groups (T2, T3, and T4) compared with the control ($P \leq 0.01$), indicating an attenuation of heat-induced physiological stress. Lymphocyte percentages were significantly higher in T2 and T4 than in T1. Of particular note, T4 exhibited the lowest H/L ratio among all groups, differing significantly from both T2 and T3 ($P \leq 0.01$), suggesting a superior stress-mitigating effect attributable to the combined intervention.

Erythrocytic parameters were also significantly improved following treatment. Red blood cell (RBC) counts and hemoglobin (Hb) concentrations increased significantly ($P \leq 0.01$), and packed cell volume (PCV) was significantly elevated ($P \leq 0.05$) in T2, T3, and T4 relative to T1. Platelet (PLT) count and mean corpuscular volume (MCV) were unaffected by any of the treatments. Mean corpuscular hemoglobin (MCH) was significantly

ISSN 2072-3857

highest in T2 and lowest in T3 ($P \leq 0.01$), whereas T1 and T4 did not differ significantly from one another.

Table 1. Hematological responses of Ross 308 broilers to aspirin supplementation under cyclic heat stress at 35 d of age (Mean ± SEM)

Treatment	WBC $10^3/\mu$□	HeterophiL %	Lymphocyte %	H / L	R BC $10^3/\mu L$	Hb g/100 ml
T1	23.69 b	22.73 a	65.60 c	0.35 a	2.23 c	10.40 b
	0.33	0.17	1.15	0.003	0.06	0.40
T2	24.90 a	21.43 b	69.10 ab	0.31 b	2.46 b	11.33 a
	0.46	0.13	0.61	0.01	0.04	0.09
T3	23.97 ab	20.82 bc	66.85 bc	0.31 b	2.61 b	11.70 a
	0.16	0.39	0.66	0.01	0.07	0.10
T4	24.55 ab	20.43 c	70.30 a	0.29 c	2.91 a	11.97 a
	24.55	0.09	0.70	0.003	0.03	0.20
Significant	*	**	**	**	**	**

Means in a row with different superscripts differ significantly (*$P \leq 0.05$, **$P \leq 0.01$); NS= not significant. T1= Control; T2= Feed deprivation (11:00–17:00 h); T3= Aspirin (100 mg L□¹, 11:00–17:00 h); T4= Combined deprivation +aspirin.

Fasting exerts a protective role against the adverse effects of heat stress on the immune system, particularly on leukocyte populations. By reducing metabolic heat production associated with feed intake, fasting attenuates the secretion of stress hormones-epinephrine, norepinephrine, and corticosterone-thereby preventing their well-documented inhibitory effects on leukocyte activity. Concurrently, aspirin, owing to its anti-inflammatory, antioxidant, and antipyretic properties, contributes to mitigating heat stress-induced immune dysregulation and restoring leukocyte homeostasis, as reflected in the near-normal white blood cell counts recorded across all treatment groups. The observed reduction in heterophil (H) counts in fasted birds may be attributed to the upregulation of heat shock protein 70 (HSP70), which attenuates excessive inflammatory responses and maintains heterophil numbers within physiologically normal ranges [14,15,16].

The highly significant decrease ($P \leq 0.01$) in heterophil counts and the corresponding increase in lymphocyte (L) counts in T4 - combining fasting and aspirin administered at different time points- corroborate the findings of Sweidan and Abuajamieh [17], and collectively represent a robust physiological indicator of the efficacy of this combined strategy in alleviating heat stress burden in broiler chickens. Regarding erythrocytic parameters, the significant improvements in red blood cell (RBC) count and hemoglobin (Hb) concentration ($P \leq 0.01$), as well as packed cell volume (PCV; $P \leq 0.05$), observed in T2, T3, and T4 relative to the control may be explained by the following mechanisms (Table 2). Fasting (T2) reduces metabolic heat generated by feed digestion and limits feed-associated water consumption, thereby preventing the hemodilution typically associated with

ISSN 2072-3857

elevated body temperature and compensatory water intake for thermoregulatory purposes.

Table 2. Blood biochemistry and leukocyte differential count in Ross 308 broilers supplemented with aspirin under cyclic heat stress at 35 d of age (Mean ± SEM)

Treatment	PCV %	PLT $10^3\mu$□	MCV Fl	MCH Pg/Cell	MCHC g/dl
T1	27.87 b	33.83	124.67	46.53	37.33 b
	1.15	9.33	1.91	0.61	0.09
T2	29.95 a	38.77	121.62	46.03	37.85 a
	0.13	6.69	1.31	0.53	0.18
T3	30.34 a	38.07	127.10	46.40	36.53 c
	0.23	5.20	2.19	0.61	0.15
T4	31.40 a	43.00	123.10	45.73	37.17 b
	0.32	1.62	0.12	0.23	0.19
Significant	*	N. S	N. S	N. S	*

Means in a row with different superscripts differ significantly (*P ≤ 0.05, **P ≤ 0.01); NS= not significant. T1= Control; T2= Feed deprivation (11:00–17:00 h); T3= Aspirin (100 mg L□¹, 11:00–17:00 h); T4= Combined deprivation +aspirin.

This results in relative hemoconcentration, evidenced by elevated RBC counts, Hb concentration, and PCV [18,19]. Aspirin further supports erythrocytic function through vasodilation and improved blood rheology, facilitating microcirculatory flow, enhancing gas exchange efficiency, and optimizing oxygen delivery to peripheral tissues. Its antipyretic action additionally prevents heat stress-induced hemodilution [20,21]. The combined application of fasting and aspirin in T4 acts synergistically to reduce core body temperature under heat stress conditions, producing favorable and additive effects on RBC count and Hb concentration.

Collectively, the combined treatment (T4) yielded the most favorable hematological profile under heat stress conditions, as evidenced by the pronounced reduction in the H/L ratio and the consistent improvement in erythrocytic indices.

Table 3. Serum biochemistry and antioxidant responses of Ross 308 broilers to aspirin supplementation under cyclic heat stress at 35 d of age (Mean ± SEM)

Treatment	TP g/dl	Alb g/dl	Glo g/dl	Cho mg/dl	Glu mg/dl	UA mg/dl	SOD ul ml	T. Ant. mmol Fe^{+2}/L
T1	3.05 b	1.59 a	1.46 b	124.33 a	281.67 a	7.17 a	350.74 b	143.05 b
	0.02	0.02	0.01	2.91	6.36	0.34	7.75	4.19
T2	3.63 ab	1.34 bc	2.29 ab	118.67 a	253.67 b	6.40 b	360.48 b	177.22 a
	1.15	0.05	0.13	1.20	7.21	0.15	8.24	5.35
T3	3.58 ab	1.46 ab	2.13 ab	115.67 b	241.00 c	5.83 c	387.77 a	154.26 b
	0.14	0.06	0.09	0.67	1.53	0.14	10.60	7.88

ISSN 2072-3857

T4	3.91 a 0.44	1.19 c 0.05	2.72 a 0.47	120.00 a 2.81	255.67 b 8.09	5.93 c 0.13	393.02 a 5.95	180.5 a 5.80
Significant	*	**	*	*	**	**	*	*

Means in a row with different superscripts differ significantly (*P ≤ 0.05, **P ≤ 0.01); NS= not significant. T1= Control; T2= Feed deprivation (11:00–17:00 h); T3= Aspirin (100 mg L□¹, 11:00–17:00 h); T4= Combined deprivation +aspirin.

Table (3) shows a significant increase (P ≤ 0.05) in total protein and globulin concentrations in birds across all treatment groups compared to the control group (T1). Additionally, albumin concentration exhibited a significant reduction in the T2 and T4 treatment groups relative to both the control (T1) and T3 groups. Cholesterol concentration was significantly lower (P ≤ 0.05) in the T3 group compared to the T1 and T4 groups, with no significant difference observed between the T2 and T4 groups. Furthermore, a highly significant decrease (P ≤ 0.01) was recorded in glucose and uric acid concentrations across all treated groups (T2, T3, and T4) compared to the control group (T1). Regarding oxidative enzymes, birds in the T3 and T4 groups demonstrated significantly higher (P ≤ 0.05) superoxide dismutase (SOD) activity than those in the T1 and T2 groups. Total antioxidant capacity was also significantly elevated (P ≤ 0.05) in the T2 and T4 groups compared to both the control (T1) and T3 groups.

The results indicate that all supplementation treatments-namely, deprivation and aspirin (T2, T3, and T4)-contributed to alleviating elevated body temperatures, as illustrated in Figure (1). A highly significant reduction (P ≤ 0.01) in body temperature was observed in birds from the T2, T3, and T4 groups compared to the control group (T1) at the peak ambient temperature (1400 h) and again at 2200 h. Consequently, these treatments mitigated the adverse effects of heat stress experienced by birds in the T2, T3, and T4 groups relative to the control (T1), as evidenced by the significant decrease in the heterophil-to-lymphocyte (H/L) ratio in the aforementioned treatment groups compared to the control (Table 1).

This reduction is indicative of diminished corticosterone secretion associated with acute stress, thereby attenuating protein catabolism. Corticosterone mobilizes endogenous nutrient reserves to sustain prolonged energy demands, primarily through gluconeogenesis from non-carbohydrate sources, particularly amino acids derived from protein breakdown [22]. The significant elevation in albumin concentration observed in the control group (T1) may be attributed to heat stress exposure. EL Shoukary et al [23] noted that plasma albumin serves as a reserve protein during abnormal physiological conditions in avian species, facilitating the transport of minerals, vitamins, fatty acids, and hormones including thyroxine and corticosterone. The highly significant increase (P ≤ 0.01) in uric acid concentration in the control group (T1) compared to the T2, T3, and T4 groups is attributable to severe heat stress exposure, corroborated by the elevated H/L ratio (Table 1) a marker of chronic stress. Under such conditions, circulating corticosterone levels rise [3,23,24] promoting proteolysis to support gluconeogenesis.

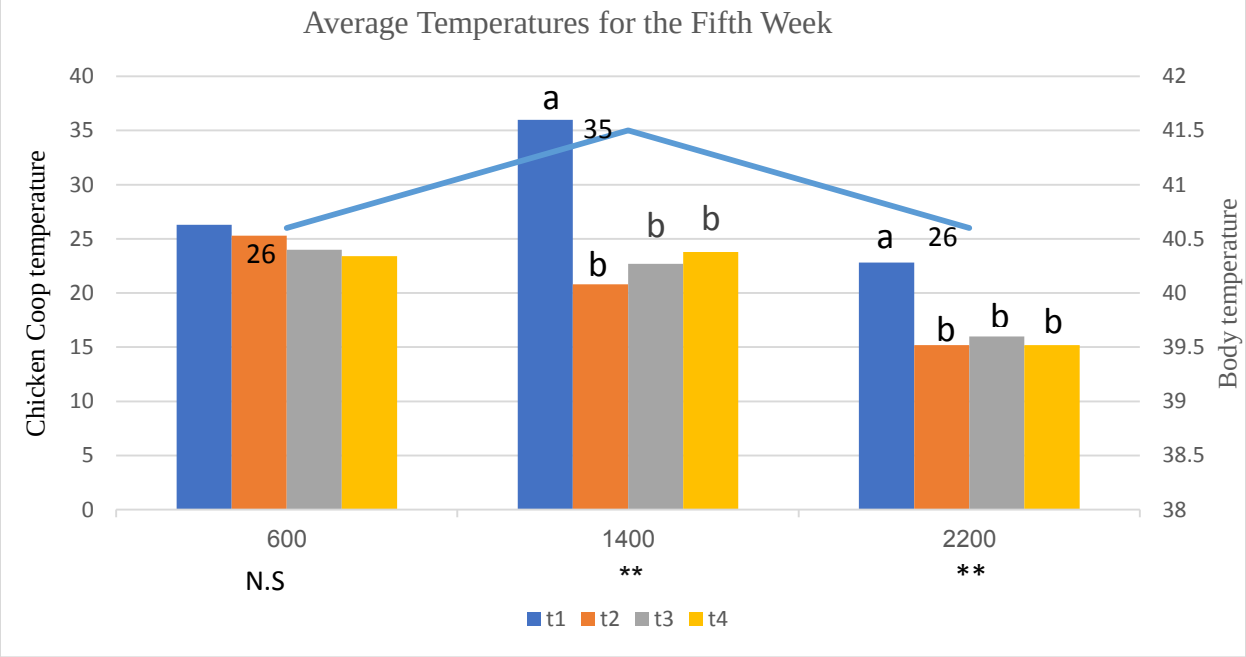
This process induces negative nitrogen balance, resulting in increased uric acid concentration concomitant with decreased total protein and elevated plasma glucose levels [3,14]. Both deprivation and aspirin

ISSN 2072-3857

represent effective interventions for ameliorating the deleterious physiological impacts of heat stress, thereby reducing free radical generation and oxidative damage incurred by birds under field rearing conditions [2,8]. These beneficial effects were reflected in the enhanced concentrations of antioxidant enzymes superoxide dismutase (SOD) and total antioxidant capacity in the T2, T3, and T4 groups compared to the control (T1), further substantiated by the significant reduction in the H/L stress index across all treatment groups relative to the control.

As shown in Figure (1), dietary supplementation with aspirin and deprivation influenced body temperature in broiler chickens subjected to cyclic heat stress at 5 weeks of age. No significant differences in body temperature were observed at 0600 h, when the ambient temperature was 26 °C. However, at 1400 h, when the ambient temperature reached 35 °C, and again at 2200 h, a highly significant decrease ($P \leq 0.01$) in body temperature was recorded in the deprivation (T2), aspirin (T3), and combined deprivation–aspirin (T4) groups compared with the control group (T1).

Figure 1. Body temperature of Ross 308 broilers supplemented with aspirin under cyclic heat stress at 5 wk. of age



Means in a column with different superscripts differ significantly ($P \leq 0.01$); NS = not significant. T1= Control; T2= Feed deprivation (11:00–17:00 h); T3= Aspirin (100 mg L□¹, 11:00–17:00 h); T4= Combined deprivation +aspirin.

The reduction in body temperature observed in the T2 group may be attributed to the thermoregulatory effect of deprivation in mitigating heat increment resulting from digestive and metabolic processes, thereby alleviating the internal heat load [8,9]. Conversely, the significant decrease in body temperature in the T3 group is ascribed to the pharmacological action of aspirin, specifically its active component, salicylic

ISSN 2072-3857

acid, which possesses potent antipyretic properties. Salicylic acid exerts its hypothermic effect primarily through the inhibition of prostaglandin synthesis via suppression of cyclooxygenase (COX) enzyme activity, thereby preventing the conversion of arachidonic acid into prostaglandins that mediate pyrexia [25]. Notably, the T4 group exhibited the most pronounced reduction in body temperature, which can be attributed to the synergistic thermoregulatory interaction between deprivation and aspirin in attenuating thermal stress.

Conclusions:

The combined strategy of feed deprivation during peak thermal hours followed by aspirin supplementation effectively mitigates cyclic heat stress in Ross 308 broilers. This integrated approach significantly reduces body temperature, attenuates stress indicators (H/L ratio), improves erythrocytic function, enhances serum protein profiles, and boosts antioxidant defenses. These findings support the adoption of feed deprivation combined with aspirin as a practical, non-invasive management tool to sustain broiler health and productivity under heat-stress conditions in tropical and subtropical regions.

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ISSN 2072-3857