https://doi.org/10.48047/AFJBS.6.10.2024.6093-6103

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Effects of early age conditioning and linseed supplementation (*Linum usitatissimum*) on biochemical and haematological parameters in heat-stressed broiler chickens at different ages

Z. Bengharbi[1], D. Benabdelmoumene[2], S. Dahmouni[3], N. Benguenouna[4], M-K. Fodil[5], F. Soltani[6]

[1-6]Applied animal physiology, Abdelhamid Ibn Badis university. Mostaganem, Algeria.
Corresponding author: djilali.benabdelmoumene@univ-mosta.dz

Article History
Volume 6, Issue 10, 2024
Received : 17 Apr 2024
Accepted : 02 Jun 2024
doi: 10.48047/AFJBS.6.10.2024.6093-6103

Abstract

This study investigates the effects of early age conditioning (EAC) and linseed (*Linum usitatissimum*) supplementation (LS) on the biochemical and haematological parameters of broiler chickens exposed to chronic heat stress and coccidiosis. Hypoproteinaemia, a significant metabolic characteristic of coccidiosis, was addressed by EAC and LS through their influence on metabolic balance and stress response mechanisms. Chronic heat stress typically reduces protein synthesis, yet EAC and LS helped maintain protein levels by mitigating stress-induced cortisol secretion and protein catabolism. Both treatments significantly reduced cholesterol and triglyceride levels, crucial markers of metabolic health under thermal stress, and increased blood glucose levels, improving homoeostasis disrupted by coccidiosis.

The treatments also normalised cholesterol and triglyceride levels in untreated infected chickens, mitigating the adverse effects of coccidiosis on lipid homoeostasis. Urea levels, a byproduct of protein degradation, were increased at the end of the rearing period, indicating enhanced proteolysis without adverse effects on renal function. The observed increase in blood pH suggested a beneficial effect of EAC and LS in preventing acid-base imbalances caused by heat stress and coccidiosis.

Additionally, EAC and LS improved fluid balance and mineral concentrations, enhancing Na+ and K+ levels, crucial for maintaining homoeostasis under heat stress. In haematological changes, such as reduced haemoglobin levels and erythrocyte counts, were mitigated by EAC and LS. EAC reduced haematocrit and blood viscosity, improving circulation and thermolysis. The treatments' combined effects significantly increased erythrocyte counts, countering reductions caused by coccidiosis and enhancing overall health.

The reduction in leukocyte counts at T3 in treated groups indicated a localised and effective immune response against parasites, highlighting the anti-coccidia, anti-inflammatory, and healing properties of linseed. EAC and LS also promoted red blood cell maturation and increased mean corpuscular volume (MCV), suggesting enhancing resilience and health status in broilers under stress conditions.

These findings provide valuable insights into the potential of EAC and LS to enhance the health and performance of broiler chickens under challenging environmental conditions, offering practical strategies for improving poultry management and welfare.

Key words: Early age conditioning, Linseed, heat stress, Broilers, blood

Introduction

Heat stress poses a significant challenge to the poultry industry, affecting the growth performance, health, and overall well-being of broilers. Elevated temperatures can lead to oxidative stress, altered haematological and biochemical parameters, and reduced productivity. To mitigate these adverse effects, various dietary interventions have been explored. One promising approach is the inclusion of linseed in the diet, which is rich in omega-3 fatty acids and possesses anti-inflammatory properties.

Several studies have investigated the impact of dietary supplements on broilers under heat stress conditions. For instance, Ding et al. (2020) demonstrated that fumaric acid supplementation improved growth performance and stabilised haematological and biochemical profiles in broilers exposed to chronic heat stress. Similarly, Hasan et al. (2015) reported that probiotics enhanced growth performance and haem-biochemical parameters during heat stress.

The genotype and sex of broilers also influence their response to dietary interventions and heat stress. Benabdelmoumene et al. (2016) highlighted that lipid oxidation and fatty acid profiles vary significantly between different chicken genotypes and sexes, impacting meat quality and stress response. Additionally, Jaiswal et al. (2017) found that thermal stress significantly alters serum biochemical and haematological parameters in broilers, underlining the need for effective dietary strategies.

Recent research by Jimoh et al. (2022) emphasised the beneficial effects of phytogenic feed additives on performance and oxidative stress markers in broilers during heat stress. Nwaigwe et al. (2020) evaluated haematological and biochemical stress markers, providing insights into the physiological impact of heat stress on broilers.

Further, Biswas et al. (2024) demonstrated that dietary ascorbic acid improves production performance and modulates haematological and biochemical parameters in heat-stressed broilers. Attia et al. (2024) explored the role of dietary electrolyte balance and arginine to lysine ratio in enhancing haematological, antioxidant, and immunological traits under cyclic heat stress.

The role of vitamins and minerals has also been studied extensively. Singh et al. (2023) showed that supplementation with vitamins E, C, and selenium positively affects blood haematological, biochemical parameters, liver enzymes, serum electrolytes, and antioxidant profiles in heat-stressed broilers. Swain et al. (2023) examined the impact of heat stress on haematological and biochemical parameters and corticosterone levels, indicating the potential for dietary interventions to alleviate stress effects.

Oganija and Apata (2022) found that linseed oil supplementation improved haematological parameters, lipid profiles, and hormonal balance in heat-stressed broilers. Studies on linseed have shown promising results; Sepehr et al. (2021) reported that different forms of flaxseed positively affect egg performance, lipid components, and fatty acid concentrations in commercial laying hens.

Hosseini et al. (2022) demonstrated that an additive mix could alleviate the adverse effects of stress on performance and health in farmed hens. Bengharbi et al. (2016) and Zineb et al. (2021) highlighted the benefits of early-age acclimation and linseed (*Linum usitatissimum*) dietary inclusion on fat deposition, fatty acids' meat traits, and thermal resistance in heat-stressed broilers.

Overall, the integration of linseed and other dietary supplements into the diet of broilers offers a viable strategy to counteract the detrimental effects of heat stress (Zineb et al., 2024), enhancing growth performance, and improving haematological and biochemical health markers.

The objective of this study is to evaluate the effects of linseed dietary supplementation on changes in blood and haematological parameters of heat-stressed broilers. Specifically, the study aims to compare these effects in broilers that have undergone early-age acclimation to induced thermoresistance. The evaluation will be conducted at three different slaughter ages: T1 (30 days), T2 (43 days), and T3 (53 days).

Material and methods

The study involved four hundred ISA15 chicks, one-day old, from the ORAVIO (Office Régional Avicole de l'Ouest) hatchery in Ain Nouissy Mostaganem. Upon arrival, the chicks were divided into two groups, each housed separately in two buildings at the UMAB University of Mostaganem's poultry farming unit, under identical environmental conditions. Both feeding and watering were provided ad libitum. Three types of feed (Table 1) were used: starter, grower, and grower supplemented with 5% ground linseed.

Table 1. Composition and calculated analyses of the experimental diets (S: starter, G: grower and 5% LS: 5% linseed (*Linum usitatissimum*) supplemented diet)

Feedstuff composition	S	G	5%LS
Ingredient (%)			
Yellow Corn	22	24	23
Wheat	39,8	40,6	36,56
Soyabean meal	29,1	27,50	27,50
Wheat Bran	5,15	4,74	4,74
Linseed	0	0	5
Methionine	0,03	0,00	0,04
MVC[1]	1	1	1
Anti stress	1	0	0
Calculated analyses[2]			
ME (Kcal/Kg)	2887,06	2887,06	2887,06
Moisture %	11,59	10,62	10,62
CP %	21,86	19,95	20
Ether extract %	2,68	2,86	4,91
Ash (% of DM)	6,97	6,52	6,52
Fibre (% DM)	2,87	3,02	4,62
Calcium %	1,22	1,31	1,31
Available P %	0,69	0,63	0,63

[1]Supplied per kilogram of diet: *7000 IU, vit A; 1400 ICU, vitD3; 20 IU, vit E; 50 mg, vit C; 2.3 mg, vit K; 1.8 mg, vit B1; 5.5 mg, vit B2; 2.3 mg, vit B6; 0.011 mg, vitB12; 27.5, mg Niacin; 0.90 mg, Folic acid; 7 mg, PA; 0.092 mg, Biotin; 50 mg, Antioxidant; 8.5mg, copper; 0.35 mg, Iodine; 0.26 mg, Iron; 0.45 mg, Manganese; 0.2 mg, Selenium; 45 mg, Zinc.*

[2] *Laboratories of Animal Nutrition and Feed Technology, Institute of Animal Science (IAS), Bulgaria*

Ambient Temperature Monitoring and Experimental Conditions

Ambient temperature was recorded daily using a thermo-hygrometer, three times a day at the same hours, under static ventilation. Heating was provided by standard gas radiators.

On the 5th day of age, the ambient temperature in one of the two buildings was increased to raise the living area temperature for the chicks to 40 ± 1°C, exposing them to 24 hours of thermal stress. The following day, temperatures in the buildings were returned to normal and maintained between 24°C and 26°C during both the growth and finishing phases to conduct the study under conditions of chronic high temperatures.

At 17 days of age (growth phase), the acclimated group was further divided into two subgroups of 100 chicks each: one subgroup was fed a diet supplemented with 5% ground linseed until the end of the finishing phase (53 days), and the other was fed a standard diet. The second control group was similarly subdivided and treated in the same manner.

From day 17 until the end of the finishing phase, our chicks were divided into four groups of 100 individuals each, as follows: Control group fed a standard diet (C), Control group fed a standard diet supplemented with 5% ground linseed (CL), Acclimated group fed a standard diet (Ac) and the fourth group is the acclimated group fed a standard diet supplemented with 5% ground linseed (AcL).

To test the effect of our different treatments on the resistance of broiler chickens to thermal shocks in adult age, the four groups were exposed at 53 days to thermal shocks of 38 ± 1°C for 6 hours.

Slaughter, Sampling, and Sample Processing

Three slaughters were performed at 30 (T1), 43 (T2), and 53 (T3) days of age. These allowed us to monitor the effects of our treatments on broiler resistance to chronic high temperatures and sudden temperature spikes during hot seasons. Haematological, and biochemical parameters were evaluated to provide scientific foundations for the observed treatment effects.

At each slaughter, a random sample of 60 subjects (15 subjects per group) was taken, weighed, then bled and plucked. During bleeding, blood from each animal was immediately collected in heparinised tubes (for biochemical evaluation) and EDTA tubes (for haematological parameter evaluation). The tubes were placed in a cooler to be kept chilled and immediately transported to the analysis laboratories.

Study of Biochemical and Haematological Parameters

To study the biochemical and haematological parameters and estimate their variations in response to our different treatments, the following assays were performed. Leukocytes, erythrocytes (Schalm et al., 1975), haemoglobin, MCV (Mitruka and Rawsley, 1977), thrombocytes, haematocrit, and MCHC were manually estimated according to Ogunwole et al. (2017). Glucose, blood pH, cholesterol, triglycerides, total proteins (including albumin), urea, creatinine, Na+, and K+ were measured using the enzymatic colorimetric method (Nanbol et al., 2016).

Statistical analysis

The data were analysed using a two-way analysis of variance (ANOVA) procedure. The model incorporated the main effects of early-age thermal conditioning (EAC), 5% dietary linseed supplementation (LS), and their interaction. Significance levels were designated as follows: * p<0.05, ** p<0.01, *** p<0.001. Statistical analyses were conducted using the JMP v. 7 software package.

Results and discussion

Biochemical and haematological parameters

At T1, Acclimation significantly increased cholesterol, glucose, and creatinine levels while reducing triglycerides (p<0.05). There was no effect on protein, albumin, urea, pH, and Na^+ and K^+ concentrations (Table 2). However, linseed supplementation significantly reduced cholesterol, triglycerides, and protein levels while increasing glucose, creatinine, pH, and K^+ concentration at T1. The interaction of both treatments resulted in a marked reduction in cholesterol, triglycerides, proteins, and a less pronounced decrease in Na^+ concentration (Figure 1).

Linseed supplementation had no effect on leukocyte count, haematocrit, mean corpuscular volume (MCV), or mean corpuscular haemoglobin concentration (MCHC). Nevertheless, this treatment significantly reduced erythrocytes count and haemoglobin levels while significantly increasing platelet count. The interaction of both treatments significantly reduced erythrocytes count and haemoglobin levels and less markedly increased platelet count at T1.

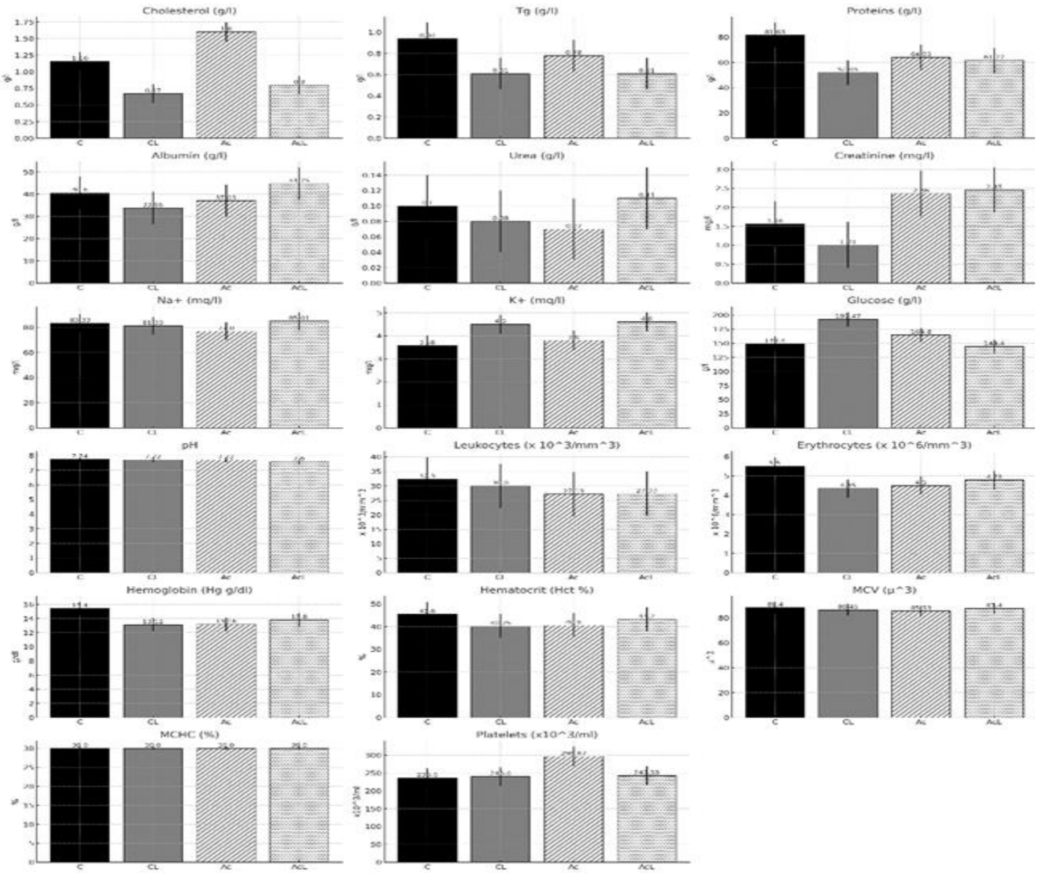

Figure 01: Effects of incorporating flaxseed (*Linum usitatissimum*) into the diet, early age acclimation and their interactions on biochemical and hematological parameters in broilers at 30 days of age (*Control group fed a standard diet: (C); (CL): Control group fed a standard diet supplemented with 5% ground linseed, (Ac): Acclimated group fed a standard diet; (AcL): acclimated group fed a standard diet supplemented with 5% ground linseed).*

At T2, Early-age conditioning (EAC) significantly reduced cholesterol and protein levels while increasing albumin, urea, creatinine, glucose, pH, and Na+ and K+ concentrations. Triglyceride levels were unaffected (Figure 2). Moreover, linseed supplementation at T2 significantly increased cholesterol, creatinine, and Na+ and K+ concentrations but reduced glucose and pH levels. Thus, the interaction of both treatments significantly increased protein, creatinine, and Na+ and K+ concentrations while less pronouncedly reducing cholesterol levels. Leukocytes, erythrocytes, haematocrits, haemoglobin, MCHC, and platelet counts remained unchanged.

No significant changes were observed in leukocytes, erythrocytes, haematocrit, haemoglobin, MCHC, or platelet counts.

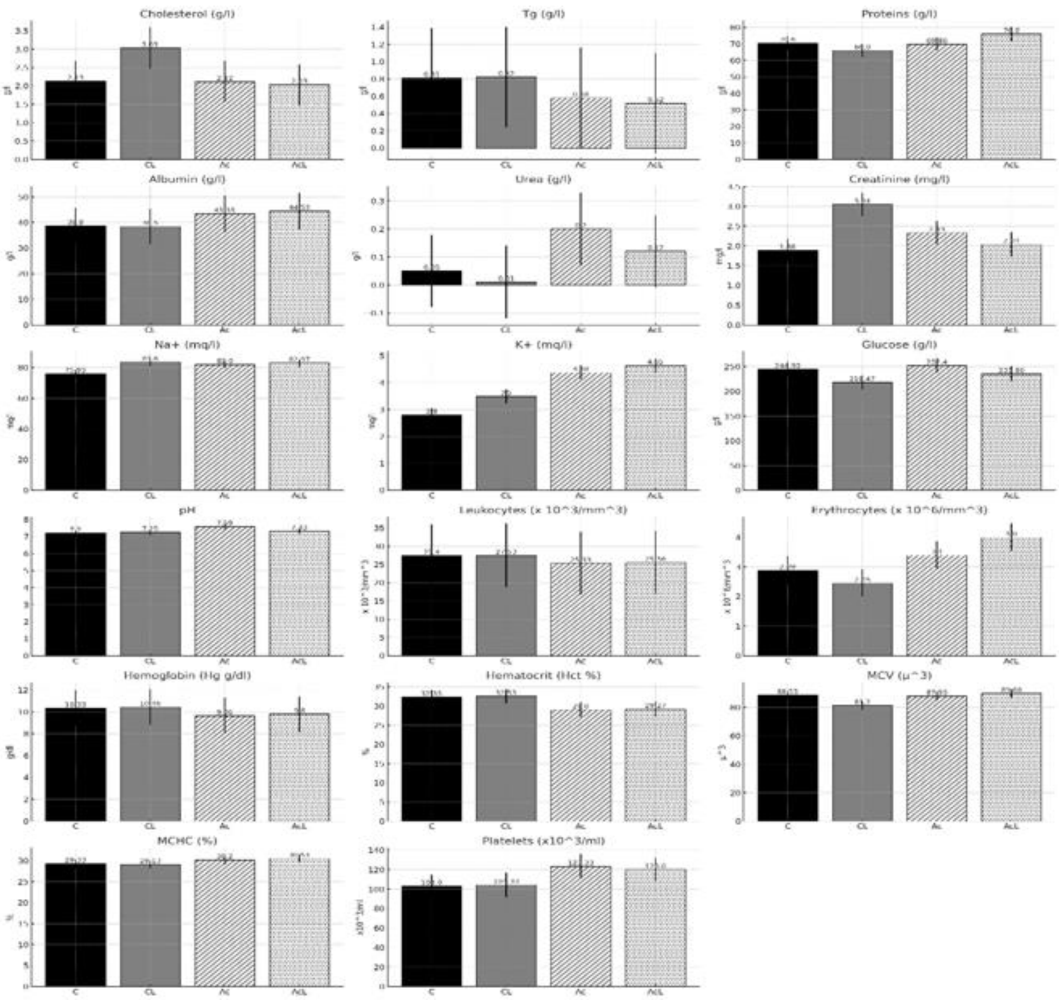

Figure 2: Effects of flaxseed (Linum usitatissimum) dietary supplementation, early age acclimation and their combination on biochemical and hematological parameters in broilers at T2 (*Control group fed a standard diet: (C); (CL): Control group fed a standard diet supplemented with 5% ground linseed, (Ac): Acclimated group fed a standard diet; (AcL): acclimated group fed a standard diet supplemented with 5% ground linseed).*

At T3, acclimation significantly reduced protein, cholesterol, and K+ concentrations ($p<0.05$) and had a lesser effect on triglycerides and creatinine ($p<0.05$), while increasing the pH, glucose, and urea levels. Albumin levels were unaffected (Figure 3). Nevertheless, linseed supplementation at T3 significantly reduced protein, triglycerides, glucose, creatinine, cholesterol, and Na+ concentrations but significantly increased urea and pH levels. The interaction of both treatments markedly reduced cholesterol and urea levels and, to a lesser extent, creatinine. Platelet counts were reduced at T3, whereas leukocytes, erythrocytes, haemoglobin, haematocrit, MCV, and MCHC counts were unaffected.

Acclimation significantly reduced protein, cholesterol, and K+ concentrations while increasing pH, glucose, and urea levels. The effect on triglycerides and creatinine was less pronounced. Albumin levels remained unchanged. Linseed supplementation significantly reduced protein, triglycerides, glucose, creatinine, cholesterol, and Na+ concentrations but significantly increased urea and pH levels. The interaction of both treatments markedly reduced cholesterol and urea levels and, to a lesser extent, creatinine. Platelet counts were reduced, whereas leukocytes, erythrocytes, haemoglobin, haematocrit, MCV, and MCHC counts were unaffected.

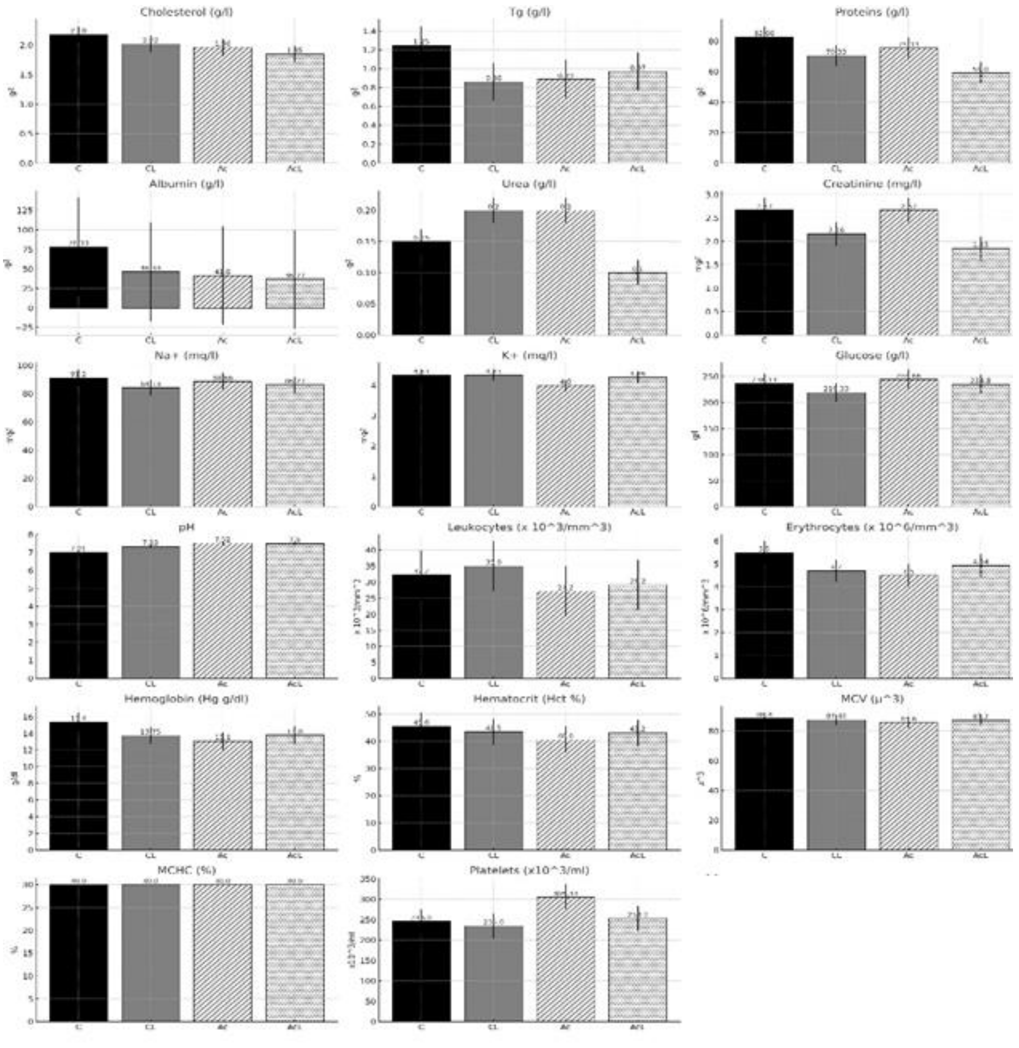

Figure 03: Impact of flaxseed (*Linum usitatissimum*) dietary incorporation, early acclimation as well as their interaction on biochemical and hematological parameters at T3 (*Control group fed a standard diet: (C); (CL): Control group fed a standard diet supplemented with 5% ground linseed, (Ac): Acclimated group fed a standard diet; (AcL): acclimated group fed a standard diet supplemented with 5% ground linseed).*

Early-age conditioning (EAC) showed its effects prominently at T3 by significantly reducing leukocyte counts. At T1, EAC significantly increased erythrocyte counts, but this effect was reversed at T2 and T3, where erythrocyte counts were markedly reduced. EAC had no effect on haemoglobin levels until T3, where a significant reduction was observed. The effect on mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) was notable at T2 but disappeared by T3.

EAC consistently and significantly increased platelet counts. The interaction of both treatments (EAC and linseed supplementation) significantly increased erythrocyte counts and MCV at T2. By the end of the rearing period, this interaction significantly reduced leukocyte and erythrocyte counts and less pronouncedly increased platelet counts.

Discussion

The study's findings highlight the multifaceted impacts of early age conditioning (EAC) and linseed supplementation (LS) on broiler chickens, particularly under conditions of chronic heat stress and coccidiosis infestation. Hypoproteinaemia, a common consequence of coccidiosis due to reduced feed intake and acute intestinal haemorrhage, was mitigated by EAC and LS through their effects on metabolic balance and stress response mechanisms. Chronic heat stress typically reduces protein synthesis, yet our study found that these treatments helped maintain protein levels, likely due to reduced cortisol secretion and protein catabolism, as supported by previous research (Kumar-Mondal et al., 2011; Temim et al., 1998).

EAC and LS also influenced lipid metabolism, with both treatments reducing cholesterol and triglyceride levels, crucial markers of metabolic health under thermal stress. This is consistent with findings by Ahmed et al. (2024) and Benabdelmoumene et al. (2016), who noted similar effects of dietary oil supplementation on lipid profiles. Furthermore, the significant increase in blood glucose levels observed with both treatments suggests an improvement in homoeostasis disrupted by coccidiosis, aligning with results from studies by Amer et al. (2021) and Jaiswal et al. (2017).

The coccidiosis-induced disruptions in lipid homoeostasis were evident in untreated infected chickens, reflected by elevated cholesterol and triglyceride levels, which were normalised by both EAC and LS. This normalisation mitigates one of the adverse effects of coccidiosis, supporting findings from Bengharbi et al. (2016) and Hosseini et al. (2022). Additionally, the treatments' impact on urea levels, a byproduct of protein degradation, indicates enhanced proteolysis and suggests no adverse effects on renal function, corroborating results by Attia et al. (2024).

The observed increase in blood pH indicates a beneficial effect of EAC and LS in preventing acid-base imbalances caused by heat stress and coccidiosis. This finding is supported by studies on the metabolic impacts of dietary interventions under stress conditions (Biswas et al., 2024; Rezar et al., 2023). Moreover, EAC and LS improved fluid balance and mineral concentration, enhancing Na^+ and K^+ levels, which is crucial for maintaining homoeostasis under heat stress (Leskovec et al., 2018; Oganija and Apata, 2022).

In haematological changes due to coccidiosis, such as reduced haemoglobin levels and erythrocyte counts, were also mitigated by EAC and LS. EAC's role in reducing haematocrit and blood viscosity improves circulation and thermolysis, which is critical for broilers under thermal stress (Yahav et al., 1997; Zhou et al., 1997). The treatments' combined effects significantly increased erythrocyte counts, countering the reductions caused by coccidiosis and enhancing overall health, as noted by Sur et al. (2023) and Singh et al. (2023).

The reduction in leukocyte counts at T3, particularly in treated groups, indicates a localised and effective immune response against parasites, highlighting the anti-coccidia, anti-inflammatory, and healing properties of linseed. This reduction, coupled with improved recovery, aligns with findings by Jimoh et al. (2022) and Swain et al. (2023). Overall, EAC and LS promote red blood cell maturation and increase MCV, suggesting enhancing resilience and health status in broilers under stress conditions.

Conclusion

This study highlights the significant effects of early age conditioning (EAC) and linseed supplementation (LS) on the biochemical and haematological parameters of broiler chickens exposed to chronic high temperatures and coccidiosis. EAC effectively mitigated the adverse effects of heat stress by enhancing erythrocyte and platelet counts and normalising blood pH, thereby improving thermoregulation and reducing intestinal haemorrhages. LS demonstrated its potential as a dietary supplement by significantly reducing cholesterol, triglycerides, and protein levels, while increasing glucose and creatinine levels, contributing to better metabolic balance and resilience against coccidia infestation.

The combined treatments (EAC and LS) further amplified these beneficial effects, significantly increasing erythrocyte counts and maintaining protein synthesis under stress conditions. The treatments also demonstrated a pronounced ability to stabilise mineral concentrations, particularly Na^+ and K^+, suggesting improved mineral retention and fluid balance during heat stress. The significant reduction in leukocyte counts at T3 indicates a localised and effective immune response, promoting recovery and overall health.

This study provides compelling evidence for the efficacy of EAC and LS in enhancing the health and performance of broilers under challenging environmental conditions, offering practical insights for improving poultry management and welfare.

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