Research Article

Impact of Melatonin on Improving Productive Traits of Broiler Exposed to Environmental Stress

Hashim Hadi Al-Jebory[1*], Mohammed Khalil Ibrahim Al-Saeedi[2], Majeed Ajafar[1], Nihad Abdul-Lateef Ali[1]

[1]*Department of Animal Production- college of agriculture-Al-Qasim Green University- Babylon province, Iraq;* [2]*Department of Environment - College of Environmental Science -Al-Qasim Green University- Babylon province, Iraq.*

Abstract | During a five-week trial, this study investigated the impact of various levels and methods of adding melatonin to water and diets on the growth performance of broiler chickens. One day old, 400 Ross 308 broiler chicks were randomly allocated into five random groups, with each group being quadruplicated and 20 birds allocated to each replication. Melatonin was supplemented in the G1 control group. G2 received 10 mg of melatonin per kg of diet, G3 received 20 mg of melatonin per kg of diet, G4 received 10 mg of melatonin per liter, and G5 received 20 mg of melatonin per liter, respectively. All groups' chicks were exposed to heat stress (30-35-30 °C) during the breeding period. Our results showed a significant increase ($p \leq 0.01$) for the G5 group in live body weight (LBW) and cumulative weight gain (W.G.). The study compared the feed intake (F.I.) and relative growth rate (RGR) of this group with those of other groups. It found a significant improvement in feed intake (F.I.) for all melatonin additions. When comparing total mortality to the G1 group, there was a significant improvement for the G3, G4, and G5 groups, with rates of 1.449, 1.467, and 1.425 respectively, in feed conversion ratio (FCR) compared to the G1 group, which had a rate of 1.563.

Keywords | Broiler, Environmental, Heat stress, Melatonin, Productive performance.

Received | January 03, 2024; **Accepted** | February 05, 2024; **Published** | February 26, 2024
***Correspondence** | Hashim Hadi Al-Jebory, Department of Animal Production- college of agriculture-Al-Qasim Green University- Babylon province, Iraq; **Email:** hashimhadi@agre.uoqasim.edu.iq
Citation | Al-Jebory HH, Al-Saeedi MKI, Ajafar M, Ali NAL (2024). Impact of melatonin on improving productive traits of broiler exposed to environmental stress. Adv. Anim. Vet. Sci. 12(4): 775-781.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.4.775.781
ISSN (Online) | 2307-8316

INTRODUCTION

It is well-documented that heat stress can impact various tissues of a bird's body. It can compromise the integrity of the intestines by reducing oxygen levels in the intestinal epithelium (Dokladny *et al*., 2016). This can lead to disruptions in the digestion and absorption of nutrients, as well as an imbalance in the microbial population within the intestinal tubule (Burkholder *et al*., 2008; Deng *et al*., 2012). This, in turn, leads to a decrease in the birds' productivity, as well as an imbalance in their physiological state, as the hormone corticosterone increases during stress, impacting the depletion of energy sources and lowering the birds' immunity (Al-Jebory *et al*., 2023; AL-Jaryan *et al*., 2023). Melatonin, a hormone secreted from the pineal gland, is known for its antioxidant properties, which help capture free radicals both inside and outside the body (Bonnefont-Rousselot *et al*., 2011). In addition to its antioxidant properties and ability to scavenge free radicals, melatonin protects cells both inside the body (*in vivo*) and outside the body, directly and indirectly. Melatonin's antioxidant capacity is attributed to its amphiphilic nature, allowing it to traverse physical barriers and mitigate oxidative damage in both fat cells and cells with high water content (Jou, 2010). This is accomplished through various mechanisms, including direct effects as an antioxidant and the neutralization of the toxic effects of active oxygen species, as well as indirect effects through the activation of antioxidant enzymes, which efficiently reduce the activity of enzymatic oxidants (Russel *et al*., 2016), additionally, melatonin plays a significant part in removing the harmful effects of certain heavy metal elements that are involved in Haber-Weiss and Fenton reactions (Taha *et al*., 2020; Al-Samrai *et al*., 2023). Sahin *et al*. (2003) noted supplemental melatonin significantly increased live weight gain and carcass characteristics under stress conditions heat exposure increased the excretion of N, Ca, P, Zn, Fe, and Cr and decreased retention rates for them, and dietary melatonin supplementation returned these values to normal, while Kadim and Alhamdani, (2023) observed that adding melatonin to the feed reduced the negative effects of heat stress on broilers when added at a concentration of 0.50 mg/kg feed. Consequently, the current study investigates the impact of varying amounts of the hormone melatonin on enhancing the productive performance of broilers under ambient heat stress. Melatonin can thereby mitigate the detrimental effects of heat stress.

MATERIALS AND METHODS

Four hundred broiler chickens (Ross 308) were utilized in this experiment, which was carried out at the Al-Anwar Poultry Company farm in the Babylon/Al-Muradiyah Governorate between July 25, 2023, and August 29, 2023. Each group had four replicates, and each one had 20 chicks. Melatonin was added to the diet and drinking water according to the following groups: G1 control group, G2 added 10 mg melatonin/ kg diet, G3 added 20 mg melatonin/ kg diet, G4 added 10 mg melatonin/ litter drinking water, G5 added 20 mg melatonin/ litter drinking water, Diets (The composition and nutrient content) were designed to meet the nutrient requirements for the birds during stages of growth (starter, grower, and finisher) based on NRC, (1994). All groups of chicks were exposed to heat stress (Table -1- °C) during the breed period.

Table 1: Explain the temperature (°C) during the breed period (time/ week).

Weeks	Hour			
	6 morning	12 evening	6 evening	12 morning
1	34.12	35.61	34.21	34.26
2	31.22	35.87	35.36	33.12
3	32.56	34.36	35.42	34.57
4	32.33	35.91	35.33	33.21
5	31.46	35.19	35.16	33.69

Performance traits

The LBW, WG, feed intake, FCR, and mortality were measured according to (Al-Fayadh and Naji, 1989). The RGR was measured according to (Regassa *et al*., 2014).

Live body weight and weight gain

The body weight and weight gain were calculated according to:

LBW= birds weight/ birds count

WG= final weight- initial weight

Relative growth rate

The relative growth rate was measured by:

RGR = ((W2-W1) / (0.5 (W2+W1)*100 as W1 = initial weight and W2 = final weight.

Feed intake

Feed intake per week was indicated according to:

FI= feed intake/ birds count

Feed conversion ratio

The feed conversion ratio was indicated according to:

FCR= weight gain/ feed intake×100

Mortality %

The mortality was indicated throughout the whole study and calculated as follows.

Total mortality percent = (The number of mortality birds during the experiment period)/(the total number of birds) X100

The data were analyzed using a completely randomized design (CRD) to study the effect of the studied parameters on the various characteristics, and the significant differences between the means were compared using the multinomial test (Duncan, 1955). The program SAS (2012) was used in statistical analysis according to the following mathematical model:

$Y_{ij} = \mu + T_i + e_{ij}$

So: Yij: the value of the jth view belonging to transaction i.

μ: the general average of the trait.

Ti: The effect of treatment i (as the study included the effect of the 5 aforementioned treatments).

eij: Random error that is normally distributed with a mean of zero and a variance of 2 e Ϭ.

RESULTS AND DISCUSSION

Live body weight

The effect of study groups on LBW is presented in Table 2. The results show no significant difference in initial weight; in the first week, the G3 groups demonstrated a significant

Table 2: Effect of melatonin in LBW of broiler exposed to heat stress (mean ± standard error)

Groups	Initial weight	1st W	2nd W	3rd W	4th W	5th W
G1	37.61±0.23	159.97± 2.75 b	450.36± 4.07 b	846.16± 1.32 c	1398.44± 15.90 b	2007.44± 3.54 c
G2	37.82±0.39	168.33± 1.02 ab	457.34± 1.34 b	873.34± 1.26 b	1429.97± 15.11 ab	2075.43± 1.89 bc
G3	38.38±1.21	169.18± 2.24 a	470.02± 0.60 a	914.44± 2.93 a	1466.71± 12.67 a	2156.24± 6.44 ab
G4	37.94±0.14	167.34± 3.35 ab	472.54± 3.49 a	931.30± 2.73 a	1447.86± 6.07 a	2169.94± 2.84 ab
G5	38.52±0.72	167.50± 1.24 ab	479.29± 3.01 a	892.54± 4.23 b	1459.79± 11.17 a	2207.14± 3.27 a
Significant	N.S	*	**	**	**	**

N.S. not significant, * at level (0.05≤p), ** at level (0.01≤p).

Table 3: Effect of melatonin in weekly W.G. of broiler exposed to heat stress (mean ± standard error)

Groups	1st W	2nd W	3rd W	4th W	5th W	Cumulative W.G.
G1	122.36± 2.95	290.39± 3.23 b	395.79± 1.95 b	552.75± 4.51 ab	608.52± 2.96 c	1969.83± 3.75 c
G2	130.51± 0.99	289.01± 3.15 b	415.99± 8.34 b	556.63± 2.89 ab	645.45± 6.23 bc	2037.60± 1.66 bc
G3	130.80± 1.43	300.83± 2.40 ab	444.42± 1.25 a	552.26± 1.42 ab	689.53± 4.58 abc	2117.86± 4.98 ab
G4	129.39± 4.30	305.20± 4.75 ab	458.75± 1.57 a	516.55± 2.21 b	722.08± 5.93 ab	2131.99± 2.77 ab
G5	128.97± 2.03	311.79± 3.11 a	413.24± 2.69 b	567.45± 3.15 a	747.35± 3.39 a	2168.82± 3.45 a
Significant	N.S.	*	**	*	*	**

N.S. not significant, * at level (0.05≤p), ** at level (0.01≤p).

Table 4: Effect of melatonin in RGR of broiler exposed to heat stress (mean ± standard error)

Groups	1st W	2nd W	3rd W	4th W	5th W
G1	123.85±2.53	95.15±0.01 a	61.04±0.31 bc	46.57±1.25 a	35.73±0.33 b
G2	126.62±2.64	92.38±0.21 b	62.52±0.09 b	48.33±1.13 a	36.82±0.27 b
G3	126.05±2.35	94.12±0.30 ab	64.20±0.19 ab	28.90±1.07 b	38.06±0.04 ab
G4	126.06±1.79	95.39±0.11 a	65.37±0.72 a	43.42±1.23 a	39.91±0.61 ab
G5	125.20±1.85	96.41±0.31 a	60.24±0.49 c	48.24±1.15 a	40.76±1.00 a
Significant	N.S.	*	*	*	*

N.S. not significant, * at level (0.05≤p).

Feed intake

superiority ($p \le 0.05$) compared to the G1 group, while in the second week, there was a significant increase ($p \le 0.01$) in the G3, G4, and G5 groups compared to the G1 and G2 groups. By the third week, there was a significant increase ($p \le 0.01$) for the G2, G3, G4, and, G5 groups compared to the G1 group. The fourth week also showed a significant superiority ($p \le 0.01$) in the G3, G4, and G5 groups compared to the G1 group. Additionally, in the fifth week, there was a significant increase ($p \le 0.01$) in the G5 group compared to the G1 and G2 groups.

Weekly weight gain

Table 3 shows the effect of the experiment on weekly weight gain. The first week noted no significant difference between treatments. Second week appeared significant increase ($p \le 0.05$) for the G5 group compared to G1, and, G2 groups, in the third week had significant superiority ($p \le 0.01$) for the G3, and, G4 groups compared to the G1, G2, and, G5 groups, while in fourth the G5 groups had significant superiority ($p \le 0.05$) compared to G4 group and not a significant difference between the G1, G2, G3, and, G4 groups, the G5 group appear height significant increase compared to G1, and, G2 groups in the fifth week and cumulative weight gain.

Relative growth rate

The effect of different methods of adding melatonin to the RGR is noted in Table 4. There was no significant difference between groups in the first week the second week showed a significant increase ($p \le 0.05$) for the G1, G4, and G5 groups on the G2 group. In comparison, the G4 group had a higher significance ($p \le 0.05$) compared to the G1, G2, and G5 groups in the third week, in the fourth had were significant increase ($p \le 0.05$) for the G1, G2, G4, and, G5 groups on G3 group, as well in fifth week noted significant increase ($p \le 0.05$) for the G5 group compared to G1, and, G2 groups.

Table 5 shows the effect of the study on F.I. During the first week, there was a significant increase ($p \le 0.01$) for the G1, G2, G4, and G5 groups on the G3 group, second

Table 5: Effect of melatonin in F.I. of broiler exposed to heat stress (mean ± standard error)

Groups	1st W	2nd W	3rd W	4th W	5th W	Total F.I.
G1	129.36± 1.89 a	312.71± 2.34 b	618.02± 8.56	964.61± 7.92 a	1400.87± 6.75 b	3425.58± 36.18
G2	130.01± 0.34 a	332.10± 1.12 a	611.06± 9.36	947.08± 12.21 ab	1468.34± 14.83 a	3488.61± 31.84
G3	122.66± 1.28 b	333.91± 2.48 a	593.27± 3.88	916.17± 13.12 b	1465.25± 12.98 a	3431.27± 17.85
G4	128.36± 0.90 a	339.15± 2.36 a	594.63± 7.21	964.91± 19.95 a	1483.90± 10.49 a	3510.96± 23.30
G5	127.52± 0.86 a	331.34± 1.00 a	597.49± 13.38	929.69± 10.42 ab	1487.38± 4.87 a	3473.43± 45.38
Significant	**	**	N.S.	*	*	N.S.

N.S. not significant, * at level (0.05≤p), ** at level (0.01≤p).

Table 6: Effect of melatonin in FCR of broiler exposed to heat stress (mean ± standard error)

Groups	1st W	2nd W	3rd W	4th W	5th W	Total FCR
G1	1.081± 0.05 a	1.112± 0.02 ab	1.561± 0.03 a	1.748± 0.05 ab	2.314± 0.12	1.563± 0.02 a
G2	0.996± 0.01 ab	1.149± 0.02 a	1.470± 0.03 ab	1.707± 0.06 ab	2.279± 0.07	1.524± 0.02 ab
G3	0.937± 0.01 b	1.110± 0.02 ab	1.386± 0.03 bc	1.664± 0.06 b	2.148± 0.13	1.449± 0.03 bc
G4	0.994± 0.03 ab	1.113± 0.02 ab	1.296± 0.02 c	1.868± 0.04 a	2.068± 0.10	1.467± 0.01 bc
G5	0.988± 0.01 b	1.062± 0.02 b	1.437± 0.03 b	1.639± 0.04 b	1.998± 0.08	1.425± 0.02 c
Significant	*	*	**	*	N.S.	**

N.S. not significant, * at level (0.05≤p), ** at level (0.01≤p).

and fifth weeks appeared to significant increase ($p \leq 0.01$) for the G2, G3, G4, and, G5 groups other G1 group, while in the third week and total F.I. there was no significant difference among groups, in fourth week shown significant increase ($p \leq 0.01$) for the G1 and G4 groups on the G3 group.

Feed conversion ratio

The effect of the experiment in FCR is shown in (Table 6) during the first week significant improvement ($p \leq 0.05$) for the G3 and G5 groups on the G1 group, and in the second week appeared to be a significant improvement ($p \leq 0.05$) for the G5 group on the G2 group, while in the third week and total FCR significant improvement ($p \leq 0.01$) for the G3, G4, and G5 groups on G1 group, in fourth-week significant improvement ($p \leq 0.05$) for the G3, and G5 groups on G4 group, meanwhile no significant difference in the fifth week.

Table 7: Effect of melatonin in total mortality of broiler exposed to heat stress (mean ± standard error)

Groups	Total mortality
G1	18.75±6.25 a
G2	11.25±7.50 b
G3	12.50±6.25 b
G4	10.00±2.50 b
G5	12.50±1.25 b
Significant	*

* at level (0.05≤p).

Total mortality

From 1-5 weeks of chick's age (Table 7), the mortality was significantly higher ($p \leq 0.05$) in the G1 group compared to the G2, G3, G4, and G5 groups.

Melatonin is important for circadian rhythms, and intestinal mucosal immune cells, this explains the productive performance improvement in melatonin groups. Melatonin can potentially support intestinal disease therapy significantly, but its exact mechanism remains unknown (Gil-Martín *et al*., 2019), because melatonin in the colon typically does not enter the systemic circulatory system, melatonin is a multifunctional chemical with a special place in the bidirectional communication between the neuroendocrine and immunological systems (Paulose *et al*., 2016). It seems intestinal melatonin has a localized influence on the mucosal immune system. Mucosal epithelial tissue, gut-associated lymphoid tissues, and commensal microbiota make up the intestinal mucosal immune system, which acts as the first line of defense against gastrointestinal infections and carries out the mucosal local defense to preserve immunological homeostasis (Nie *et al*., 2019). Gut melatonin, which is impacted by feed intake, plays a significant role in the function of the gut epithelium (Ma *et al*., 2017). The location of immune cells, microbial colonization, and nutrition digestion occur in the gastrointestinal intestine tract (Arnoldini *et al*., 2018; Ma *et al*., 2018). The intestinal epithelial barrier forms the mucosal immune system, shields surrounding tissues from intestinal contents and the luminal environment, and allows specific molecules to be absorbed for nutritional purposes; if this intestinal balance is upset, an overreaction to the interaction of gut microbes with the host barrier created by intestinal epithelial cells may result in the development of several gut-related disorders (Sommer *et al.*, 2017), This could explain why the melatonin addition groups' productive performance improved due to better intestinal health, epithelium histological features, and the microbial community, which enhanced nutrient absorption and digestion and consequently enhanced chick growth. Prior research on chickens has also demonstrated the connection between cellular oxidative stress and heat stress (Estévez, 2015; Surai *et al.*, 2019) since excess free radicals produced during oxidative stress damage DNA, lipids, proteins, and other components of every cell. Depending on how severe the oxidative stress is, the results might range from minor, reversible alterations to apoptosis and cell death (Lennon *et al.*, 1991). Therefore, in poultry, oxidative stress is linked to biological harm, severe health conditions, slower growth rates, and financial losses (Estévez, 2015). Melatonin functions by controlling the activity of mitochondria to lower the temperature of the cell and mitochondria, which lowers the generation of free radicals and lessens the impact of oxidative stress, studies have shown a decrease in the physiological and productive performance of birds raised at high temperatures, suggesting that the control group birds' worsening productivity may have been caused by heat stress (Ohtsu *et al.*, 2015), since the alterations are also caused by the chronic heat stress-related process of thermoregulation, behavioral and physiological harm to several tissues and organs, including the liver (Jastrebski *et al.*, 2017) and heart tissue (Aengwanich and Simaraks, 2004). The liver is crucial for preserving the body's metabolic equilibrium and plays a significant role in synthesizing antioxidants, contributing to the liver's low heat stress tolerance (Santana *et al.*, 2021). Apart from the fact that stress raises the hormone corticosterone, which in turn causes growth hormone to drop due to feedback, the hormone corticosterone also directs the body's energy resources to resist stress, which also results in lower growth (Jastrebski *et al.*, 2017).

Melatonin is usually associated with reduced oxidative damage (Taha, 2016). Melatonin's metabolites could eliminate reactive oxygen species and active nitrogen types, setting it apart from other antioxidants. This ongoing defense mechanism provided by melatonin and its metabolites is known as free radical defense (Hardeland and Pandi-Perumal, 2020), this means that even at low concentrations, melatonin is highly effective at protecting cells from oxidative damage (Tan *et al.*, 2015). One of the activities of melatonin is its antioxidant potential, which is attribute to two side chains: an indoleamine, a 3-amide group, and a 5-methoxy group (Galano *et al.*, 2016). Because of its high lipophilicity and hydrophobicity, melatonin and its metabolites can easily cross cell membranes, making them significant for direct and indirect antioxidant activities (Tamura *et al.*, 2012). Melatonin shields many biomolecules from damage caused by reactive oxygen species, nitrogen species, and free radicals through direct scavenging in oxidative stress situations; this reduces the quantity of glutathione in many cells (Pandi-Perumal *et al.*, 2013). The actions of enzymes that increase intracellular levels of decreased GSH are preserved by melatonin; glutathione is oxidized to its disulfide glutathione, which is swiftly reduced back to GSH by glutathione reductase; melatonin stimulates these enzymes, which is one of the critical ways that melatonin reduces oxidative stress, a nuclear binding site appears to be the site of action for melatonin's ability to modulate enzyme activities, which is one of the antioxidant activities that regulate the GSH/GSSG balance (Reiter *et al.*, 2018), as a result of increased melatonin, numerous GSH-metabolizing enzymes, such as CAT (Marshall *et al.*, 1996). Furthermore, melatonin increases gamma glutamylcysteine synthesizes to regulate glutathione formation, which considerably raises glutathione levels (Karasek and Winczyk, 2006), so that, in contrast to the traditional antioxidants (vitamins C and E), one melatonin molecule can scavenge up to ten ROS (Taha *et al.*, 2020; Al-Samrai *et al.*, 2023).

CONCLUSION

Overall, heat stress decreased broiler production, while the concentration of 20 mg melatonin/liter of drinking water gave the best results in terms of improvement in LBW, WG., and relative growth efficiency, at the same time the other melatonin groups improved in productive performance compared to the control group.

ACKNOWLEDGEMENTS

Authors would like to express their special thanks and gratitude to the Department of Animal production/College of agriculture/ Al-Qasim Green University, and Al-Anwar company for all their kind and helpful support during the study period.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This study is the first one by using melatonin hormone in Iraq/ Babylon government, to decrease heat stress acute in broiler.

AUTHORS CONTRIBUTION

All authors contributed equally for this work.

ETHICAL STATEMENT

All animals in this study were handled and cared for according to the appropriate biosecurity procedures. The study was performed by the rules of the 'Guide for the Care and Use of Laboratory Animals, and Broiler ROSS 308 guide that were approved by the Ethics Committee of the College of Agriculture, Al-Qasim Green University, Iraq (Number 20 A.P. at 20/6/2023) before starting this study.

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