

Melatonin Modulates Immunity in Broilers Exposed to Heat Stress

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ABSTRACT: Previously thought to be a pharmaceutical for regulating sleep and rhythm, **Published Online:** Melatonin has demonstrated its potential as a co-adjuvant treatment by modulating mast cells, **April 06, 2024**

macrophages, dendritic cells, and T/B cells. Melatonin is immunomodulatory, which means it regulates T-cell differentiation, interrupts T/B cell interaction, and reduces the production of pro-inflammatory factors; it acts as an antioxidant through specific receptors. Therefore, this study investigates the effect of Melatonin in improving broiler immunity using four hundred broiler chicks divided into five treatments. There was a significant increase ($p \leq 0.05$) for the T2, T3, T4 and T5 treatments in total protein, albumin, globulin and immunoglobulins compared to the T1 treatment. Significant increase ($p \leq 0.05$) for the T3, T4 and, T5 treatments in antibody titer for Newcastle (ND), infection bronchitis (IB), influenza daises (INFLU). Significant improvements ($p \leq 0.05$) for the T2 and T4 treatments in the relative weight of Fabricius gland and Fabricius index.

KEYWORDS: Melatonin, immunity, heat stress, broiler, immunoglobulins.

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INTRODUCTION

Because of climate and environmental changes, the poultry industry faces a constant challenge in terms of heat stress, which causes poor productive, immune and physiological performance of broilers; the hormone corticosterone also causes the destruction of all energy stores in the body, leading to fatigue and death (Al-Saeedi et al., 2023; Al-Jebory et al., 2023 a,b). The pineal gland secretes Melatonin, a significant hormone molecular product that controls circadian cycles and the immune system, into the bloodstream (Auld et al., 2017). It has also been studied recently as a co-adjuvant therapy for several gastrointestinal disorders. Melatonin is a multifunctional chemical that acts as a critical signal that mediates microbial metabolism, circadian rhythms, and intestinal mucosal immune cells. It has the potential to significantly improve intestinal illness therapy, albeit its exact mechanism is yet unknown (Gil-Martín et al., 2019). Due to its multi-functionality, Melatonin is exceptional in the reciprocal communication between the immunological and neuroendocrine systems.

Since Melatonin produced in the intestines typically does not reach the systemic circulatory system, the potential impact of intestinal Melatonin on the mucosal immune system is limited. Mucosal epithelial tissue, gut-associated lymphoid tissues, and commensal microbiota make up the intestinal mucosal immune system, which acts as the first line of defence against gastrointestinal infections and carries out the mucosal local defence to preserve immunological homeostasis (Paulose et al., 2016; Ma et al., 2017; Nei et al., 2019). When Melatonin was added to feed and drinking water for broilers under heat stress, Al-Jebory et al. (2024) observed an increase in the productive traits of the birds. Thus, the current experiment aimed to determine how Melatonin could strengthen broiler immunity in situations where there is ambient heat stress.

MATERIALS AND METHODS

This experiment was conducted using four hundred broiler chicks from July 25, 2023, to August 29, 2023, at the Al-Anwar Poultry Company farm in the Babylon/Al-Muradiyah Governorate (Ross 388). There were four duplicates and one 20-chick per group. By the following categories, Melatonin was introduced to the food and water: during the breeding phase, chicks in the T1 control treatment, T2 added 10 mg of melatonin/kg meal, T3 added 20 mg of melatonin/kg diet, T4 added 10 mg of melatonin/litter drinking water, and T5 added 20 mg of melatonin/litter drinking water were all exposed to heat stress (30–35–30 c).

Studied traits

The studied traits were calculated according to Luico and Hitchner (1979); Synder et al., (1984); Al-Khafaji and AL-Jebory (2019); Al-Khafaji et al., (2022); Al-Saedi et al., (2021). The data analysis was done by SAS (2012), and the test was used by Duncan (1955).

RESULTS AND DISCUSSION

1- Biochemical traits

Table 1 shows the effect of the study on some biochemical traits; in 21 days of chick's age, the total protein significantly increased ($p \leq 0.05$) in T3 treatment compared to T1 and T5 treatments, while there was no significant difference in albumen and globulin. At 35 days of the chick's age, the T1 treatment reached the highest level ($p \leq 0.05$) in total protein and globulin in albumin. The T2, T3, T4 and T5 treatments increased ($p \leq 0.05$) more than the T1 treatment.

21 days			
Treatments	Total protein	Albumen	Globulin
T1	2.88±0.29 c	1.61±0.07	1.27±0.28
T2	3.48±0.08 ab	1.60±0.10	1.87±0.14
T3	3.73±0.14 a	1.71±0.04	2.01±0.18
T4	3.22±0.12 abc	1.63±0.15	1.59±0.27
T5	3.02±0.04 bc	1.71±0.13	1.31±0.16
Significant	*	N.S	N.S
35 days			
Treatments	Total protein	Albumen	Globulin
T1	5.24±0.58 a	1.23±0.40 c	4.01±0.13 a
T2	4.66±0.75 b	1.63±0.38 b	3.03±0.28 b
T3	3.91±0.68 c	2.01±0.12 a	1.90±0.19 d
T4	4.72±0.51 b	2.13±0.2 a	2.59±0.35 c
T5	4.46±0.63 b	2.09±0.03 a	2.37±0.33 c
Significant	*	*	*
NS is not significant. * at level ($p \leq 0.05$)			

2- Immunoglobulins

The immunoglobulin changes shown in (table 2), in 21 days, noted a significant increase ($p \leq 0.05$) for all melatonin addition treatments compared to T1 treatment in IgM, IgG and IgA. Meanwhile, in 35 days, there was a significant increase ($p \leq 0.05$) for T2 and T4 IN IgM and IgG, respectively, and there was no significant difference in IgA among treatments.

21 days			
Treatments	IgM	IgG	IgA
T1	1.67±0.02 c	2.17±0.05 c	1.24±0.28 c
T2	2.31±0.03 a	2.86±0.09 b	1.93±0.10 b
T3	2.41±0.01 a	3.21±0.11 a	2.00±0.15 b
T4	1.92±0.08 b	3.14±0.21 ab	2.17±0.08 ab
T5	2.07±0.11 ab	2.98±0.07 b	2.25±0.20 a
Significant	*	*	*
35 days			
Treatments	IgM	IgG	IgA
T1	1.36±0.05 b	1.53±0.08 c	1.01±0.11
T2	2.18±0.14 a	1.75±0.06 b	0.98±0.09
T3	2.08±0.08 b	1.69±0.10 b	1.06±0.10
T4	2.16±0.07 b	1.91±0.12 a	1.05±0.10
T5	2.27±0.11 b	1.87±0.01 ab	1.02±0.06
Significant	*	*	N.S
NS is not significant. * at level ($p \leq 0.05$)			

3- Antibody titer

Table 3: explain the study impact on antibody titer in 21 days significant improvement ($p \leq 0.05$) for all melatonin addition treatments compared to T1 treatment. In 35 days of chicks age in ND significant increase ($p \leq 0.05$) for T1, T3, T4 and T5 treatments on T2 treatment, while in IB and INFLU, titer significant increase ($p \leq 0.05$) for T2, T3, T4 and T5 treatments compared to T1 treatment.

Table 3: Effect of Melatonin in antibody titer of broiler exposed to heat stress (mean±S.E)			
21 days			
Treatments	ND titer	IB titer	INFLU titer
T1	4230.15±75.12 d	5017.20±142.36 c	4369.10±150.21 d
T2	4425.00±100.10 b	5190.32±69.14 c	4536.21±142.39 c
T3	4615.40±50.14 a	6010.52±105.12 a	4752.18±127.20 a
T4	4375.51±121.36 c	5522.13±111.31 b	4632.89±96.41 b
T5	4469.21±82.69 b	5560.89±147.30 b	4589.17±114.70 c
Significant	*	*	*
35 days			
Treatments	ND titer	IB titer	INFLU titer
T1	3612.02±63.12 b	4521.23±42.31 d	5120.33±126.10 c
T2	3598.21±55.42 c	4821.00±36.17 b	5430.17±205.14 b
T3	3930.14±75.14 a	4698.23±60.89 c	5379.10±149.58 b
T4	3874.91±87.10 ab	4560.74±87.01 d	5389.36±29.85 b
T5	3739.55±25.36 b	5070.89±204.20 a	5670.41±146.78 a
Significant	*	*	*
* at level ($p \leq 0.05$)			

4- Relative weight of Fabricius gland and Fabricius index

Table 4 shows the treatment's effect on the relative weight of the Fabricius gland and Fabricius index; there was a significant increase ($p \leq 0.05$) for T2 and T4 in the relative weight of the Fabricius gland and Fabricius index on other groups.

Table 4: Effect of Melatonin in the relative weight of Fabricius gland and Fabricius index of broiler exposed to heat stress (mean±S.E)		
35 days		
Treatments	Relative weight of Fabricius gland	Fabricius index
T1	0.296±0.02 c	1.00±0.02 c
T2	0.335±0.01 a	1.13±0.04 a
T3	0.324±0.03 b	1.09±0.03 b
T4	0.334±0.01 a	1.12±0.05 a
T5	0.317±0.02 b	1.07±0.06 b
Significant	*	*
* at level ($p \leq 0.05$)		

Exposure to heat stress causes an increase in the concentration of the hormone corticosterone, which decomposes lymphocytes. Therefore, immunity is impaired in broilers exposed to heat stress (Xu et al., 2018). Also, heat stress in birds stimulates the secretion of the hormones epinephrine and norepinephrine from the adrenal medulla and then increases their level in the blood, which will destroy lymphocytes (McCorkle and Talor, 1993). The improvement in the immune characteristics of broilers may be due to the influential role of Melatonin in supporting immunity; as a critical element of neuro-immuno-modulation, intestinal Melatonin specifically has immune-regulating qualities in the gut, including regulating intestinal inflammation, functioning as a suitable immune system participant, or acting as a natural antioxidant for immune stimulation, different mechanisms are employed to carry out these modulatory acts, such as the immune system's ability to regulate and modify different cells and cytokines and its amphiphilic characteristics, which make it resistant to attacks by free radicals, Melatonin has the potential to be developed as a medicine that targets the gut-brain axis in addition to relieving the symptoms of intestinal illnesses (Huang et al., 2013; Chen et al., 2017). T-cell proliferation, differentiation, and activation are all regulated by Melatonin. T cells possess four melatonin-synthesizing enzymes (AADC, TPH, ASMT, and AA-NAT) as well as four melatonin receptors (MT1, MT2, RZRβ, and RORα,β,γ). Melatonin regulates these T cell receptors in several ways, such as suppressing Th1 differentiation by decreasing IFN-γ production, enhancing Th2 differentiation by increasing IL-4 and IL-10 production, preventing Th17 cell differentiation, and lowering the number of Treg

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cells restricted in immunosuppressive environments, Melatonin stimulates the differentiation of B cells. It raises the cytokine IL-4, which is produced by Th2 cells. The promotion is restricted to bursa B cells, where Melatonin mediates the proliferation of B lymphocytes triggered by light. Melatonin, however, does not directly affect other B cell differentiation, but it generally has the potential to co-occur with Th2 cells and stimulate the synthesis of IgG1. Melatonin balances the intracellular and extracellular amounts of Melatonin, regulates the inflammatory response, and functions as an injury sensor in mast cells; with the help of AA-NAT, the activated NF- κ B pathway during the inflammatory response causes the mast cell to create pro-inflammatory cytokines like TNF- α and IL-6, it also increases the manufacture of En-Mel and releases more of it for its paracrine effects, this ultimately causes an increase in Ex-Mel and interferes with the produced process in the opposite way, Mel has a broad spectrum of inhibitory actions on macrophage inflammatory responses, including the suppression of TFs, HiFs, STAT-related signalling, and IRFs (Ha et al., 2006; Ma et al., 2019).

CONCLUSION

Melatonin has contributed to improving the immunity of chickens through its effect on lymphocytes and phagocytes, reducing the severity of stress and its effect in improving the immunity of chickens, as during stress the hormone corticosterone is secreted, which works to decompose lymphocytes, which are considered the immune arm in the body, which reduces the immune system and at the same time melatonin. It has four receptors on lymphocytes, which enhances their role and immune effectiveness when broilers are exposed to stress.

REFERENCES

1. Al-Jebory HH, Al-Saeedi MKI, Ajafar M, Ali NAL (2024). The effect of Melatonin on improving the productive traits of broilers exposed to environmental stress. *dv. Anim. Vet. Sci.* 12(4): 775-781.
2. Al-Jebory, H.H. and S.A.H. Naji. 2021. effect of Pelleted Fermented Feed in Production Performance of Laying Hens. fourth International Conference for Agricultural and Sustainability Sciences IOP Conf. Series: Earth and Environmental Science 910 (2021) 012007 IOP Publishing doi:10.1088/1755-1315/910/1/012007.
3. Al-Jebory, H.H., M. K. I. Al-Saeedi., I. L. Al-Jaryan., and F.R. Al-Khafaji., 2023 a. Impact of Neem (*Azadirachta Indica*) leaves powder on growth performance of broiler (Ross 308) exposed to HS *Research Journal of Agriculture and Biological Sciences*, 15(2): 1-5. DOI: 10.22587/rjabs.2023.15.2.1
4. AL-JEBORY, H.H., H.F.A.-H. AL-JEWAHERY, MKI. AL-SAEEDI, I.M.A Abd ALZAHRA , I.H. KADEM, N.A.-L. ALI. 024. The Antibacterial effects and chicks quality response of copper nanoparticles of Japanese quail in hatching. *or. . Agri. Sci.* 5 (1): 40-44. <https://doi.org/10.5281/zenodo.10826294>.
5. Al-Jebory, H.H., M.A. Elsaygher, H.Q. Baqer, M.K.I. Al-Saeedi, I.L. Al-jeryan, and F. Al-Khafaji. 2023 b. histological Study of Jejunum in Broiler Chicks Fed in the Embryonic Period with Silver Nanoparticles and Exposed to Heat Stress. *Syrian Journal of Agricultural Research – SJAR.* 0(5): 138-149.
6. Al-Jebory, H.H., M.K.I. Al-Saeedi, S.A. Sakr, F.R. Al-Khafaji, N.A.L. Ali, B.A.M. Lehmoed, H. Taheri. AA A. Qotbi, and S. Ghazi. 023 c. Improving Chicken Growth Performance with Nano Silver Added to Drinking Water. *International Journal of Scientific Research in Biological Sciences.* 0 (6): 01-04.
7. Aljebory, H.H.D S.A.H. Naji. 2021. effect of Pelleted Fermented Feed-in Egg Quality of Laying Hens. *iyala Agricultural Sciences Journal* 13 (1): 41-57. <https://dx.doi.org/10.52951/dasj.21130105>.
8. Al-Khafaji, F. R., and AL-Jebory, H. H. 2019. Effect Of Injection In Hatching Eggs With Different Concentrations Of Nanosilver At 17.5 Days Age In Some Hatching Traits And Blood Parameters For Broiler Chickens (Ross 308). *Iant Arch.*, 19(2):1234–1238.
9. Al-Khafaji, F.R.A., H.H.D. Al-Gburi, and N.M.A. Al-Gburi. 2022. Effect of injecting hatching eggs with different concentrations of nanosilver at the age of 17.5 days from the age of the embryos on the qualitative traits for the carcass and some lymphatic organs for the broiler chickens (Ross 308). *euroQuantology.* 20(11): 2768 -2774. doi: 10.14704/NQ.2022.20.11.NQ66281.
10. AL-SAEEDI, MKI, HH AL-JEBORY, and M. AJAFAR. 023. Effect of in Ova Injection with Nano-copper in Productive Performance of Japanese Quail Exposed to Pathological and Environmental Challenges. *nnals of Agri-Bio Research* 28 (2): 361-366.
11. Al-Saeedi, M.K.I., H.H. Dakhil, and F.R.A. Al-Khafaji. 2021. Effect of adding Silver Nanoparticles with drinking water on some Lymphatic Organs and Microflora in the intestinal for broiler Chickens (ROSS 308). *st INTERNATIONAL VIRTUAL CONFERENCE OF ENVIRONMENTAL SCIENCES IOP Conf. Series: Earth and Environmental Science* 722 (2021) 012004 IOP Publishing doi:10.1088/1755-1315/722/1/012004.
12. Auld F, Maschauer EL, Morrison I, Skene DJ, Riha RL. Evidence for the efficacy of Melatonin in the treatment of primary adult sleep disorders. *leep Med Rev.* 2017;34:10-22.

13. Chen X, Eslamfam S, Fang L, Qiao S, Ma X. Maintenance of gastrointestinal glucose homeostasis by the gut-brain axis. *urr Protein Pept Sc.* 2017;18(6):541-547.
14. Duncan, D.B. 1955. ultiple ranges test and Multiple F – test. *Biometrics.* 11:1-42.
15. Gil-Martín E, Egea J, Reiter RJ, Romero A. The emergence of Melatonin in oncology: focus on colorectal cancer. *ed Res Rev.* 2019. <https://doi.org/10.1002/med.21582>
16. Ha E, Han E, Park HJ, et al. Microarray analysis of transcription factor gene expression in melatonin-treated human peripheral blood mononuclear cells. *Pineal Res.* 2006;40(4):305-311.
17. Huang H, Wang Z, Weng SJ, Sun XH, Yang XL. Neuromodulatory role of Melatonin in retinal information processing. *rog Retin Eye Res.* 2013;32:64-87.
18. Lucio, B., and S.B. Hitchner . 1979 . nfection bursal disease emulsified vaccine ; Effect upon neutralizing-antibody levels in the dam and subsequent protection of the progeny . *Avian Dis.,*23:466-478.
19. Ma N, Tian Y, Wu Y, Ma X. Contributions of the interaction between dietary protein and gut microbiota to intestinal health. *urr Protein Pept Sc.* 2017;18(8):795-808.
20. Ma, N., J. Zhang, R.J. Reiter, X. Ma. 2019. Melatonin mediates mucosal immune cells, microbial metabolism, and rhythm crosstalk: A therapeutic target to reduce intestinal inflammation. *ed Res Rev.* 2019;1-27.
21. McCrokle, F.M., and RL Talor, 1993. Biogenic amines regulate at avian immunity . *oult. Sci.,*72(7):1285-1288.
22. Nie P, Li Z, Wang Y, et al. Gut microbiome interventions in human health and diseases. *ed Res Rev.* 2019. [tps://doi.org/10.1002/med.21584](https://doi.org/10.1002/med.21584)
23. Paulose JK, Wright JM, Patel AG, Cassone VM. human gut bacteria are sensitive to Melatonin and express endogenous circadian rhythmicity. *LOS One.* 016;11(1):e0146643.
24. SAS. 2012. tatistical Analysis System, Users Guide. tatistical. Version 9.1 th ed. AS.Inst . Inc.Cary. C USA.
25. Synder, E.L., P.M. Ferri, and D.F. Mosher. 984. ibronectin in liquid and frozen stored blood components. *ournal of Applied Psychology,*24(1): 53-56.
26. Xu, Y., X. Lai, Z. Li, X. Zhang, and Q. Luo, 2018. ffect of chronic heat stress on some physiological and immunological parameters in different breed of broilers. *oult.Sci.,* 97(11):4073–4082.