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EXOGENOUS MELATONIN ENHANCES CARCASS TRAITS, LITTER CONDITION, AND WELL-BEING OF BROILERS EXPOSED TO HEAT STRESS

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Abstract

One of the main goals of producing broilers successfully is to maintain dry litter. Exposure to heat stress may be affected carcass traits and litter condition in poultry houses. This study was conducted to evaluate the using exogenous melatonin for elevate the negative effects of heat stress on carcass traits and litter for broiler chickens (Ross 308). 400-one day old broiler chicks were randomly split into five equal groups with four replicates, each with 20 chicks. The first group (C) fed on basal diet without melatonin and served as control. While the second (M1) and third (M2) groups received 10 and 20mg of melatonin / kg diet, respectively. And the 4th and 5th received 10 and 20mg of melatonin in drinking water / Liter, respectively. All birds were subjected to heat stress during the experimental period (1-35 d). Results indicated that carcass yield, breast, thigh and back percentages were significantly ($P<0.05$) increased for birds received melatonin hormone compared with control. Feces weight was significantly ($P<0.05$) decreased in birds received melatonin compared to the control group at the 2nd, 4th and 5th weeks of age. Data showed that using of melatonin significantly ($P<0.05$) improved litter condition (moisture, pH and bacterial count) at heat stress. Also, adding melatonin significantly ($P<0.05$) improved feather hygiene and decreased footpad dermatitis%. Finally, we can conclude that administration melatonin in diet or drinking water for heat-stressed broilers at levels 10 or 20mg significantly improved carcass traits, litter condition with beneficial effects on well-being of birds.

KEYWORDS: melatonin, litter, well-being, carcass, heat stress, broilers.

Introduction

Heat stress (HS) is an important factor affecting the poultry industry, especially the welfare of birds. A negative balance between the net energy leaving the animal's body and entering its surroundings and the energy the animal produces as heat is what causes heat stress in animals (**Lara and Rostagno, 2013**). It can cause imbalances in the bacteria community within the intestinal tubule and disturbances in the digestion and absorption of nutrients (**Deng *et al.*, 2012**). The effects of heat stress on a bird's body and its tissues are widely known. According to **Donkoh (1989),** heat stress decreases feed intake, live weight gain, and feed conversion ratio, which all have an impact on broiler performance. By lowering the oxygen levels in the intestinal epithelium, it may compromise the integrity of the intestines (**Dokladny *et al.*, 2016**). As a result, birds become less productive and exhibit physiological imbalances. This is because stress causes the hormone corticosterone to rise, which impacts the depletion of energy sources and the immune system of the birds (**Al-Jubouri *et al.*, 2023; Al-Jaryan *et al.*, 2023**). According to **Zhang *et al.*, (2012),** when broiler chickens are subjected to high temperatures throughout their growth phase, heat stress results in inferior meat characteristics and ultimately lower meat quality. The efficiency of nutrition, metabolism, hormones, and the immune system is all decreased by heat stress (**Zhao *et al.*, 2023**). Furthermore, heat stress produces inflammation, which disrupts the microbiome (**Zwirzitz *et al.*, 2023**).

The pineal gland secretes the hormone melatonin, which is well-known for its antioxidant qualities that aid in scavenging free radicals from the body's exterior **(Bonnefont-Rousselot *et al.*, 2011)**. Because of its strong ability to scavenge free radicals, melatonin is a potent antioxidant **(Reiter, 1995)**. Apart from its potential to neutralize peroxyl and hydroxyl radicals as well as other reactive oxygen species, melatonin plays a crucial role in numerous physiological processes, including energy metabolism, temperature control, and immunological response **(Sahin *et al.*, 2004)**. Additionally, according to **Pieri *et al.*, (1994),** melatonin increases the activity of a number of enzymes connected to the antioxidant defense system. Additionally, **Zeman *et al*, (2001)** revealed that using melatonin in diets of broiler led to a significant reduction in their heat production. As reported by **Kadim and Alhamdani, (2023),** melatonin reduced the negative effects of heat stress on broilers when administrated in the diet at 0.50 mg/kg feed. Furthermore, **Sahin *et al.*, (2003)** stated that melatonin significantly increased live weight gain and carcass characteristics under heat stress.

The present work examines the effects of using different melatonin levels in diet or drinking water for broilers on some carcass traits and litter conditions in ambient heat stress.

MATEIAL AND METHODS

Place and objective of experiment:

The present study was carried out at the Al-Anwar Poultry Company farm in the Babylon, Al-Muradiyah Governorate, Iraq to evaluate the effects of administration melatonin in diet or drinking water for heat-stressed broilers (**Ross 308**) on some carcass traits and litter condition during the period from July 2023 to August 2023.

Birds' husbandry, diets and experimental design:

A total number of 400-one day old broiler chicks (Ross 308) were randomly assigned into five equal groups. Each group had four replicates, each with 20 chicks. The first group (C) fed on basal diet without melatonin and used as control. While the second (M1) and third (M2) groups were fed on basal diets supplemented with melatonin at levels of 10 and 20mg/kg diet, respectively. On the other hand, the fourth (M3) and fifth (M4) groups were fed on basal diets and received melatonin in drinking water at levels of 10 and 20mg/ Liter, respectively. All chicks were reared in floor pens at the same space with wood shavings as litter.

For three days after hatching, the newly hatched chicks were kept under constant lighting, 24 hours a day. After that, the photoperiod was progressively shortened (one hour/week) to 20 hours at light intensities of 5 and 10 Luxes, respectively.

Based on **NRC (1994),** diets were designed to satisfy the nutrient needs of the birds at each stage of growth. Starter diets from 0 to 21 days of age containing 23% crude protein, 3000 Kcal/kg metabolic energy, 3.84% fat, 3.44% crude fiber, 0.51% available phosphorus and 1.05% calcium, while grower diets from 22 to 35 days of age containing 21% crude protein, 3100 Kcal/kg metabolic energy, 5.86% fat, 3.49% crude fiber, 0.45% available phosphorus and 0.93% calcium.

For the duration of the trial, all birds were raised under identical management circumstances, fed the experimental diets ad libitum, and allowed unrestricted access to water. Throughout the trial, every group of chicks was subjected to heat stress as presented in **Table (1)**.

Table (1): Average ambient Temperature (°C) during the experimental period (Time/ day).

Time (Hour)	**Age (Day)**				
	0-7	**8-14**	**15-21**	**22-28**	**29-35**
6 Am	34.12	31.22	32.56	32.33	31.46
12 PM	35.61	35.87	34.36	35.91	35.19
6 PM	34.21	35.36	35.42	35.33	35.16
12 AM	34.26	33.12	34.57	33.21	33.69

Studied traits:

At the end of the trial (35 days of age), 8 birds were randomly taken from each group, slaughtered; plucked, and eviscerated then the dressed weight was obtained. Dressed carcasses were weighed and calculated relative to live body weight. Breast, thigh, and back were separated, weighed, and calculated as relative to live body weight.

For microbiological studies, three pooled litter samples were taken on days 21 and 35 from each replicate. Using sterile plastic bags, litter samples were taken from every replicate. Samples were put on ice and transported in an ice box to the lab where they were analyzed bacteriologically in less than two hours. A total of sixty litter samples were used for bacterial testing (**Klement *et al*., 1990**). Colony forming units (CFU) per gram were calculated as described by **Sahoo *et al*., (2017).** Litter moisture was determined as described by (**Sluiter *et al*., 2008**). According to **Hoskins *et al*., (2003)**, pH of litter was measured three times weekly then the average was taken. At the same time, feces weight was obtained using paper litter and then treated according to (**AOAC, 2019**). Foot pad dermatitis and feather hygiene percentages were evaluated as described by **Kaukonen *et al*., (2016)** and **Karcher *et al*., (2013),** respectively**.**

Statistical analysis:

General Liner Model (GLM) procedure of **SAS (1998) was** used for analyzing our results by using one-way ANOVA according to the following model:

$$\mathbf{Y_{ij} = \mu + T_i + E_{ij}}$$

Where, Y_{ij} = an observation; μ = the overall mean; T_i = effect of experimental treatments; (i= 1,2;) E_{ij} = Random error.

Duncan's multiple range tests at the level of P<0.05 (**Duncan, 1955**) used for determining significant differences of obtained means. A level of probability (P. value) of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

1- Carcass traits:

Broilers who received 20mg melatonin hormone in their diets or drinking water had the highest (P<0.05) values of carcass yield and breast percentages, followed by those who received melatonin at 10mg (M1 and M3 groups), but the lowest (P<0.05) values were found in the control group (**Table 2**). Data explained that the differences were not significant between M1 and M3, also between M2 and M4 groups in percentages of carcass yield and breast. Our findings in **Table 2** illustrated that administration of melatonin at different levels in diet or drinking water for broilers significantly (P<0.05) improved thigh and back percentages compared with control, without any significant differences among M1, M2, M3, and M4 treatments.

Our findings are in harmony with **Kucuk *et al*., (2003)** and **Akşit *et al*., (2006)**. Furthermore, Sahin et al., (2003) showed that carcass characteristics were significantly (P<0.01) improved for Japanese quails that received melatonin in their diets at 40mg/kg under heat stress conditions.

Additionally, **Zeferino *et al*., (2016)** reported that heat stress causes unfavorable effects on carcass characteristics, reduces its quality, and reduces breast meat percentage. According to **Lara and Rostagno (2013)**, high ambient temperatures may lead to physiological modifications that change carcass traits and degrade meat quality. Moreover, **Ahmed *et al.,* (2019)** recorded that carcass weight and eviscerated carcass weight for broilers reared under heat stress were significantly (P<0.05) decreased. However, our findings in contrast with **Chen *et al.*, (2023),** who showed that Melatonin supplementation had not significant effects on carcass characteristics. In Mongolian cashmere goats**, Duan *et al.*, (2019)** revealed that carcass weight and dressing percentage were not significantly affected by Melatonin implantation. In lambs, **Kanyar and Karadas (2023)** indicated that melatonin hormone significantly (P<0.05) improved hot carcass weight and dressing percentage for Awassy lamb when injected daily with melatonin at level 18 and 36mg/head as compared to those in the control group.

It is well known that one of the main issues facing the poultry industry is heat stress. It is reported by **Sahin *et al.*, (2003)** that heat stress resulted in notable reductions in carcass characteristics and supplementing with melatonin lessened these negative effects. Broiler chickens are sensitive to heat stress (**Yousaf *et al.*, 2019**). The low quality of the carcass for stressed birds could be the result of decreased feed intake and impaired utilization of nutrients **(Ahmed *et al.*, 2019)**. It has been shown that using melatonin hormone in diets for broiler chicks will enhance their growth and feed efficiency (**Osei *et al*., 1989**), thereby improving carcass characteristics. Several studies illustrated that supplementation of melatonin in feed or drinking water had positive effects on health, growth performance, feed intake, and feed efficiency **(Brennan *et al*., 2002; Abbas *et al*., 2007; Akbarian *et al*., 2014)** consequently improving carcass traits. Also, **Relić *et al*., (2022)** presented that melatonin significantly (P<0.05) increased final body weight and gain in broilers. According to **Ghanima *et al*., (2021),** the higher pre-slaughter weight, which has a strong correlation with dressing yield, could be the cause of the increased dressing and breast percentage. As well as **Elsagheer *et al*., (2022)** reported that the improvement in dressed carcass, breast, and thigh percentages might be attributed to increased edible muscle mass or an improvement in the utilization of nutrients.

Table (2): Effect of melatonin administration on carcass traits for broilers (Ross 308) exposed to heat stress at 35 d of age.

Item / Group	Carcass yield, %	Breast, %	Thighs, %	Back, %
C	70.55± 0.14[c]	31.30±0.02[c]	18.63±0.24[b]	9.57±0.28[b]
M1	73.11± 0.35[b]	32.14±0.01[b]	20.94±0.71[a]	10.77±0.32[a]

M2	74.32± 0.09^{a}	33.81±0.03^{a}	21.26±0.64^{a}	11.02±0.41^{a}
M3	73.16± 0.51^{b}	32.33±0.04^{b}	21.00±0.72^{a}	10.80±0.61^{a}
M4	74.29± 0.11^{a}	33.94±0.01^{a}	21.52±0.81^{a}	10.91±0.72^{a}
Significant	*	*	*	*

$^{a,b\text{ and }c}$:Means with different superscripts in the same column are significantly different (P< 0.05).

C= Control, **M1**= Melatonin at 10mg/Kg diet, **M2**= Melatonin at 20mg/Kg diet, **M3**= Melatonin at 10mg/Liter of drinking water, **M4**= Melatonin at 20mg/Liter of drinking water, ***=** Significant

Feces weight (g):

As shown in **Table (3)**, data illustrated that feces weight was significantly ($P<0.05$) decreased by using melatonin hormone during the second, fourth, and fifth weeks of age, while melatonin hormone had not any significant effect on feces weight during the first and third weeks of age. Our results revealed that broilers who received melatonin at a level of 20mg in diet or drinking water had the lowest ($P<0.05$) feces weight, followed by those who received 10mg melatonin, while the highest ($P<0.05$) values of were observed in the control group, without any significant differences between M1 and M3 or between M2 and M4 groups at the same weeks.

Using melatonin hormone for broiler chickens enhanced nutritional absorption and digestion (**Al-Jebory et al., 2024**). That could be the cause of declining feces weight (**Al-Jebory and Naji, 2021a**). Furthermore, by prolonging the feed's residence time in the chicken's digestive tract, enhancing the microbiome in the colon will enhance the preparedness and digestibility of the eaten feed and lower fecal excretion (**Al-Jebory and Naji, 2021b**). According to **Burkholder *et al*., (2008)**, heat stress is linked to increased fecal shedding, which raises the possibility of contamination in poultry products. This explains the increased amount of feces in the control group. **Kadim and Alhamdani, (2023)** indicated that negative effects of heat stress on broilers were significantly decreased by using melatonin hormone in diets at 0.50 mg/kg feed. Additionally, Melatonin has been shown by **Bermudez *et al*., (1983)** to decrease feed intake and increase feed efficiency. That explain the significantly decreasing in feces weight for birds received melatonin compared with those in control. It is reported that intestinal secretion was increased with heat stress for broilers (**Al-Jebory *et al*., 2023**), who added that the highest weight of feces for broilers at heat stress may be attributed to high drinking water consumption with the decrease in feed intake.

Table (3): Effect of melatonin administration on Feces weight (g) for broilers (Ross 308) exposed to heat stress.

week / Group	First	Second	Third	Fourth	Fifth
C	8.86±0.87	26.36± 1.22^{a}	38.56±2.01	44.63±1.31^{a}	50.81±1.31^{a}
M1	8.01±1.03	22.14±1.13^{b}	38.01±1.52	42.69±1.08^{b}	47.89±1.10^{b}
M2	7.99±1.12	21.16±0.98^{c}	37.69±1.84	41.12±1.00^{c}	46.38±1.22^{c}
M3	8.50±0.98	23.14±1.34^{b}	38.12±1.76	43.12±1.42^{b}	48.00±1.04^{b}
M4	8.65±1.08	21.69±1.28^{c}	37.81±1.11	41.50±1.29^{c}	46.57±1.06^{c}
Significant	**N.S**	*	**N.S**	*	*

[a,b and c]:Means with different superscripts in the same column are significantly different (P< 0.05).
C= Control, **M1**= Melatonin at 10mg/Kg diet, **M2**= Melatonin at 20mg/Kg diet, **M3**= Melatonin at 10mg/Liter of drinking water, **M4**= Melatonin at 20mg/Liter of drinking water, ***=** Significant, **NS**= Non-significant.

Litter condition:

3-1- **litter moisture percentage (%)**

Data in **Table (4)** showed that using melatonin in diets or drinking water at different levels significantly (P<0.05) decreased moisture percentage as compared to the control group. Our results explained that there were no significant effects among M1, M3, and M4 groups in the 1st and 5th weeks, among M2, M3, and M4 groups in the 2nd week, also among M1, M2, M3, and M4 groups in the 3rd and 4th weeks of age.

There is a relationship between feces weight and humidity of the litter **(Al-Jebory *et al.*, 2023**), who obtained that the high moisture in the litter for heat-stressed broilers could be attributed to increase in the moisture content in feces due to the high-water consumption. The decreasing in litter moisture percentage for melatonin groups may be attributed to the low weight of the feces produced from birds received melatonin in their diets. **Rodriguez *et al.*, (2019)** reported that increased fecal moisture is associated with heat stress. As well as **Kuter *et al.*, (2023)** noted that heat stress increased the litter moisture for broiler chickens (P<0.001) for broilers, which could be explained by the higher excretion of water and undigested feed. According to **Lott (1991)**, broilers exposed to acute heat stress consume more water. This may clarify the increase in litter moisture in the control group. **Gamba *et al.*, (2015)** illustrated that a proportionate rise in fecal moisture that occurs with increased water consumption for broilers.

Table (4): Effect of melatonin administration on litter moisture percentage (%) for broilers (Ross 308) exposed to heat stress.

week / Group	First	Second	Third	Fourth	Fifth
C	10.32 ± 0.75^{a}	20.25 ± 1.00^{a}	28.31 ± 0.18^{a}	31.45 ± 0.75^{a}	40.23 ± 1.20^{a}
M1	8.52 ± 0.90^{c}	15.69 ± 0.75^{c}	25.88 ± 0.37^{b}	28.21 ± 1.00^{b}	35.42 ± 1.25^{b}
M2	9.00 ± 0.63^{b}	18.23 ± 0.10^{b}	26.00 ± 0.42^{b}	27.60 ± 0.62^{b}	32.15 ± 2.84^{c}
M3	8.45 ± 0.55^{c}	18.11 ± 0.25^{b}	26.33 ± 0.21^{b}	29.00 ± 1.23^{b}	36.60 ± 2.36^{b}
M4	8.20 ± 0.55^{c}	18.56 ± 1.01^{b}	26.12 ± 0.21^{b}	28.00 ± 0.80^{b}	36.00 ± 2.00^{b}

Significance	*	*	*	*	*

[a,b and c]:Means with different superscripts in the same column are significantly different (P< 0.05).

C= Control, **M1**= Melatonin at 10mg/Kg diet, **M2**= Melatonin at 20mg/Kg diet, **M3**= Melatonin at 10mg/Liter of drinking water, **M4**= Melatonin at 20mg/Liter of drinking water, ***=** Significant

3-2- Litter bacterial contamination:

The effects of melatonin on litter bacterial contamination at the end of the experiment (35 days of age) are shown in **Table (5)**. Data explained that melatonin administration in diets or drinking water at different levels significantly (P<0.05) increased Lactobacillus bacteria compared with the control group. Data revealed that the differences in Lactobacillus bacteria were not significant between M1 and M3, also between M2 and M4 groups. However, the highest (P<0.05) values of E. coli bacteria and total bacterial count were observed for a litter of control group as compared to other groups that received melatonin whether in drinking water or diets. There were no significant differences among M1, M2, and M4, and also between M3 and M4 groups in E. coli bacteria. Additionally, results stated that the total bacterial count was not differ between M1 and M4, also between M2 and M3 groups.

Research has demonstrated melatonin's exceptional antibacterial properties, which include defense against bacterial infections. The results of **Akbarian *et al*., (2014)** reported that in birds treated with exogenous melatonin, ileal coliform count decreased while lactobacillus counts considerably rose. Also, **He *et al*., (2021)** reported that melatonin exerts antibacterial activities not just traditional gram-negative and -positive bacteria. There is evidence that melatonin can change the composition of the gut microbiome (**Yildirim *et al*., 2019**). In particular, it increases the relative abundance of Lactobacillus and decreases that of Clostridiales (**Jing *et al*., 2019**), which may result in improvement in microbial contamination in the litter of broilers that received melatonin in their diets**. Zheng *et al*., (2013)** stated that melatonin activates the antioxidant enzymes either directly or indirectly in broilers when there is heat stress. Additionally**, Kadim and Alhamdani (2023)** found that the addition of melatonin in diets for broilers significantly improved antioxidant Condition. Because melatonin has antioxidant properties, it helps broiler chickens' intestines stay healthier. Its antioxidant qualities, however, provide a viable explanation for why melatonin administration increases the Lactobacillus numbers in the broiler ileum (**Akbarian *et al*., 2014**). Strong antioxidant properties of melatonin have been documented (**Pieri *et al*., 1994**). This leads to a balance in the microbiota, an increase in good bacteria, and a decrease in bad bacteria (**Al-Jebory *et al*., 2023**). An increase in beneficial bacteria, particularly lactobacilli, lowers the pH in the intestine, which inhibits the growth of harmful bacteria like Salmonella (**Beal *et al*., 2006**), Campylobacter, and coliforms (**Heres *et al*., 2004**). Lactic acid, which is produced by lactobacillus bacteria, can enter the bacterial cell and damage nucleic acid and protein (**Moran *et al*., 2006**). According to research by **Al-Fataftah and Abdelqader (2014)**, birds exposed to heat stress showed lower (P<0.05) viable counts of Lactobacillus and Bifidobacterium, which explain the decrease in Lactobacillus count, also increasing total count and *E.* coli bacteria in the control group. **Xing *et al.*, (2019)** found that heat stress significantly decreased Lactobacillus bacteria and increased Coliforms bacteria in laying hens. That clarifies the highest values of Coliforms and the lowest values of Lactobacillus in litter for the control group. Increased moisture content in the litter promotes microbial growth, which raises microbial activity in the moist litter (**Kuter *et al.*, 2023**).

Table (5): Effect of melatonin administration on total litter bacterial contamination for broilers (Ross 308) exposed to heat stress.

Item / Group	Lactobacilli (log^{10} CFU/g)	E. coli (log^{10} CFU/g)	Total count (log^{10} CFU/g)
C	6.18 ± 0.15^{c}	2.72 ± 0.01^{a}	10.14 ± 0.02^{a}
M1	7.32 ± 0.16^{b}	1.38 ± 0.02^{b}	9.68 ± 0.04^{b}
M2	8.29 ± 0.22^{a}	1.63 ± 0.08^{b}	9.11 ± 0.02^{c}
M3	7.98 ± 0.09^{b}	0.92 ± 0.04^{c}	9.12 ± 0.03^{c}
M4	8.27 ± 0.25^{a}	1.02 ± 0.03^{bc}	9.52 ± 0.01^{b}
Significance	*	*	*

$^{a,b\ and\ c}$:Means with different superscripts in the same column are significantly different (P< 0.05).
C= Control, **M1**= Melatonin at 10mg/Kg diet, **M2**= Melatonin at 20mg/Kg diet, **M3**= Melatonin at 10mg/Liter of drinking water, **M4**= Melatonin at 20mg/Liter of drinking water, *= Significant

Litter pH:

From observations in **Table (6)**, we can report that litter pH values were significantly (P<0.05) increased in the litter for birds that received a control diet during the entire weeks of the experiment except for the first week of age; data showed that the melatonin administration had no significant effect on litter pH at the first week of age. At the 2nd and 4th weeks of age, data showed that the differences were not significant between M1 and M2 groups, also between M3 and M4 groups. Also, there were no significant differences among M1, M2, M3, and M4 groups at the 3rd week of age. There were no significant differences between M3 and M4 groups at the 5th week of age.

The highest value in litter pH for the control group was in harmony with results obtained by **Al-Jebory *et al.*, (2023)**, who also indicated that lactobacillus as a beneficial bacteria leads to decrease in the pH of the intestine. While, according to **Brauer-Vigoderis *et al.*, (2014)**, E. coli bacteria is known to raise the pH of the litter in chicken houses. **Hsu *et al.*, (2019)** suggested that the lactic acid bacteria may have beneficial uric acid–lowering effects. Moreover, the decrease in litter pH of the melatonin groups can be explained by the action of lactic acid on lowering the pH inside the bacterial cell (**Al-Jebory *et al.*, 2023**), which stops the action of enzymes and protein synthesis and its damage or may cause the outer wall of the bacteria to rupture, causing its death (**Alakomi *et al.*, 2000**). When heat stress was applied, the broiler litter's pH (P = 0.001) rose (**Kuter *et al.*, 2023**).

Table (6): Effect of melatonin administration on litter pH for broilers (Ross 308) exposed to heat stress.

week / Group	First	Second	Third	Fourth	Fifth
C	6.62±1.00	7.22±0.01^{a}	8.62±0.11^{a}	9.11±0.20^{a}	8.09±0.12^{a}
M1	6.54±1.20	6.75±0.09^{b}	7.13±0.10^{b}	8.23±0.27^{b}	6.89±0.10^{c}
M2	6.60±1.13	6.82±0.07^{b}	7.17±0.13^{b}	8.10±0.31^{b}	7.17±0.16^{b}
M3	6.71±1.41	6.11±0.04^{c}	7.09±0.12^{b}	7.25±0.21^{c}	6.31±0.01^{d}
M4	6.51±1.25	6.05±0.06^{c}	7.11±0.15^{b}	7.32±0.30^{c}	6.24±0.02^{d}
Significant	**N.S**	*	*	*	*

$^{a,b,c \text{ and } d}$:Means with different superscripts in the same column are significantly different (P< 0.05).
C= Control, **M1**= Melatonin at 10mg/Kg diet, **M2**= Melatonin at 20mg/Kg diet, **M3**= Melatonin at 10mg/Liter of drinking water, **M4**= Melatonin at 20mg/Liter of drinking water, *= Significant, **NS**= Non-significant.

Birds' well-being

3-3- **Foot pad dermatitis (%)**:

The present study revealed that using melatonin for broilers had significant effects on foot pad dermatitis (**Table, 7**). Our results indicated that birds received melatonin hormone in diets or drinking water had the lowest (P<0.05) values of foot pad dermatitis percentages compared with those in the control group. Data illustrated that the differences were not significant among M1, M3 and M4, also among M1, M2, and M4 groups in foot pad dermatitis %.

3-4- **Feather hygiene (%):**

The effects of melatonin administration on feather hygiene are shown in **Table (7)**. Our findings reported that the best (P<0.05) percentages of feather hygiene were observed for birds in the M2 and M4 groups, followed by M1 and M3 groups, while birds in the control group had the lowest (P<0.05) feather hygiene %. Data stated that there were not significant differences between M1 and M3, also between M2 and M4 groups in percentages of feather hygiene.

Footpad dermatitis is a skin ailment that affects the plantar surface of the footpads in broiler chickens and turkeys. The study's findings demonstrated a significant improvement in the percentage of footpad dermatitis and the standard of feather hygiene in the melatonin treatments. This can be attributed to melatonin's direct and indirect role in enhancing microbial and litter traits, as well as its ability to be added to reduce the effects of HS. According to **Kuter *et al*., (2023)**, broiler hens exposed to prolonged heat stress had a higher incidence and severity of footpad dermatitis (P < 0.01). Footpad dermatitis in broiler chickens is brought on by increased litter moisture (**Vieira *et al*., 2013**; **de Jong *et al*., 2014**), which results in significant financial losses for the global poultry sector. Numerous risk factors, such as moisture of the litter and environmental factors like high temperatures have been linked to the development of footpad dermatitis in broiler chickens (**Cengiz *et al*., 2011; 2013; 2018; Sevim *et al*., 2021**). Each of these elements has a negative impact on the growth and well-being of broiler chickens. None of the earlier research (**Mayne *et al*., 2007; Zhao *et al*., 2010; Saenmahayak *et al.,* 2010; Cengiz *et al*., 2011; Abd El-Wahab *et al*., 2013; Cengiz *et al*., 2018; Sevim *et al*., 2021**) has been able to completely prevent the development of footpad dermatitis. However, it's possible that melatonin reduces the development of footpad inflammation by lowering factors that exacerbate it, like the amount of moisture in the litter. According to **Garces *et al*., (2013)**, a drop in the litter's pH value is correlated with a drop in ammonia and uric acid, improving the welfare of the hens. Heat stress causes broiler chicks to have poor feather hygiene and more footpad dermatitis (**Al-Jebory *et al*., 2023**).

Table (7): Effect of melatonin administration on foot pad dermatitis and feather hygiene percentages for broilers (Ross 308) exposed to heat stress.

Item / Group	Lactobacilli (log10 CFU/g)	E. coli (log10 CFU/g)	Total count (log10 CFU/g)
C	6.18 ± 0.15^{c}	2.72 ± 0.01^{a}	10.14 ± 0.02^{a}
M1	7.32 ± 0.16^{b}	1.38 ± 0.02^{b}	9.68 ± 0.04^{b}
M2	8.29 ± 0.22^{a}	1.63 ± 0.08^{b}	9.11 ± 0.02^{c}
M3	7.98 ± 0.09^{b}	0.92 ± 0.04^{c}	9.12 ± 0.03^{c}
M4	8.27 ± 0.25^{a}	1.02 ± 0.03^{bc}	9.52 ± 0.01^{b}
Significance	*	*	*

$^{a,b \text{ and } c}$:Means with different superscripts in the same column are significantly different ($P< 0.05$).

C= Control, **M1**= Melatonin at 10mg/Kg diet, **M2**= Melatonin at 20mg/Kg diet, **M3**= Melatonin at 10mg/Liter of drinking water, **M4**= Melatonin at 20mg/Liter of drinking water, ****=** High Significant

CONCLUSION

It is well known that heat stress has negative effects on birds, which is reflected in carcass characteristics, and the quality of litter deteriorates while birds are exposed to heat stress. Therefore, from the results of the current study, it can be recommended to add melatonin, whether in the feed or drinking water of broiler birds, because it has a good effect in alleviating the negative effects of heat stress. It also improves the health condition of the birds, the quality of the carcass, and the performance of the birds during heat stress. It was also noted that the use of melatonin led to improve the litter condition by reducing moisture, feces weight, and bacterial count in the litter, which reflects on the well-being of the birds.

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