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EFFECTS OF DIETARY SUPPLEMENTATION OF MELATONIN ON GROWTH PERFORMANCE IN BROILER CHICKENS

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Abstract: The present study was undertaken to evaluate the effect of the dietary melatonin supplementation on serum antioxidant profile, immune status and growth performance in broiler chickens. Two hundred forty day old commercial broiler chicks weighing around 45 to 55g were procured and reared in common for 14 days. After 14 days of rearing, the broilers were divided randomly into four groups in triplicates with 20 birds in each group in three replicates. They were allotted to different dietary supplements; Group I comprised of control group fed basal diet; for Group II, the basal diet supplemented with Melatonin @ 20 mg/kg feed; for Group III, with Melatonin @ 30 mg/kg feed and for Group IV, with Melatonin @ 40 mg/kg feed. Daily feed consumption was recorded by subtracting the left-over feed from the feed offered every day for each group. A representative of two birds was slaughtered on 14th, 28th and 42nd day for recording the weight of immune organs. Growth performance, feed intake, and feed conversion ratio (FCR) were calculated weekly during the study period. Dressing percentage was also calculated by slaughtering five birds from each study group on day 42. Dietary melatonin supplementation has significantly improved body weight, average weekly body weight gain, feed conversion efficiency and dressing percentage in supplemented groups. It is concluded that the supplementation of melatonin in the diet have positive influence on growth performance in broiler chickens.

Keywords: Melatonin, body weight, FCR, growth, broiler chicken.

I. Introduction

India is fifth in meat production, according to production figures from the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2023)[1]. The nation produced 9.77 million tons of meat in 2022–2023, up from 6.69 million tonnes in 2014–2015. An estimated 5 million tonnes (MT) of broiler chicken meat are produced in the nation each year. With an average annual growth rate of 8% for poultry meat between 2014-15 and 2021-22, the India's poultry industry has demonstrated remarkable progress. From 2024 to 2032, the Indian poultry industry is expected to expand at a compound annual growth rate (CAGR) of 8.9%, from its

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2023 valuation of Rs. 2,099.2 billion. India earned USD 134 million by exporting poultry and poultry products to 64 countries in 2022-2023. The demand for chicken as a protein source has increased due to factors such as growing urban populations and changing dietary choices, which include a tendency towards more processed and ready-to-eat poultry items.

The poultry industry is concentrating on increasing operational efficiencies by boosting feed conversion ratios (FCR), which indicate higher bird weight as a percentage of feed utilized, in the light of high cost of feed. Melatonin's many biological roles, which go beyond its well-known regulatory role in circadian rhythms, have made supplementing with this hormone more significant in recent years. Nearly all living things, including microbes, plants and animals contain melatonin (N-acetyl-5-methoxy tryptamine) (Mannino *et al.,* 2021)[2].

According to research, melatonin has a variety of biological roles in the body, such as scavenging free radicals, promoting sleep, controlling reproductive processes, boosting immunological physiology, modifying biological rhythms, inhibiting the development of cancer, and having anti-inflammatory, anti-obesity, and anti-aging effects (Zhao *et al.,* 2019)[3]. According to Egbuniwe and Ayo (2016)[4], broiler chickens that secrete insufficient amounts of melatonin are linked to metabolic and physiological disorders that result in breeding diseases and deaths, as well as financial losses and a number of detrimental aspects of animal welfare, such as fearful reactions in the birds. Calislar *et al.* (2018)[5] reported similar findings. The most significant function of melatonin in the body is its involvement in the antioxidant system and defence of cells against the damaging effects of free radicals (Zhao *et al.,* 2019)[3]. Numerous studies have demonstrated that, under controlled lighting conditions, adding melatonin through chicken feed, drinking water, or parenteral administration has improved the health and immune status of the broiler chickens. It also positively influences growth performance, feed intake and feed conversion efficiency, the morphology of intestinal mucosa and circulating growth hormone levels (Zeman *et al.,* 1999; Brennan *et al.,* 2002; Abbas *et al.,* 2007; Sinkalu, 2012 and Akbarian *et al.,* 2014)[6,7,8,9,10]. Melatonin has been linked in a number of studies to changes in chicken growth and metabolism. Melatonin promotes feed conversion, according to several research (Osei *et al.,* 1989)[11], however the exact mechanisms of action are unknown. Melatonin may influence the effectiveness of nutrition utilization at multiple levels. This hormone can modulate intermediary metabolism (Zeman *et al.,* 1993)[12], interact with transcriptional factors (Chuang *et al.,* 1996)[13], impact thermoregulatory mechanisms (Rozenboim *et al.,* 1998)[14], influence energy metabolism (Apeldoorn *et al.,* 1999)[15] and affect growth hormone production (Zeman *et al.,* 1999)[6].

Perusal of literature revealed that there are meagre studies on dietary melatonin supplementation on growth performance. Hence, in this scenario, this study on the effects of dietary supplementation of melatonin on growth performance in broiler chickens was undertaken with the following objectives.

II. Materials and Methods

The present study was undertaken to evaluate the effect of dietary melatonin supplementation on antioxidant profile, immune status and growth performance in broiler chickens. The rearing of broiler chickens was carried out in the poultry house of the Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bengaluru, during September and October, 2023 for a period of 42 days (6 weeks) and the estimation were carried out in the Department of Veterinary Physiology and Department of Veterinary Public Health and Epidemiology, Veterinary College, Hebbal. Melatonin extra pure, 99% (25 grams) crystalline powder was obtained from Sisco Research Laboratory (SRL) stored at -20 °C until utilised.

Day old commercial broiler chicks (240 numbers) weighing around 45 to 55g were reared in common for 14 days for acclimatization period on broiler diet (as per BIS, 2007)[16]. After 14 days of rearing, the broilers were divided randomly into four groups in triplicates with 20 birds in each group in three replicates. They were allotted to different dietary supplements; Group I comprised of control group fed basal diet; for Group II, the basal diet was supplemented with Melatonin @ 20 mg/kg feed; for Group III, the basal diet supplemented with Melatonin @ 30 mg/kg feed and for Group IV, the basal diet supplemented with Melatonin @ 40 mg/kg feed. Institutional Animal Ethical Committee (IAEC) permission was obtained to carry out the study (No. VCH/IAEC/2023/21 dated 17.08.2023). All the broiler chicks were provided *ad-libitum* feed and fresh water (NRC, 1994)[17]. Daily feed consumption was recorded by subtracting the left-over feed from the feed offered every day for each group. Two birds from each group and each replicate were slaughtered on 14th, 28th and 42nd day and the weight of immune organs such as thymus, spleen and bursa of Fabricius were recorded. Growth parameters such as body weight, weekly body weight gain, feed intake, and feed conversion ratio (FCR) were recorded weekly during the study period. Dressing percentage was measured by slaughtering five birds from each group at the end of the study.

2.1. Measurement of Growth Performance

2.1.1. Body Weight (g)

The body weight of individual birds was recorded at the beginning of the experiment and at the end of each week till six weeks of age to monitor the pattern of body weight gain. The weighing

of the birds was done in the early hours of the day before feeding using digital electronic pan top balance.

2.1.2. Weekly Body Weight Gain (g)

The growth rate of the birds was reflected through the weekly live weight gain. Individual body weight of the birds from each group was taken at weekly interval, starting from the day-old stage. The birds were weighed during morning hours before feeding. Electronic weighing balance with a least count of 0.5 g was utilized for weekly weighing of body weight of chicks as well as the amount of feed supplied and consumed by the chicks. However, the separate weighing scales were used for weighing. The average weekly weight gain of the birds of the different groups was calculated by subtracting the previous week average weight of the group of the birds from the present weekly average weight of the group of birds.

2.1.3. Feed Intake (g)

The daily feed consumption of each group was estimated as differences between the total quantity of feed offered and quantity of feed left over during 24 hours period. Feed consumptions recorded were added together for seven days of the week and was considered as weekly feed consumption.

2.1.4. Feed Conversion Ratio (FCR)

The amount of feed consumed per unit gain (feed conversion ratio) was calculated as the ratio of feed consumed to weight gain (Maurya *et al.,* 2016)[18] during the experimental period. The feed consumption and weight gain for each week worked out for each treatment separately.

$$\textbf{FCR} = \frac{\text{Average feed consumption per bird during the week (kg)}}{\text{Average weight gain per bird during the week (kg)}}$$

2.1.5. Dressing Percentage

The per cent of the live animal that ends up as carcass after defeathering and removal of offal's were calculated.

$$\text{Dressing percentage} = (\text{Carcass weight} / \text{Live weight}) \times 100$$

2.2. Statistical Analysis

The data obtained were analysed statistically by two-way ANOVA with the application of Bonferroni post-test using 'GraphPad Prism' version 9.0.0 (121) computerized software, San Diego, CA, USA. The values were expressed as Mean ± Standard Error and the level of significance or non-significance was determined at P value of 0.05, as per the methods described by Snedecor and Cochran (1994)[19].

III. Results and Discussion

In the poultry sector, where maintaining animal health and welfare while meeting high production targets is demanded, defining the ideal light and nutritional management remains a difficulty. To mitigate the effects of stressors that birds encounter in intensive production settings, several authors have suggested oral administration of melatonin and its precursor tryptophan (Abbas *et al.,* 2007; Akbarian *et al.,* 2014; Calislar *et al.,* 2018 and Moore and Siopes, 2000)[8,10,5,20].

3.1. Body weight (g) and Weekly body weight gain (g)

In the present study, the mean body weights were significantly (P<0.05) higher in all melatonin supplemented groups compared to the control group on day 28, day 35 and day 42 (Table 1). The average weekly body weight gain did not vary significantly between the control and melatonin supplemented groups during week 1 and week 2. At week 3 the average weekly body weight gain was significantly higher (P<0.05) in Group III and Group IV when compared to control group. At week and week 5 the average weekly body weight gain was significantly (P<0.05) higher in Group IV when compared to control group. However, at week 6 average weekly body weight gain was significantly (P<0.05) higher in Group III when compared to the control group and other melatonin supplemented groups (Table 2).

Table 1. Body weight (g) in different groups of broiler chickens supplemented with melatonin

Period / Group	Day Old	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	44.60^{a} ±0.42	167.37^{a} ±3.67	460.70^{a} ±4.43	968.80^{a} ±5.25	1491.23^{a} ±14.86	2026.87^{a} ±14.80	2436.60^{a} ±15.22
Group II	44.97^{a} ±0.34	170.17^{a} ±0.98	460.43^{a} ±3.26	988.83^{ab} ±9.68	1546.13^{b} ±19.62	2098.30^{b} ±16.24	2550.63^{b} ±19.48
Group III	45.23^{a} ±0.35	171.77^{a} ±1.20	461.03^{a} ±3.38	1034.17^{bc} ±8.68	1598.03^{bc} ±22.10	2186.23^{c} ±21.04	2732.17^{c} ±26.86
Group IV	45.00^{a} ±0.28	173.63^{a} ±1.20	468.50^{a} ±2.34	1043.80^{c} ±11.04	1632.30^{c} ±22.02	2249.13^{d} ±20.63	2688.10^{c} ±26.44

Note: The values bearing different superscripts between groups (a, b, c) differ significantly (P≤0.05).

Table 2. Average weekly body weight gain (g) in different groups of broiler chickens supplemented with melatonin

Period / Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group I	122.77^{a} ±3.66	293.33^{a} ±5.62	508.10^{a} ±7.39	522.43^{a} ±14.90	535.63^{a} ±20.66	409.73^{a} ±21.05
Group II	125.20^{a} ±1.02	290.27^{a} ±3.39	528.40^{ab} ±9.57	557.30^{ab} ±19.70	552.17^{a} ±22.06	452.33^{a} ±16.01
Group III	126.53^{a} ±1.29	289.27^{a} ±3.80	573.13^{b} ±9.55	563.87^{ab} ±18.34	588.20^{ab} ±25.22	545.93^{b} ±30.31
Group IV	128.63^{a} ±1.31	294.87^{a} ±2.64	575.30^{b} ±10.68	588.50^{b} ±18.93	616.83^{b} ±17.73	438.97^{a} ±29.10

Note: The values bearing different superscripts between groups (a, b, c) differ significantly ($P \leq 0.05$).

These results were in agreement with the reports of Osei *et al.* (1989)[11], Patil *et al.* (2013)[21], Akbarian *et al*. (2014)[10], Calislar *et al.* (2018)[5], Sinkalu *et al.* (2020)[22], Relic *et al.* (2022)[23], Al-Samrai *et al*. (2023)[24] and Al-Jebory *et al.* (2024)[25] in broiler chicken and Sahin *et al*. (2004a)[26] in Japanese quails who reported that dietary melatonin supplementation has significantly improved body weight and average weekly body weight gain in these birds. Melatonin has a beneficial influence on broiler growth performance (Calislar *et al.,* 2018)[5] and energy metabolism (Zeman *et al*., 1999)[6] by stimulating the pituitary gland's production of growth hormone (GH) and its release into the bloodstream through its action on the hypothalamus. Shortening the photoperiod during the third week of the experiment resulted in decreased broiler activity, which has an impact on body weight gain (Apeldoorn *et al.,* 1999)[15]. The potential advantages of enhancing poultry growth performance under heat-stress conditions are further demonstrated by the fact that melatonin administration in heat-stressed quails enhanced the final body weight and liver weight (Sahin *et al.,* 2004a)[26].With the exception of a decrease in the incidence of sudden death syndrome, melatonin supplementation had no impact on body weight and feed consumption in broilers grown in appropriate temperature and illumination conditions (Clark and Classen, 1995)[27].

In a comparable manner, Akbarian *et al.* (2014)[10] observed that melatonin acts on the hypothalamus to stimulate the pituitary gland's production of growth hormone (GH) and its

release into the bloodstream. This has a favourable impact on growth performance, feed intake and conversion, intestinal mucosal morphology, and growth hormone levels (Zeman *et al.,* 1999)[6]. According to Sahin *et al.* (2004a)[26], melatonin treatment corrected the impairment in live weight and restored mineral status in Japanese quails, supporting their hypothesis that ascorbic acid and melatonin prevent heat-induced performance inhibition and oxidative stress. It has been observed that adding melatonin to the diet helps Japanese quail overcome the detrimental effects of heat stress on performance metrics. According to Patil *et al.* (2013)[21], the addition of melatonin to an aflatoxin-incorporated diet led to a considerably greater weight gain at the end of the three-week trial than the toxin-only treated group suggesting that it plays a favourable effect. In their study of the effects of dietary melatonin on broiler chickens exposed to continuous lighting for the first two weeks of their lives, Relic *et al.* (2022)[23] found that, on day 42, the body weight of the broiler chickens fed melatonin (30 mg/kg feed) significantly increased in comparison to the control group. They came to the conclusion that adding melatonin to broiler feed improved the birds' growth and productivity while reducing the negative impacts of constant lighting.

Nonetheless, Taha and Mawlood (2019)[28] showed how adding varying amounts of melatonin affected some physiological and productive traits of quail birds at 35 days of age. They also found that the addition of melatonin had no discernible impact on the live body weight or body weight gain between 7 and 35 days of age. According to reports, the hypothalamus-pituitary-adrenal axis is triggered during stressful situations, causing the adrenal gland to secrete glucocorticoids. Stress is characterized by an abundance of glucocorticoids (Osho and Adeola, 2020)[29]. Glucocorticoids are hormones associated with stress that help manage a stressful disposition in addition to preserving natural growth and metabolism of carbohydrates. Additionally, glucocorticoids have a catabolic effect on skeletal muscles, which results in a drop in muscle weight and, eventually, a decrease in growth by preventing the synthesis of proteins and accelerating the breakdown of muscle fibers (Furukawa *et al.,* 2016; Wang *et al.,* 2020)[30,31]. When glucocorticoids are released in response to stress, they generate free radicals, which are the cause of membrane lipid degradation, meat quality loss, immune system deterioration, muscle fibre atrophy, and decreased production (Furukawa *et al.,* 2016)[30].

3.2. Feed intake (g) and Feed conversion ratio

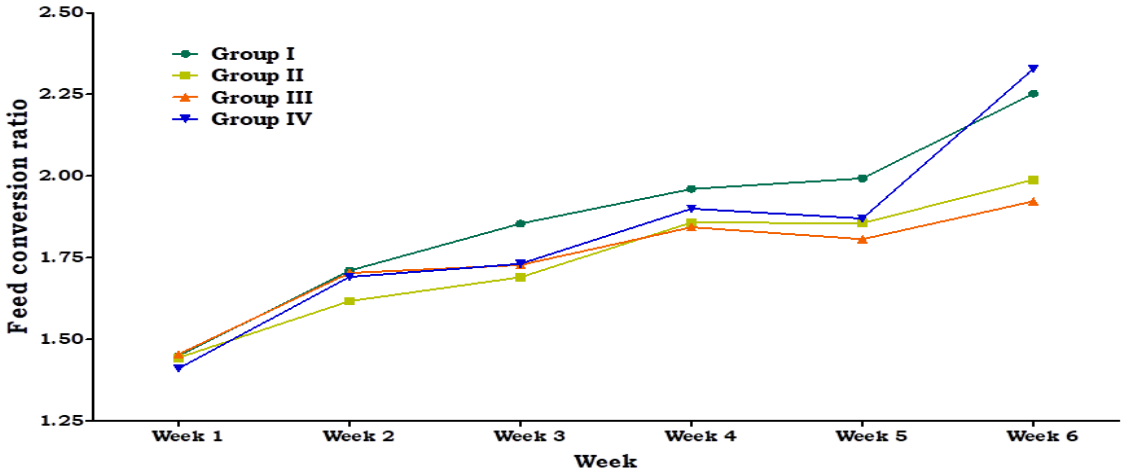
In the present study the feed intake was highest in melatonin supplemented Group IV and lowest was in Group III compared to control (Group I) during six weeks of rearing (Table 3).

At the end of the study period (Week 6) the FCR is better and lower in Group III when compared to the Group I, II and IV (Fig. 1). These results were in conformation with the study reports of Osei *et al.* (1989)[11], Clark and Classen (1995)[27], Akbarian *et al*. (2014)[10], Jalili Kolavani *et al.* (2015)[34], Sinkalu *et al.* (2020)[9], Relic *et al.* (2022)[23] and Fathi *et al*. (2023)[33] who reported that the dietary melatonin supplementation in broilers reduced feed intake and improved feed conversion efficiency when compared to the unsupplemented controls. Similar results have also been reported in rats and layer chickens (Ishibashi *et al.,* 1966; Bermudez *et al.,* 1983)[34,35]. Dietary melatonin increased feed efficiency and energy retention in broiler chicks by an average of 19%, according to Osei *et al.* (1989)[11]. Clark and Classen (1995)[27] examined the effects of feeding broilers melatonin constantly or intermittently on their health and performance. They discovered that during the first three weeks, feed consumption decreased linearly as melatonin levels rose. Additionally, the diurnal birds ate more than the 20 ppm chicks that were fed the 20 ppm melatonin meals in a daily rhythm. Birds fed the 40-ppm diet showed improved feed efficiency at 8 to 14 and 0 to 21 days compared to the control diet. Although there was no difference in the mean results for the 20 and 40 ppm diets, a linear improvement was also shown. From 8 to 14 days, feed efficiency was likewise lower under the diurnal diet compared to 20 ppm, and from 0 to 42 days, it was lower than both the control and 20 ppm diets. Performance from 0 to 21 days was unaffected by diurnally fed melatonin when compared to the control group.

Table 3. Weekly feed intake (g) per bird in different groups of broiler chickens supplemented with melatonin.

Period / Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
Group I	181.38	496.44	979.78	1092.40	1100.44	1018.84	4869.29
Group II	182.67	467.73	968.62	1047.47	1091.60	1085.60	4843.69
Group III	184.00	492.58	990.18	1039.24	1062.76	1049.76	4818.51
Group IV	181.44	498.60	995.73	1118.20	1153.69	1022.11	4969.78

Fig. 1. Feed conversion ratio in different groups of broiler chickens supplemented with melatonin.



The use of melatonin improved feed utilization (Clark and Classen, 1995; Apeldoorn *et al.,* 1999) [27,15] and reduced feed intake and heat production because it reduced locomotor activity in broiler chickens (Zeman *et al.,* 2001; Brennan *et al.,* 2002)[36,7]. Melatonin's hypothermic impact is the reason why it lowers body basal heat (George, 1999)[37]. By lowering energy expenditure for physical activity, melatonin supplementation in the diet may have improved feed conversion (Apeldoorn *et al.,* 1999)[15]. Melatonin supplementation suppressed spontaneous and serotonin-induced smooth muscle contraction in the gut (Bubenik, 2002)[41], which may have led to comparatively slower feed transit and the decreased feed intake in melatonin-supplemented groups. According to Thakur (2004)[39], melatonin administration was also associated with enhanced activity of digestive enzymes, which may have helped the melatonin-supplemented groups achieve higher FCR. Leptin, a satiety signal that regulates feed intake, has been demonstrated to rise in response to melatonin (Montano *et al.,* 2010)[40]. However, Apeldoorn *et al.* (1999)[15] found that adding melatonin to the meal had no effect on feed intake or feed conversion. Taha and Mawlood (2019)[28] showed how adding varying amounts of melatonin affected some of the productive and physiological traits of quail birds at 35 days of age. They also found that the addition of melatonin had no discernible effect on feed consumption or the feed conversion ratio between 7 and 35 days of age. The impact of melatonin on broiler productivity was examined by Al-Samrai *et al.* (2023)[24], who found no discernible variations in the broiler's feed conversion ratio or feed consumption rate.

3.3. Dressing percentage

In the present study the dressing percentages on day 42 were significantly higher in Group III when compared to Group IV and non-significantly higher when compared to Group I and II

(Table 4). These results were in conformation with the study reports of Sahin *et al*. (2004b)[41], Ahmed *et al.* (2006)[42] and Yang *et al.* (2015)[43] who reported that dietary melatonin supplementation in broilers improved carcass characteristics and its weights.

Table 4. Dressing percentage in different groups of broiler chickens supplemented with melatonin.

Period / Group	Day 14	Day 28	Day 42
Group I	$61.79 \pm 0.46^{a, x}$	$64.27 \pm 0.43^{a, y}$	$72.53 \pm 0.32^{ab, z}$
Group II	$60.70 \pm 0.44^{a, x}$	$65.62 \pm 0.61^{a, y}$	$75.04 \pm 0.60^{ab, z}$
Group III	$61.88 \pm 0.79^{a, x}$	$65.74 \pm 0.90^{a, y}$	$75.84 \pm 1.06^{b, z}$
Group IV	$61.56 \pm 0.70^{a, x}$	$66.75 \pm 0.53^{a, y}$	$73.57 \pm 0.38^{a, z}$

Note: The values bearing different superscripts between groups (a, b, c) and between periods (x, y, z) differ significantly ($P \leq 0.05$).

According to Sahin *et al.* (2004b)[41], melatonin treatment corrected the impairment in carcass characteristics and ascorbic acid and melatonin prevent oxidative stress and heat-induced performance inhibition in Japanese quails. It has been observed that adding melatonin to the diet helps Japanese quail overcome the detrimental effects of heat stress on performance metrics. Melatonin administration enhances poultry performance by promoting the absorption of fats, proteins, and carbohydrates into tissues (Ahmed *et al.,* 2006)[42]. However, Al-Samarai *et al.* (2023)[24] reported that the melatonin administration and the intermittent lighting regime had no discernible impact on the carcass's features when compared to the control treatment. According to Chen (2023)[44], melatonin administration had no discernible effect on the carcass features, pH values, drip loss, or cooking loss of breast meat; nevertheless, it did considerably alter the meat colour.

IV. Conclusion

Dietary melatonin supplementation has significantly improved body weight and average weekly body weight gain and dressing percentage in broilers. Dietary melatonin supplementation in broilers reduced feed intake and improved feed conversion efficiency when compared to the control group.

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