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Exogenous melatonin improves growth performance, intestinal microbiota, and morphology in temporarily feed restricted broilers

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ABSTRACT

In birds, exogenous melatonin possesses a wide spectrum of biological effects and can interfere with a wide range of behavioral and physiological processes including growth but the underlying mechanisms under feed restriction conditions have not been well defined. This study was carried out to investigate the effects of melatonin on growth performance, gut health, and some biochemical parameters of broilers subjected to feed restriction. Eleven-day-old male Ross 308 broilers were randomly distributed to 3 dietary treatments with 6 replicates of sixteen chicks each as follows: (1) control (CON); (2) 20% feed restriction (d 11–18; FR); and (3) the same as FR group, but melatonin was supplemented to drinking water (30 μg/ml, d 11–35; MFR). All birds had access to feed and water from 18 to 35 d of age. At d 35, broilers were sampled for quantification of blood components, intestinal morphology, and microbial counts. Supplementation of melatonin improved feed conversion rate of feed restricted birds ($P<0.05$). The MFR group had higher plasma growth hormone level as compared with CON ($P<0.05$). The villus height of duodenum was increased in MFR group as compared with those of CON and FR groups ($P<0.05$). Melatonin supplementation increased the jejunal villus height of chickens in FR group ($P<0.05$). Compared to FR group, ileal coliform counts decreased in birds treated with melatonin and CON group ($P<0.05$ and <0.01, respectively). An increment in ileal *Lactobacillus* counts was found in MFR group compared to CON ($P<0.05$). No significant differences were noticed in microbial counts of cecum among treatments. Results revealed that administration of melatonin to chickens seems to improve performance, gut histomorphology, and microbiota under temporarily feed restriction conditions.

1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is synthesized in the gastrointestinal tract, in addition to the pineal gland. The hormone is rapidly carried to other organs via blood or cerebrospinal fluid. It is highly lipophilic, which allows it to cross cell membranes easily and to reach all subcellular compartments, including mitochondria (Ozen et al., 2009). In birds, exogenous melatonin, possesses a wide spectrum of biological effects and can interfere with a wide range of behavioral and physiological processes (Moore and Siopes, 2000). Several studies suggest that melatonin modulates intermediary metabolism, growth, and improves feed conversion rate (Kvetnoy et al., 2002; Apeldoorn et al., 1999), interacts with transcription factors and growth hormone (GH) production, affects thermoregulatory mechanisms, and

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influences energy metabolism (Rozenboim et al., 1998; Zeman et al., 1999). Because of its implication in growth control and nutrient repartitioning, GH is likely a potential candidate for explaining some of melatonin effects (Canpolat et al., 2006). John et al. (1990) reported that melatonin administration stimulates GH secretion in pigeons. Zhan et al. (2007) noted that GH and 3,5,3′-triiodothyronine (T_3) are reduced during the period of feed restriction. Furthermore, as reviewed by Bubenik (2008), a relationship between feed intake and melatonin production in the gastrointestinal tract has been demonstrated in pigs, rats, mice, and monkeys. Bermudez et al. (1983) reported that melatonin depresses feed intake and improves feed efficiency.

The increase in the growth rate of modern broilers has been associated with increased fat deposition and high incidences of skeletal and metabolic diseases (Tahmoorespur et al., 2010). These situations most commonly occur with broilers under *ad libitum* feeding. Studies have demonstrated that early-life feed restriction followed by full-feeding is a potential way to reduce these problems and to improve feed efficiency in broilers (Cangar et al., 2008). Hereby, we hypothesized that melatonin—a hormone associated with photoperiod- supplementation might provide some insight into the physiological responses of feed restricted chickens and exert positive effects on performance of broilers, which implies its potential to apply at a larger scale, *i.e.*, in commercial broiler farms.

The practice of using feed withdrawal to initiate molting may result in a decrease in gastrointestinal melatonin, thereby increasing harmful microbial colonization in the hen intestine, indicating the potential of melatonin in reducing harmful microbial population (Moore and Siopes, 2000). Melatonin prevents deterioration of cellular membranes and membrane lipid destruction through its potent antioxidant activity (Antolin et al., 1996). It has been proposed that antioxidant materials act as a protective agent for the gastrointestinal tract. Recently, we have shown that antioxidant supplementation to feed could exert beneficial effects on gut health parameters of broiler under challenging / stressful conditions (Akbarian et al., 2013a, 2013b). From another view and in another work, we showed that feed withdrawal is challenging and/or stressful for chickens as shown by higher oxidative stress compared to normally raised chickens, possibly through decreasing antioxidants levels during feed restriction (Michiels et al., 2014). However, to the best of our knowledge, the underlying mechanisms of melatonin under feed restriction conditions have not been well defined. Therefore, and in view of these drawbacks, this study was designed to explore the beneficial effects of melatonin on performance and related anabolic hormones, intestinal tissue criteria, and microbial populations in broilers subjected to feed restriction.

2. Materials and methods

2.1. Animals, diets and experimental design

The experimental protocol was approved by the Animal Care Committee of the Ferdowsi University of Mashhad (Mashhad, Khorasan Razavi, Iran). A total number of 300 male one-day-old Ross 308 broiler chickens was obtained from a commercial hatchery (Seamorgh Co., Quchan, Mashhad, Iran) and raised for 7 d. In order to accustom to experimental cages, a 3 d adaptation period (d 8–11) was included prior to the commencement of the experiment, *i.e.*, starting the feed restriction. On d 11, the birds were individually weighed, and 108 birds around the average live weight were selected and placed in experimental cages to have 6 replicates of 6 birds each. Chickens were vaccinated for Infectious Bronchitis on d 1, for Newcastle Disease and Avian Influenza on d 7 and for Infectious Bursal Disease on d 14 of age. Ambient temperature on d 1 was set at 32 °C and was gradually reduced to 22 °C by d 21. Birds received a commercial grower and finisher diet according to the Ross 308 Management Guide (Aviagen, 2009) from d 11 to 35 of age. The nutrient composition of the grower and finisher diets are shown in Table 1.

A completely randomized design was used with 3 dietary treatments replicated in 6 cages of 6 birds. The three experimental groups were as follows: (1) Control (CON; *ad libitum* feeding, no melatonin supplementation), (2) 20% feed restriction (*i.e.*, 80% of the feed consumed *ad libitum* by the control group during the previous day) for 1 week (d 11—18) without supplementation of melatonin in drinking water (FR group) and (3) 20% feed restriction for 1 week (d 11–18) and melatonin (Sigma-Aldrich, Chemie GmbH, Germany) supplementation (30 μg/ml; d 11–35 of age) to drinking water (MFR group). All birds were fed *ad libitum* after the restriction period. In light of avoiding effects of lighting program on chickens' physiology and

Table 1
The composition of grower and finisher diets.

Item	Grower (11–24 d)	Finisher (25–35 d)
Ingredients (%)		
Corn	56.19	61.81
Soybean meal	36.07	30.99
Vegetable oil	4.00	3.60
Limestone	1.12	1.11
Dicalcium phosphate	1.47	1.36
Common salt	0.43	0.43
L-Lysine HCL	0.10	0.09
DL-Methionine	0.12	0.11
Vitamin and mineral premix[a]	0.50	0.50
Calculated composition		
ME (MJ/kg)	12.56	12.76
Crude protein (%)	20.70	19.30
Calcium (%)	0.86	0.81
Ava. phosphorus (%)	0.43	0.40
Lysine (%)	1.05	0.92
Methionine (%)	0.40	0.36
Met + Cys (%)	0.80	0.72
DCAB[b] (mEq/kg)	228	211

[a] Vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 9790 IU; vitamin E, 121 IU; B12, 20 μg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamin, 4 mg; zinc sulfate, 60 mg; copper sulfate, 100 mg; selenium (Sodium selenate), 0.2 mg; iodine, 1 mg; manganese oxide, 60 mg.

[b] DCAB = Na + K − Cl.