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Effects of a Diet Low in Vitamin and Mineral Complex and Darkness on the Growth Performance, Mineralization and Femur Histological Structure of Broiler Chickens

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ABSTRACT

Background: The aim of this study was to investigate the effects of dietary vitamin and mineral deficiency and darkness on growth performance, femur size, mineralization and bone microstructure in broiler chickens.

Methods: 120 male Cobb 500 chicks were randomly assigned to three groups, including a control group and two experimental groups (1st group on a vitamins and minerals deficient diet, 2nd group reared in total darkness), with 40 subjects per group from day 7. Daily calculations of average weight and weight gain were performed and morphological and histomorphometric measurements of the femur were performed on days 28, 35 and 42.

Result: The results revealed that the experimental groups showed a significant decrease in growth performance, body weight and weight gain ($P<0.05$) compared to the control group. In addition, a reduction in bone mineralization (weight and ash percentage) and altered bone microarchitecture were observed in the experimental groups. These results indicated that vitamin and mineral complex deficiencies in feed and darkness negatively affected growth performance and trophic and morpho-histological aspects of long bones in broilers. The results of this study may have significant implications for the poultry industry, as they highlights the need to consider factors such as nutrition and lighting conditions when rearing broiler chickens for optimal growth and health. In conclusion, the present study provides valuable insights into the effects of dietary deficiencies of the vitamin-mineral complex and darkness on broiler growth and bone development.

Key words: Broiler, Darkness, Dietary deficiencies, Growth performance, Long bones, Microstructure.

INTRODUCTION

The rapid growth of modern broilers has led to skeletal problems, particularly in the leg bones such as the femur, tibia and metatarsus, which can hinder growth and increase mortality in broilers (Han *et al*., 2015). The lower marketing age of broilers and the disproportionate increase in muscle mass are responsible for the high incidence of skeletal deformities (Pitsillides *et al*., 1999). The skeletal system is susceptible to the effects of physical, dietary and physiological variables (Rath *et al*., 2000). Several major nutritional deficiencies in poultry can lead to bone, joint, muscle, or nerve damage, resulting in locomotor problems (Sauveur, 1988). In the past, 23-hour photoperiods have been used to produce broilers. More than 18 hours of light from the age of 7 days has been banned in the European Union since June 2010 by new European welfare standards (Minimum Guidelines for the Protection of Chickens Raised for Meat Production) (Lewis *et al*., 2010). Light duration and intensity are essential for regulating and controlling chickens' growth, reproduction, behavior and welfare (Schwean-Lardner *et al*., 2013). Leg health and carcass quality can be used to measure the welfare of broilers (Onbasilar *et al*., 2007).

In modern poultry production systems, continuous or near-continuous lighting programs have become common to increase live weight gain and growth rate. However, rapid

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weight gain has been associated with numerous skeletal disorders in broilers (Velleman, 2000).

Histology is a valuable technique that allows the characterization of post-mortem bone changes, including those caused by trauma, pathology and ageing (Brits *et al*., 2014). Histomorphometry, on the other hand, is a quantitative assessment of microscopic organization and structure in tissues commonly used to provide information on cellular responses, tissue pathology and metabolic bone disorders (Egan *et al*., 2012). The femur is particularly susceptible to skeletal abnormalities in broilers, making it a focal point for investigations of long bones development and microarchitecture (Lilburn, 1994). Therefore, we focused on the femur to investigate its development and microarchitecture under different rearing conditions.

MATERIALS AND METHODS

Ethical approval

The national regulations on animal welfare and the institutional animal ethical committee were followed during the experiment.

Experimental design

The research was conducted within the period of April 8 to May 20, 2021, at the animal facility located within the Faculty of Biological Sciences at the University of Batna 2. At one week of age, 120 male Cobb 500 chicks were randomly divided into three groups each comprising 40 subjects (control group: Chicks were reared under normal conditions; experimental group 1: Chicks were fed a diet deficient in vitamin and mineral complex, experimental group 2: Chicks were reared in continuous darkness; treatment began on day 14 and continued until day 42). At D28, D35 and D42, five chickens per group were randomly selected and individually weighed. They were then sacrificed by cutting the neck at the jugular vein; the thighs were boned to obtain the femurs. Left femurs were frozen in labelled plastic bags and stored for metric and weight measurements.

Biochemical analysis

Following the method described by Hall *et al*. (2003); the left femurs were cleaned of all attached tissues, including connective tissue, muscle tissue and even their articular cartilage, by boiling them in an autoclave at a pressure of 6.82 bar for 8-12 min. They were cooled and weighed using a precision balance. For estimation of water content; the samples were put in porcelain crucibles and then left to dehydrate in an oven at 105°C for 24 h (Wensley *et al*., 2020). Femurs were incinerated in a muffle furnace at 550°C for 24 h to measure bone ash weight and calculate ash percentage (Pardy *et al*., 2004).

Microscopic analysis

Histological sections followed the protocols and techniques described by Carson and Hladik (2009); right femurs were immediately fixed in a 10% buffered formalin solution. After washing with running water, decalcification was performed according to the technique described by An and Martin (2003), in which a 5% formic acid solution was used. The tissues were dehydrated in graded ethanol and treated with xylene. The next step involved embedding each sample in paraffin blocks, which were then cut into 5 µm thick sections using a microtome (Debbou-louknane *et al*., 2018). The slides obtained were stained with HandE and analyzed using a Carl Zeiss Axioskop 20 optical microscope equipped with a DOM 300 digital camera (Boussouar *et al*., 2019). The images were visualized using Oasis software and analyzed with Image J.

Statistical analysis

Statistical analysis of the experimental data was performed using SPSS version 25 for windows. Means of the various parameters studies were compared by one-way analysis of variance (ANOVA), followed by pairwise comparisons between groups using Tukey's test. Quantitative data were presented as a mean ± (SD). Differences at $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Growth performances

Table 1 displays the study's findings on average body weight and weight growth variations. Table 1 showed a significant difference ($P<0.05$) between the experimental and control group's body weight on D14, D21, D28, D35 and D42.

Table 1: Body weight evolution and body weight gain of chicks (mean±SD, n=5).

Group		Control	Experimental 1	Experimental 2
Body weight (g)	D14	992.4± 3.36	289.4±2.70*	919.6±19.46*
	D21	1352±5.63	305.4±3.85*	1054±07.50*
	D28	1830±3.72	369.6±28.00*	1322±68.79*
	D35	2442±100.4	432.0±19.46*	1725±72.82*
	D42	2949±118.1	498.0±16.81*	1872±58.90*
BWG (g/d)	D14-D21	51.37	2.29*	19.20*
	D21-D28	68.29	9.17*	38.29*
	D28-D35	87.43	8.91*	57.57*
	D35-D42	72.43	9.43*	21.00*

D: Day, SD: Standard deviation, *: Different superscripts in a line indicate significant differences between the groups ($P<0.05$).

The experimental groups displayed considerably lower live weight values than the control group.

The average daily gains during [D14-D21] for the three groups were 51.37 g/d, 2.29 g/d and 19.2 g/d respectively, the BWG at [D21-D28] for the three groups were 68.29 g/d, 9.17 g/d and 38.29 g/d respectively, the BWG at [D28-D35] for the three groups were 87.43 g/d, 8.91 g/d and 57.57 g/d respectively, the BWG at [D35-D42] for the three groups were 72.43 g/d, 9.43 g/d and 21 g/d respectively.

Our results indicate that reducing the mineral and vitamin intake significantly affected the body weight and weight gain of the chickens (P<0.05) during the first period, which agrees with the findings of Jafari Sayadi *et al*. (2005). Nutritional deficiencies can affect broiler chickens weight and weight gain by interfering with their ability to grow and develop their skeletons (Ventura and Matias da Silva, 2019). A study by Xu *et al*. (2021) showed that body weight and weight gain were affected by dietary calcium and phosphorus deficiency, causing a reduction in both parameters.

Low light had a significant impact on live weight and body weight gain of chickens (P<0.05); light limited birds consumed less food, which led to a reduction in body weight, consistent with the findings of Lewis *et al*. (2009). Studies on restricted lighting consisting of long blocks of light and dark showed a decrease in body weight as the duration of darkness increased (Renden *et al*., 1992). However, Foss *et al*. (1972) found that birds housed in continuous darkness were able to gain weight almost as well as those under standard lighting systems. Low light conditions have been commonly used to increase body weight gain in chickens, but our results suggest that it may not be an effective strategy. To minimize the negative effects of low light, Buyse *et al*. (1996) recommended including a short period of darkness to habituate birds to darkness and minimize panic during a power failure.

Femur weight

Table 2 illustrates the linear increase in bone weight with ageing. This finding is coherent with the previous research published by Han *et al*. (2015), who stated that increase in bone weight was due to minerals retention and higher dietary intake.

Our study revealed that the femur weight was highest in control group and lowest in experimental group 1. Furthermore, our results indicated that chickens fed vitamin and mineral complex deficient diet had significantly lower femur weights (P<0.05) than the control group. The results are perfectly concordant with the study conducted by Hakami *et al*. (2022) who demonstrated that broiler femur weight was strongly affected by reduced dietary intake. However, our results are directly opposed to those of Williams *et al*. (2000b), who found that tibia bone weight tends to decrease with increasing dietary calcium content.

The chickens raised in total darkness had a significantly lower femur weight (P<0.05) than the control group. Our findings were consistent with the previous studies of Van der Pol *et al*. (2015), who conducted a comparative study on the tibiotarsus and noted that weak light did not increase bone weight. Similarly, our results suggest that low light influences femur weight in broilers.

Weight and ash percentage

The results of this study exhibit a linear increase in bone ash weight with increasing age which correspond to the findings of Han *et al*. (2015).

As demonstrated in Table 2, the experimental groups had considerably less bone ash weight and percentage than the control group (P<0.05).

Our results highlighted that broilers fed a diet deficient in vitamins and minerals complex had lower bone ash content than those fed a normal diet. According to research by Hall *et al*. (2003), ash weight is a stronger indicator of bone mineralization than ash percentage, as non-mineralization parameters can affect the latter.

Our study also reported that broilers on a vitamins and minerals deficient diet had lower bone ash content than broilers receiving a normal diet. This finding supports the results of Shi *et al*. (2022), who observed that reducing dietary Ca and P compromised growth and bone mineralization in broilers. Additionally, low dietary Ca and P have been reported to significantly reduce bone ash, with this study further supporting the idea that bone ash is more sensitive than growth performance to dietary Ca and P changes (Wang and Kim, 2021).

Table 2: Results of bone dimensions on D28, D35 and D42 (mean±SD, n=5).

Parameter/ group	Group	Femur length (mm)	Diaphyseal diameter (mm)	Femur weight (g)	Ash weight (g)	Ash percentage (%)
D28	Control	61.40±1.11	7.77±1.05	6.29±1.10	1.43±0.29	35.65±02.78
	Experimental 1	31.82±1.07*	5.20±1.04*	1.89±0.27*	0.35±0.29*	32.44±22.22*
	Experimental 2	52.34±1.23*	6.90±1.51*	4.58±1.12*	1.01±0.29*	43.74±07.33*
D35	Control	71.46±1.19	9.61±1.28	8.59±1.13	2.02±0.30	39.09±02.75
	Experimental 1	40.62±1.03*	5.82±1.10*	2.03±0.28*	0.36±0.29*	33.42±21.99*
	Experimental 2	61.80±1.26*	8.33±1.29*	6.52±1.12*	1.32±0.29*	32.68±01.89*
D42	Control	72.42±1.38	10.34±1.28	10.38±0.93	2.29±0.29	35.69±02.01
	Experimental 1	42.06±1.37*	6.75±1.30*	3.39±1.41*	0.38±0.29*	30.14±19.36*
	Experimental 2	62.36±1.37*	8.98±1.35*	7.28±1.14*	1.35±0.29*	31.94±01.86*

D: Day, SD: Standard deviation, *: Different superscripts in a line indicate significant differences between the groups (P<0.05).

Our findings indicate that the chickens in experimental group 2 (raised under continuous darkness) had lower bone ash than those in the control group. Furthermore, Lewis *et al*. (2009) discovered that ash content increased logarithmically with photoperiod.

Bone length and diameter

The experimental groups of chickens showed shorter femur lengths and smaller diaphyseal diameters than the control group. Specifically, on D28, the femur lengths were 61.40 mm, 31.82 mm and 52.34 mm for the control and experimental groups (1 and 2), respectively. On D35, the femur lengths were 71.46 mm, 40.62 mm and 61.80 mm for the control and experimental groups (1 and 2), respectively. On D42, the femur lengths were 72.42 mm, 42.06 mm and 62.36 mm for the control and experimental groups (1 and 2), respectively.

Table 2 indicate that bone length and diameter increased with age in broilers, consistent with the findings of Han *et al*. (2015) who found that age influenced bone length and diameter. Our results showed that the bones of broilers chickens fed a diet deficient in vitamin and mineral complexes were significantly ($P<0.05$) shorter than those of the chickens in the control group, which was consistent with the findings of Moreki *et al*. (2011). Moreover, Williams *et al*. (2000a) reported that tibia and humerus length at all ages in chickens responded significantly to calcium intake. Meanwhile, bone width increased as chickens became heavier with age and *ad libitum* feeding.

Our results also showed that the bones of chickens reared in total darkness were significantly ($P<0.05$) shorter than controls; similar reductions were revealed for femoral diaphysis diameters, which is also compatible with Fidan *et al*. (2017).

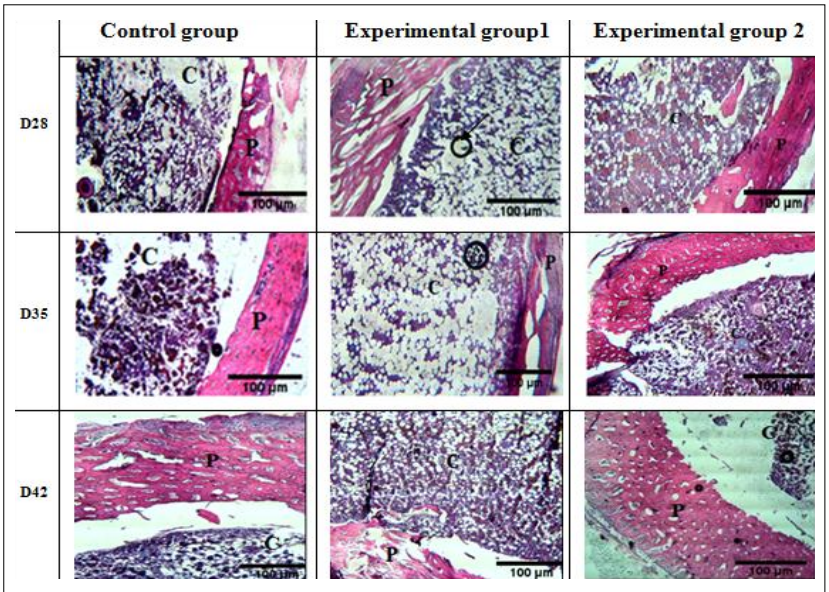
Histomorphometric measurements

The results (Table 3) indicate that the experimental groups had a thinner and less dense femoral cortex compared to the control group (Fig 1). Chickens that received a diet low

Table 3: Histomorphometric measurements of the diaphysis (mean±SD, n=5).

Parameters/group	Group	Total thickness of cortex (µm)	Bone density of cortex (%)
D28	Control	627.9±10.43	48.69±1.85
	Experimental 1	535.9±06.88*	39.06±2.04*
	Experimental 2	618.8±07.97*	43.37±2.20*
D35	Control	555.1±29.97	56.33±1.23
	Experimental 1	440.8±10.24*	47.66±1.55*
	Experimental 2	481.0±09.29*	52.75±1.51*
D42	Control	524.6± 06.15	65.31±1.46
	Experimental 1	358.5± 12.93*	52.30±1.35*
	Experimental 2	435.4± 07.40*	61.49±1.17*

D: Day, *: Different superscripts in a line indicate significant differences between the groups ($P<0.05$).



(H and E stain, X40). Scale bar=100 µm. C: Cortex, P: Periosteum.

Fig 1: Cross section of the diaphysis; decalcified femur of a chicken at D28, D35 and D42.

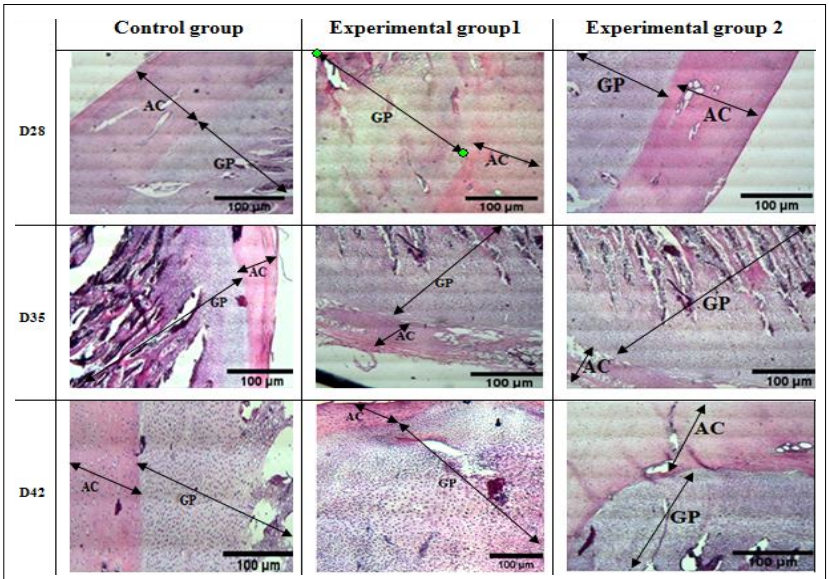
in essential elements showed a reduction in cortical thickness and density compared to the control group, consistent with the findings of Almeida Paz and Bruno, (2006) and Bai *et al*. (2022). According to Almeida Paz and Bruno, (2006), the thinning of the cortex may be attributed to the dietary calcium reduction.

Chickens in experimental group 2 exposed to low light showed a reduction in cortical thickness and density compared with the control group. However, this result conflicts with the study by Kokolski *et al*. (2017), which showed that low light in fast-growing animals does not affect cortical thickness and tibial density.

We conclude that bone density is affected by a diet deficient in vitamins and mineral complexes and by darkness; our results were similar to the previous study done by Kranioti *et al*. (2019) who demonstrated that bone density is affected by various factors, such as age, weight, diet, exercise and pathology.

Table 4 and Fig 2 illustrate that for the epiphyses at D28, D35 and D42, the chickens in experimental group 1 had a more extended proliferative zone than controls and a smaller hypertrophic zone than controls. Our data are similar to research performed by Shao *et al*. (2019), who noted a significant increase in the tibial proliferative zone length for the Ca and P -deficient group (P<0.05), as well as significant reduction in the tibial hypertrophic zone length (P<0.05). A further study by Li *et al*. (2022) suggested that dietary phosphorus deficiency affected tibial growth in goslings; the length of the proliferative zone was lower in the phosphorus-deficient group than in the control group, while the length of the hypertrophic zone was higher.

Chickens in experimental group 2 had higher lengths of both zones (proliferative and hypertrophic) compared to control chickens. This result agrees with Kokolski *et al*. (2017), who demonstrated that photoperiod significantly impacted bone growth characteristics, including growth plate



(H and E stain, X40). Scale bar=100 µm. AC: Articular cartilage, GP: Growth plate.

Fig 2: Longitudinal section of the epiphysis; decalcified femur of a chicken at D28, D35 and D42.

Table 4: Histomorphometric measurements on the epiphysis (mean±SD, n=5).

Parameters/ group	Group	Articular cartilage (µm)	Proliferative zone (µm)	Hypertrophic zone (µm)	Calcified zone (µm)	Trabecular area (%)
D28	Control	892.5±02.36	453.3±03.84	392.1±04.24	812.4±10.75	29.74±06.61
	Experimental 1	931.4±07.09*	884.3±01.42*	388.9±01.13*	491.4±08.12*	32.92±02.29*
	Experimental 2	867.5±18.51*	506.5±15.14*	410.7±11.53*	800.9±01.20*	40.21±01.22*
D35	Control	750.5±14.49	375.3±04.83	333.5±11.88	714.1±19.19	38.65±02.49
	Experimental 1	809.8±05.81*	712.8±01.69*	330.2±01.04*	440.4±10.48*	40.40±01.06*
	Experimental 2	806.6±10.54*	380.4±06.07*	348.8± 03.94*	630.3±03.13*	37.23±02.35*
D42	Control	709.3±07.53	342.3±17.42	262.9±12.00	688.3±02.02	38.09±01.21
	Experimental 1	729.8±02.54*	360.8±16.22*	259.7±13.50*	433.1±07.65*	44.59±03.00*
	Experimental 2	704.7±11.77*	347.8±04.40*	358.6±01.63*	565.8±02.78*	35.02±01.78*

D: Day, *: Different superscripts in a line indicate significant differences between the groups (P<0.05).

size. The research revealed a clear increase in proliferative and hypertrophic zones, as well as a considerable increase in overall growth plate width under short-day conditions. The researchers also concluded that the tibial bone volume and trabecular surface of short-day hamsters were much greater than those of long-day animals. The increase in total bone volume in short-day hamsters was mainly due to increased bone development rather than bone density.

We conclude that in fast-growing animals, the growth plate, particularly the proliferative and hypertrophic zone, is affected by various factors, such as diet and low light. Our results concur with findings of Thompson (2007).

CONCLUSION

Based on the results of our study, we conclude that a diet deficient in essential minerals and vitamins and exposure to low light levels, significantly inhibit growth and weight gain in broilers. Furthermore, these factors negatively affect the mineralization of the femur, reducing bone weight and percentage of bone ash. These detrimental effects are reflected in microscopic changes, mainly in the thickness of the femoral cortex and the length of the growth plate zones (proliferative and hypertrophic zones).

Conflict of interest

All authors declare that they have no conflicts of interest.

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