



A study on nutritional agents associated with leg lameness in commercial broiler chicken

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ABSTRACT

This study aimed to investigate the occurrence of leg lameness in commercial broilers and the nutritional agents associated with this condition. The study was carried out during the period from October 2022 to March 2023 in and around Puducherry. A total of 20 commercial broilers farms were investigated for the occurrence of lameness based on the gait scoring system. Birds with a score more than 3 were considered as lame bird. Out of 20, 10 farms with highest occurrence of lameness were selected for further study. The average percentage of lameness in the surveyed and selected farms was 21.1 % and 25 %, respectively. All the flocks were maintained under deep litter system with the recommended floor space on continuous lighting program and *ad libitum* feeding. None of the flocks showed lesions of foot pad dermatitis and hock burn. Though, the level of calcium and phosphorus did not differ significantly in the feed sample, magnesium and manganese contents were found to be below the recommended level of BIS, 2007. All the feed samples were positive for aflatoxin and in 50% of the samples, the level exceed 20 ppb. No significant difference was recorded in the mean values of serum phosphorous, ALT, AST and glucose between control and lame group but the serum calcium and vitamin D3 were significantly lower in latter. Bone mineral profile revealed relatively lower dry fat free bone weight and ash weight for lame group compared to control group. The bone manganese content was significantly lower in lame birds compared to the control group. The present study highlighted the importance of feeding adequate levels of manganese in the diet of broilers. In addition, the feed should be routinely screened for the levels of aflatoxins.

Keywords: Aflatoxin, Broiler, Lameness, Manganese, Vitamin D3

Broiler farming plays a key role in global market of meat industry and a pivotal role on the economy of the countries like India. Genetic selection and efficient feed conversion programs have played a key role in producing the modern-day broilers for fast growth. Compared to the early 1950s, wherein it took an average of 14 weeks to grow a broiler to a market weight of two kilograms, today it takes only 5.6 weeks (39 days) to attain the same market weight (Cicek and Tandogan, 2016). The rapid weight gain and fast muscle growth due to intense genetic selection and improved nutrition in commercial broiler strains has resulted in an increased pressure on the legs to bear the large bodyweight (Havenstein *et al.* 2003; Zuidhof *et al.* 2014) thus, making the fast-growing broilers more prone for leg weakness or lameness.

Leg weakness has been a portmanteau term used to describe a number of pathological states affecting broilers whose walking ability is impaired (Butterworth, 1999). Leg disorders can increase mortality and the number of

culls, condemnations, downgrades from trimming, reduced productive and reproductive performance and increased production costs (Morris, 1993; Rojas *et al.* 2020). Broiler lameness is often determined by evaluating the gait of individual chickens. According to Kestin *et al.* (1992) birds with score 3 or higher experience pain or discomfort. Hence, the birds with a gait score of three and above are considered as lame bird. Earlier studies found that 14% to 50% of broilers suffer from lameness with gait scores 3, 4 or 5 (Kestin *et al.* 1992; Knowles *et al.* 2008; de Jong *et al.* 2011; Bassler *et al.* 2013; Kittelsen *et al.* 2017).

The etiology of leg lameness is often multiple and include infectious as well as non infectious factors. Although high growth rate is identified as a key factor for lameness (Julian, 1998; Kapell *et al.* 2012), factors such as genotype, age, gender, litter quality, flock management, stocking density and housing condition and imbalance of macro and micro minerals, protein and vitamins are also found to be associated (Guz, 2022). Understanding the various causes can help producers identify areas for improvement and develop effective strategies to reduce the incidence of lameness. Hence, the present study aimed to investigate the occurrence and the role of certain minerals

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in the causation of leg weakness in commercial broiler chickens.

MATERIALS AND METHODS

The study was carried out in 20 commercial broiler farms located in and around Puducherry. Gait score of the birds were assessed following the methods of Kestin *et al.* (1992). In addition, the data pertaining to management such as stocking density, feeding, litter and lighting programme of farms were collected. Out of 20 farms, 10 farms with highest occurrence of leg weakness were selected for further study. From each of the 10 farms, five lame birds (gait score ≥ 3) and one control bird (gait score of 0) per farm were selected and representative samples of serum and tibial bone from each bird were collected at the time of slaughter. Approximately 500 g of feed sample was also collected from those farms for further analysis.

Determination of bone and feed ash: The left tibial bone was thoroughly cleaned manually by removing adhering muscles, connective tissues and fibula was defatted for 3 hours in petroleum ether (boiling point 80°C) using Soxhlet apparatus, dried in hot air oven (100°C) to a constant weight and weighed. A known quantity of feed sample was taken and dried in hot air oven (100°C) overnight. The feed sample was ground to fine homogenized mixture and subjected to charring under bunsen flame till no more flumes evolve. Ashing was done at 600°C for 2 hours in a muffle furnace.

Bone and feed calcium, Phosphorous, Magnesium and Manganese content: The ash samples of bone and feed were digested with dilute hydrochloric acid (1:3) and dilute nitric acid (1:3). Digested samples were quantitatively transferred to 50 ml volumetric flasks using Whatman filter paper no. 42. The residues were washed with hot distilled water two or three times and volume of the solution was made up to 50 ml using distilled water. Estimation of calcium was determined by Talapatra's method and also using Graphite furnace atomic absorption spectroscopy with flow injection analysis software (Perkin Elmer, USA), whereas magnesium and manganese were determined using Graphite furnace atomic absorption spectroscopy with flow injection analysis software (Perkin Elmer, USA). Estimation of phosphorous was determined by Fiske Subbarow method using Spectrophotometer. In addition, 250 g of feed samples were collected for estimation of aflatoxin levels.

Serum biochemical analysis: The blood samples were collected in clot activator and allowed to stand at room temperature, and then centrifuged at 2,500 rpm for 10 min to separate serum. The samples were stored at -40°C until they were analysed. The parameters *viz* aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) activities along with, bilirubin, calcium, phosphorous and glucose were estimated using EM (Erba Mannheim) 360 automated clinical chemistry analyzer. Serum vitamin D3 level was estimated by high performance

liquid chromatography.

Statistical analysis: All values were presented as means \pm SE. Data were statistically analyzed using Graph-pad prism 8.0.1.244. The level of significance between control and lame groups was tested by Student t- test (Mann Whitney test)

RESULTS AND DISCUSSION

The flock size, age and the gait score of lame birds of the surveyed farms is presented in table.1. The flock size of the farms varied from 1000 to 8000 birds. The bird's belonged to Cobb strain and age ranged from 30 to 42 days. The stocking density ranged from 14.35 to 25.83 kg/m² with an average of 20.94 kg/m². The birds were maintained under deep litter system with coconut husk as litter material with regular racking. The birds were fed with *ad libitum* feed and water. Half of the farms surveyed were of contract type.

The average occurrence of lameness was 21.10%, as indicated by a gait score of three and above (Table 1). Complete immobility was recorded in 1.35 % of the birds with the gait score of 5. The birds with the gait score of 0 to 2 was recorded in 78.90%. None of the flocks showed lesions of foot pad dermatitis and hock burn.

From these 20 farms surveyed, 10 farms with highest occurrence of lameness (GS \geq 3) were selected for further study. The average percent lameness in the selected farms was 25%. 75% of the birds had a gait score of 0 to 2 while 2.10 % of the birds had complete lameness with a gait score of 5. The various clinical signs exhibited by the birds included hock sitting posture (Fig. 1), unilateral paralysis (Fig. 2), dropped wings, unsteady gait sideways falling and wings assisted walking.

The lameness incidence observed were consistent with the results of previous studies by Kestin *et al.* (1992), Kittlesen *et al.* (2017) and Granquist *et al.* (2019) who reported lameness rates of 26%, 24.6% and 19%, respectively. Although, lameness has been observed from the first week onwards, the occurrence was frequent and well pronounced in birds between 30 to 42 days of age (Knowles *et al.*, 2008). Commercial broiler production often uses continuous lighting for increased production. On the contrary, reduction in photoperiod for one hour may limit expression of skeletal deformities (Sorensen *et al.*

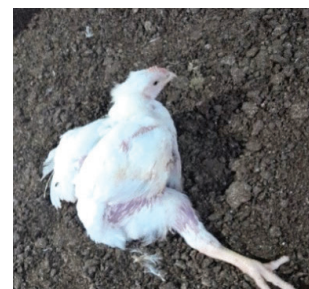


Fig. 1 Chicken sitting on its hock joint with leg stretched forward one week old

Fig. 2 Right unilateral paralysis in Broiler chicken aged 35 days

Table 1. Details of the location, age, total number of birds, stocking density, gait score and percentage of lameness of surveyed farms (n= 20)

Sl. No.	Age (days)	Total no. of birds	Stocking density (Kg/m ²)	Gait score				% of lameness (GS≥3)
				0-2	3	4	5	
1	30	8000	24.60	72	17	10	1	28
2	38	3000	21.53	79	14	7	0	21
3	38	4500	21.53	81	10	8	1	19
4	38	8000	20.26	75	15	9	1	25
5	35	3600	21.53	72	18	7	3	28
6	42	2500	17.94	73	14	10	3	27
7	36	7000	25.12	80	12	7	1	20
8	38	2000	21.53	85	8	7	0	15
9	38	3000	25.83	77	9	11	3	23
10	36	1000	14.35	78	7	13	2	22
11	38	8000	22.96	90	6	4	0	10
12	35	1000	21.53	82	11	7	0	18
13	32	2500	17.94	77	12	9	2	23
14	34	3000	21.53	80	10	8	2	20
15	32	5000	19.57	75	16	7	2	25
16	32	3000	21.53	85	8	6	1	15
17	38	2500	17.94	84	10	6	0	16
18	38	2500	21.53	75	11	12	2	25
19	35	3000	18.45	82	11	6	1	18
20	33	3000	21.53	76	10	12	2	24

1999) and reduced the prevalence of lame birds (Bassler *et al.* 2013).

In the present study, birds were fed *ad libitum* with broiler starter and finisher ration. According to Yousaf *et al.* (2021), the development of non infectious bone abnormalities can be reduced by decreasing the growth rate by dietary restriction. Leterrier *et al.* (2008) concluded that sequential feeding using diets varying in energy and crude protein reduced the prevalence of lameness in birds. Interestingly, none of the flocks showed lesions of foot pad

dermatitis and hock burn. This could be attributed to good litter quality and regular racking avoiding the accumulation of moisture.

The feed calcium and phosphorous levels recorded in the present study were within the BIS, 2007 recommended level for the broiler finisher ration (Table 2). However, the magnesium and manganese level in all the 10 farms were found to be below the recommended level of 300 mg/kg and 100 mg/kg, respectively.

The consequences of feeding magnesium and manganese

Table 2. Levels of minerals and aflatoxin in feed

Farm	Ca (g%)	P (g%)	Ca:P	Mg (mg%)	Mn (mg%)	Aflatoxin				Gait score (>3)
						B1	B2	G1	G2	
A	1.05	0.49	2.14	14.00	8.2	44	15	0	0	28
B	1.07	0.51	2.10	14.27	7.1	30	7	0	0	25
C	1.05	0.48	2.19	14.50	6.8	7	0	0	0	28
D	1.15	0.45	2.56	13.31	5.8	22	0	0	0	27
E	1.30	0.50	2.60	13.37	5.2	15	0	0	0	23
F	1.1	0.49	2.24	13.67	4.9	7	0	0	0	22
G	1.05	0.52	2.02	12.62	5.3	7	0	0	0	23
H	1.26	0.56	2.25	12.76	5.0	7	0	0	0	25
I	1.12	0.49	2.29	13.00	4.0	22	0	0	0	25
J	1.08	0.47	2.30	12.63	3.7	22	0	0	0	24

deficit diet were evident in the occurrence of lameness in the studied farms. About 22-28% of the birds exhibited lameness, either with or without visible lesions, possibly due to inadequate levels of magnesium and manganese in dietary. Previous studies by Wang *et al.* (2021) and Xia *et al.* (2022) also reported that manganese deficiency affects proliferation and differentiation of chondrocytes in the tibial growth plate, leading to tibial dyschondroplasia and lameness in broilers. It is further important to note that the dietary deficiency of magnesium did not show noticeable effects on practical feeding in previous studies (Shastak and Rodehutsord, 2014). However, the present study indicated that suboptimal levels of magnesium in the feed may indeed have an impact on the occurrence of lameness in broilers. This discrepancy might be attributed to variations in the study conditions, genetic factors, or other environmental factors that influence the susceptibility of birds to mineral deficiencies.

All the 10 feed samples analysed for the presence of aflatoxins B1, B2, G1 and G2 were positive for aflatoxins B1 and B2. In 5 samples the levels were higher than 20 ppb and corresponded to the occurrence of lameness was 24 – 28 %. Bird (1978) reported a significant interaction between AFB₁ and vitamin D₃ on the bone mineralization of white leghorn cockerels by interfering with the conversion of vitamin D₃ to its more active physiological derivatives. Huff *et al.* (1980) represented that bone ashes were decreased by dietary aflatoxin (2500 ppb and more) in Hubbard male broilers, and mentioned that aflatoxin inhibits the vitamin D₃-mediated mineralization of bones and contributes to bone development problems. Fouad *et al.* (2019) also reported that, aflatoxin increases elimination of calcium and decreases serum calcium, thereby, causing lameness in poultry. However, the bone ash content did not vary significantly in the present study. Probably higher levels aflatoxin is required to produce such effects, although, Mesgar *et al.* (2022) had reported that even a low concentration of AFB₁ can interfere with bone development and strength. As the agro-climatic condition in the study area is conducive for the growth of fungi, the feed samples should be routinely screened for mycotoxins.

Table 3. Serum Biochemical parameters for control and lame group

Parameter Mean±SE	Control group (n=10)	Lame group (n=50)	p value
Ca (mg/dL)	11.13 ± 0.3 ^b	10.21±0.1 ^a	0.0290*
P (mg/dL)	5.683 ± 0.12	5.414±0.08	0.0799
ALT (U/L)	14.00 ±1.08	13.87±0.56	0.5000
AST (U/L)	315.1 ± 35.86	290.8±10.24	0.2642
GGT (U/L)	16.53 ± 2.12 ^a	21.83 ± 0.98 ^b	0.0163**
Glucose (mg/dL)	268.7 ± 13.97	264.7 ± 5.22	0.3980

Values with the different superscript within a row, differed significantly (p<0.05), Mann Whitney U test: * Significant at p<0.05, **Significant at p<0.01

The serum biochemical and enzyme parameters for the control and lame group are presented in table 3. There was a significance difference (p<0.05) in the serum calcium and GGT level between control and lame group.

Previous studies have also emphasized the importance of serum calcium, phosphorus, and vitamin D₃ in bone development and health. The findings of Khan *et al.* (2010) and Zhang *et al.* (2020) support the notion that these minerals and vitamins play a crucial role in bone tissue formation and turnover.

Interestingly, the study also found a significant increase in serum GGT levels in lame birds compared to control birds. Elevated serum GGT levels are often associated with liver disease and aflatoxicosis in poultry. However, GGT has also been linked to oxidative stress and inflammation in the body. Both oxidative stress and inflammation have been associated with bone loss and impaired bone remodelling.

Serum vitamin D₃ level of control and lame birds are presented in Table 4 and Fig.3. The mean serum vitamin D₃ level of lame group was significantly lower than control group. The findings of this study are consistent with previous research by Elliot *et al.* (1995) and Halit *et al.* (2012), who reported decreased serum calcium and phosphorus levels in broilers with tibial dyschondroplasia

Table 4. Serum vitamin D₃ of control and lame group

Farm	Control (n=6)	lame (n=11)
A	35.93	21.11
B	43.29	22.51
C	25.98	28.43
D	32.66	23.22
E	12.77	30.63
F	27.49	25.69
Mean ± SE	29.69 ± 4.2 ^b	22.89 ± 1.37 ^a
p value		0.0491*

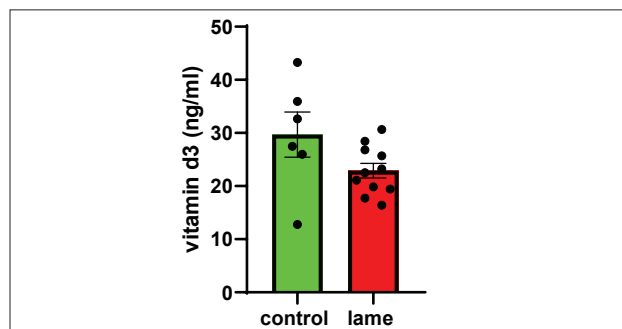


Fig. 3 comparison of serum vitamin D₃ of control and lame group with significant difference

Table 5. Bone mineral profile of control and lame group

Parameter Mean \pm SE	Control group (n=10)	Lame group (n=48)	p value
Dry fat free bone weight (g)	6.500 \pm 0.38	5.93 \pm 0.18	0.1530
Ash (g)	2.379 \pm 0.11	2.185 \pm 0.06	0.0787
Ash percentage (%)	36.85 \pm 0.8	37.11 \pm 0.54	0.4980
Bone Cal (g%)	35.29 \pm 0.66 ^a	35.49 \pm 0.48 ^a	0.2121
Bone P (g%)	16.62 \pm 0.84	17.26 \pm 0.46	0.1872
Ca:P	2.158 \pm 0.15	2.16 \pm 0.094	0.4019
Bone Mg(mg%)	14.53 \pm 0.87	14.73 \pm 0.44	0.4594
Bone Mn (mg%)	1.99 \pm 0.67 ^b (N=6)	0.61 \pm 0.05 ^a (N= 26)	0.001**

Values with the different superscript within a row differed significantly ($p < 0.05$), Mann Whitney U test. * Significant at ($p < 0.05$) **Significant at ($p < 0.01$)

associated lameness. This suggests that maintaining optimal levels of serum calcium and phosphorus is crucial for preventing the development of bone disorders in broilers. The study also highlighted the critical role of vitamin D3 in bone mineralization and bone health. The disruption of this balance due to reduced vitamin D3 can lead to abnormal bone remodelling, compromising bone strength and increasing the risk of lameness in broilers.

The bone mineral profile of control and lame group are present in table 5. Significant difference ($p < 0.05$) was observed in the manganese content between control and lame group indicating a potential association between bone mineral profile, manganese deficiency, and lameness in birds. Manganese (Mn) is an essential trace element that plays a crucial role in various enzymatic reactions and metabolic processes, including carbohydrate, lipid, and protein metabolism. It is also involved in chondroitin sulfate synthesis, which is closely linked to bone formation in broilers. Liu *et al.* (2015) and Wang *et al.* (2015) had also demonstrated that a manganese deficiency model in broilers resulted in abnormal tibial development, attributable to inhibited osteoblast vitality, reduced chondrocyte proliferation, increased chondrocyte apoptosis in the tibia, and disrupted levels of bone regulatory hormones and enzymes in the serum. Additionally, Wang *et al.* (2021) and Xia *et al.* (2022) reported that manganese deficiency affected the proliferation and differentiation of chondrocytes in the tibial growth plate, leading to the development of tibial dyschondroplasia in broilers. These findings are consistent with the present study, which identified Tibial dyschondroplasia (TD) lesions in 34% of the examined birds.

In conclusion, in the present study, 25% of the birds experienced moderate to severe degree of lameness, which clearly affected the development and end-weight of broiler chickens resulting in a significant financial loss to the farmers. Although many distinct factors impact the leg health of broiler chicken, our results suggested that the low

levels of magnesium and manganese in the feed, vitamin D and calcium in serum, manganese in bone and the presence of aflatoxins in feed may be confounding detrimental factors for lameness in broilers. Adequate supplementation of nutrients in the feed, understanding their bioavailability and routine screening of feed for toxic and anti-nutritional factors in feed are essential to control lameness in broilers.

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