Explor Anim Med Res,
Vol.12, Issue - 2, 2022, p. 246-251
DOI : 10.52635/eamr/12.2.246-251

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

RADIOGRAPHICAL AND BIOCHEMICAL STUDIES OF LEG BONES DURING POST-HATCH DEVELOPMENT OF BROILER CHICKENS

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Received 21 June 2022, revised 17 November 2022

ABSTRACT: 'Vencob' day-old broiler chicks(45 no.) were reared up to day 42 to carry outradiographical and biochemical studies on the long leg bones (femur, tibiotarsus, and tarsometatarsus) and associated growth cartilages at different ages (on days 1,7, 14, 21, 28, 35 and 42). The present study confirmed the presence of both proximal as well as distal growth plates in all these three bones from day 1 till day 42, the latter one being more authentic. In the femur, the presence of growth plates was reported for the first time. The secondary center of ossification in broiler chicken appeared in the epiphyseal ends of the tibiotarsus and tarsometatarsus (but not in the femur) on day 21. The average serum Ca and P levels in plasma and bone (%) in broiler birds gradually increased with age. The ratio between serum Ca and P level (Ca: P) and bone Ca and P percentage remained almost constant during the growing period of the broiler chickens to maintain a proper balance for adequate mineralization of bones.

Key words: Biochemical study, Radiography, Leg bones, Post-hatch broiler chickens.

INTRODUCTION

In recent years, there has been much genetic selection for faster growth and tender meat production in broilers (Williams *et al*. 2000, Manohar *et al*. 2015). The desired genetic manipulation produces early muscle growth without a simultaneous increase in skeletal development, thus resulting in leg weakness and disorders (Manohar *et al*. 2015). It is well recognized that the long leg bones (femur, tibiotarsus, and tarsometatarsus) are principal weight-bearing bones in poultry birds. The importance of legs and leg bones in birds is well documented (Sreeranjini*et al*. 2013, Behera *et al*. 2021).

The growth plate is a highly organized cartilage structure *i.e.*,a connective tissue composed of cells (chondrocytes) embedded in a highly hydrated extracellular matrix. It is entrapped between the epiphyseal and metaphyseal bone at the ends of the long bones (Getty 1975, Van der Eerden *et al*. 2003, Valteau *et al*. 2011).The same was mentioned in the text (Ghosh 2015). Growth plate cartilage is the key to the longitudinal growth (elongation) of long bones. Any sort of disruption in normal development and growth of the epiphyseal growth plate cartilage is sure to affect normal bone growth, specifically the elongation of long bones. This may lead to deformity of the bones. Some of the common health problems observed in broilers are skeletal disorders namely tibial dyschondroplasia (TD), which is due to growth plate cartilage anomaly (Williams *et al*. 2000, Farquharson and Jefferies 2000, Applegate and Lilburn 2002). There is a high prevalence of such musculoskeletal disorders at the peak of long bone growth (Naldo *et al*. 2000). An important factor in the diagnosis of skeletal abnormality is an understanding of the normal anatomy and development of the long bones. Radiography is an important tool used to document bone development and abnormality (Naldo *et al*. 2000). Moreover, epiphyseal closure of long leg bones by radiography along with other gross morphometrical parameters is still useful for age determination.

Calcium (Ca) is the most abundant mineral in the body of animals (McDonald *et al.*1995). About 90% of Ca is found in the bones (skeleton), where it combines with phosphorus (P), the second most abundant mineral in bone, to form calcium phosphate crystals or hydroxyapatite to provide structural strength and hardness to the bones (Goff

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2004). Skeletal strength and hardness are dependent on normal blood calcium and phosphorus level. Bone mineralization occurs when plasma Ca and P concentrations are normal. Under stable conditions, the rate of bone resorption is equal to the rate of bone formation so that the mineral content of the skeleton remains unchanged (Goff 2004).

Detailed radiographic and biochemical data on the long leg bones in broiler chickens are scarce. Besides, literature on these data in Vencob broilers is lacking. In the context of the aforesaid facts, the present study was undertaken on the long leg bones ofVencob broiler chickens at different ages (on days 1,7, 14, 21, 28, 35, and 42).

MATERIALS AND METHODS

For the present study, day-old Vencob broiler chicks (45 no.) were procured from Eastern Hatcheries Pvt. Ltd., Bhubaneswar, Odisha. The male and females (after conventional sexing method and wing banding) were separately reared for up to 6 weeks (*i.e.,* market age) under a deep litter system in the experimental pens of the Department of Anatomy & Histology, College of Veterinary Science & A.H., O.U.A.T., Bhubaneswar. The routine management practice was followed that included housing, vaccination, medication, and feeding. Proper ventilation and lighting arrangement were done. The bone samples (Femur, tibiotarsus and tarsometatarsus bones, and associated growth plate cartilages) were collected after the humane sacrifice of the birds.

Radiographic study

Digital radiography of the said bones (right and left side) from both the male and female birds along with associated growth or epiphyseal plates (cartilages) were performed in a local radiography centre (Auro Diagnostic Centre, Forest Park, Bhubaneswar) before and after taking the longitudinal section of these bones. The radiographic exposure factors were taken into account *i.e.,*50 kV, 0.1 seconds,15 mA, 100cm FFD, and 12 x10 film size. Some of the gross morphometrical parameters such as the width of bone and bone marrow and the thickness of cortical bone and growth plates were also recorded and analysed on radiography.

Biochemical study

The estimation of serum calcium (Ca) and phosphorus (P) levels (in mg/ dl) was done by a semi-auto analyzer (Merck Minilab 300) using a commercially available calcium kit (Coral Clinical Systems, Goa) and phosphorus kit (Coral Clinical Systems, Goa) as per the method of Leske and Coon (2002). Representative samples from the diaphysis (shaft) of all the leg bones (right) under study were used for the determination of bone ash, calcium, and phosphorus on dry, fat–free bones in the Department of Nutrition, College of Veterinary Sc. & A.H., O.U.A.T., Bhubaneswar. The total ash content was analysed and assayed for calcium and phosphorus content as per the methods of AOAC (1995) and Hamdi*et al.* (2015a).The calcium and phosphorus content of the bone was expressed in % per gram (DM basis) of the respective bone.

Statistical analysis

All the biochemical data generated were subjected to standard statistical analysis (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Radiographic study of leg bones and associated growth cartilages

Some of the gross morphometrical parameters like the width of the bones and bone marrow, and cortical bone thickness were measured and analysed on radiography of the long leg bones (Femur, tibiotarsus and tarsometatarsus) of broiler chickens at different ages during a post-hatch period and the data were compared with the gross morphometrical observations of the bones. Besides, the presence or absence of growth plates or cartilages (proximal and /or distal) in each of these bones at different ages was distinctly evident on radiography even in day-old chicks. The growth plate (cartilage) could not be traced (identified) grossly in the femur of both sides and sexes on day 1. But on the radiograph, the same was marked easily. The growth plate (cartilage) was identified on gross observation in the tibiotarsus of all the day-old chicks under study. The same was also confirmed on a radiograph of the bones.

However, from day 7 till day 42 the growth plates were easily identified radiographically for radiographic

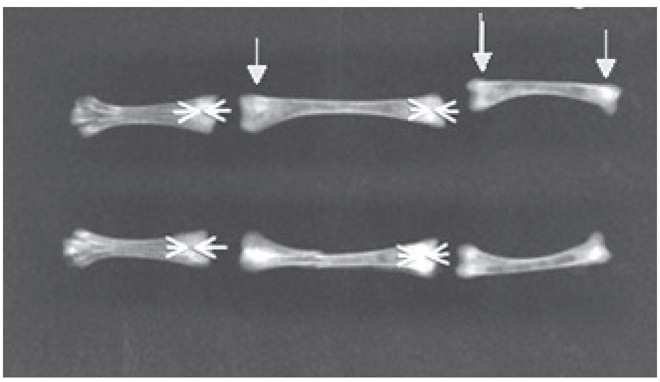

Fig. 1. Radiograph of left and right leg bones of day-oldbroiler chicks showing growth cartilages (arrow).

observations of different morphometric parameters of the bones (Fig. 1,2, 3). Though the proximal growth plate (cartilage) was identified, the distal one could not be traced (identified) grossly in the tarsometatarsus of both sides and sexes on day 1. But the growth cartilages of both ends were visible on the radiograph of the bones from day 1 till day 42. The growth cartilages in female birds appeared slightly thinner than those of male birds of respective ages. And there was a minor variation in thickness between proximal and distal growth cartilage at a given age. The growth cartilage in all the bones became a little bit thinner after day 28.

The radiographical data on some bone dimensions of the said bones under study corroborate exactly with those of gross morphometrical values. The radiographical analysis is the mere confirmation of the gross morphometrical observations in the present study. Moreover, radiography authenticates and confirms the presence of growth plates in these bones from day 1 till day 42. Hogg (1980) investigated the center of ossification in avian skeletons at and after hatching. He opined that the proximal tibial center was the only true secondary center in the long bones. However, both tibiotarsus and tarsometatarsus bones showed the presence of a secondary ossification center as revealed by the simultaneous histological observation of these bones in the present study. Sadler (1991) studied the fusion of proximal epiphysis in tibiotarsus and the development of spur core and its fusion with tarsometatarsus in domestic fowl. On the contrary, none of the tarsometatarsus bones under study showed the spur core and its fusion as also reported earlier by Sadler (1991) who observed much late appearance of the spur core and its fusion with tarsometatarsus. Different dimensions of the leg bones were radiographically analysed by Naldo *et al.* (2000), Williams *et al.* (2000) and Breugelmans*et al.* (2007) in domestic chickens. Charuta *et al.* (2013) assessed and evaluated the skeletal maturity and development in domestic ducks likewise.

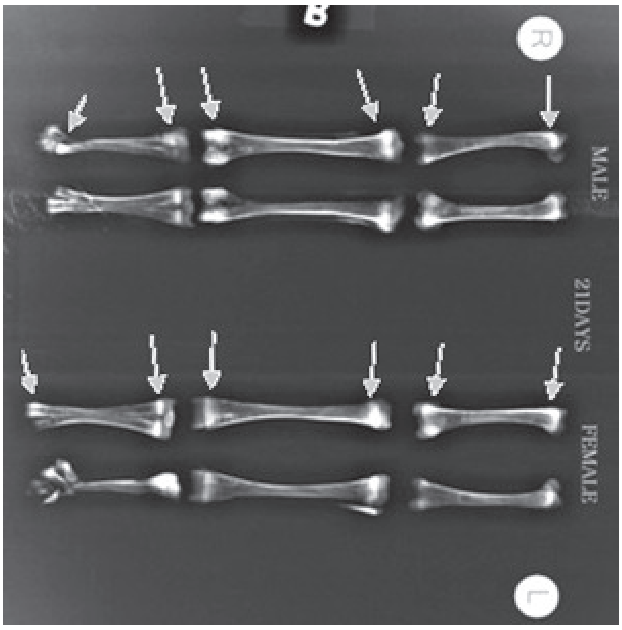

Fig. 2. Radiograph of left and right leg bones of 21 days old male and female broiler chickens showing growth cartilages (arrow).

Biochemical study

The age-related changes in serum Calcium and Phosphorus level (mg/ dl) and bone Calcium and Phosphorus level (%) in post-hatch broiler chicken at different ages were presented in Table 1 and Table 2 respectively.

Serum Ca and P level (concentration) was estimated in male and female broiler chickens during the post-hatch period to assess its effect on the skeletal morphology and integrity as well as to correlate it with the simultaneous changes in bone mineral content. The average serum Ca levels in male and female birds gradually increased from day 7 onward. The values were the maximum on day 42. However, only significant changes ($p \leq 0.05$) were noticed on Days7,35 and 42. The sex variation in the values in a particular age group was also evident. Female birds showed comparatively lower values than thoseof males.

Serum P levels in maleas well as female birds, showed a similar increasing trend with age including significant variation($p \leq 0.05$) like that of serum Ca. Male birds had comparatively higher values in an age group. The ratio between serum Ca and P level (Ca: P) varied accordingly to the age and sex of the birds.

According to Hassanabadi*et al*. (2007), serum Ca and P levels (mg/dL) in control broilers were 6.69 and 7.21 respectively on day 28. As per the observation of Rutt (2008), plasma Ca concentration (mg/dL) varied from 10.9 to 12.7 in 18 -21 days old broiler chickens with the control diet. Rani *et al.* (2011) recorded serum Ca and P levels (g %) as 10.92 and 5.41 respectively in control broiler chickens. Imik *et al*. (2012) reported serum Ca and P levels (mg/dL) to be 11.53± 0.64 and 7.30± 0.68 respectively in control (healthy) Ross 308 broiler chickens on day 42. Serum Ca and P levels (mg/dL) were recorded as 10.56 and 7.15 respectively in the control Cobb 400 broiler chickens (Vijayakumarand Balakrishnan 2014). The present observation on serum Ca and P levels (mg/ dl) in broiler chicken is well comparable with those of the said workers. A little variation in these values may be attributed to strain or breed difference of the chicken

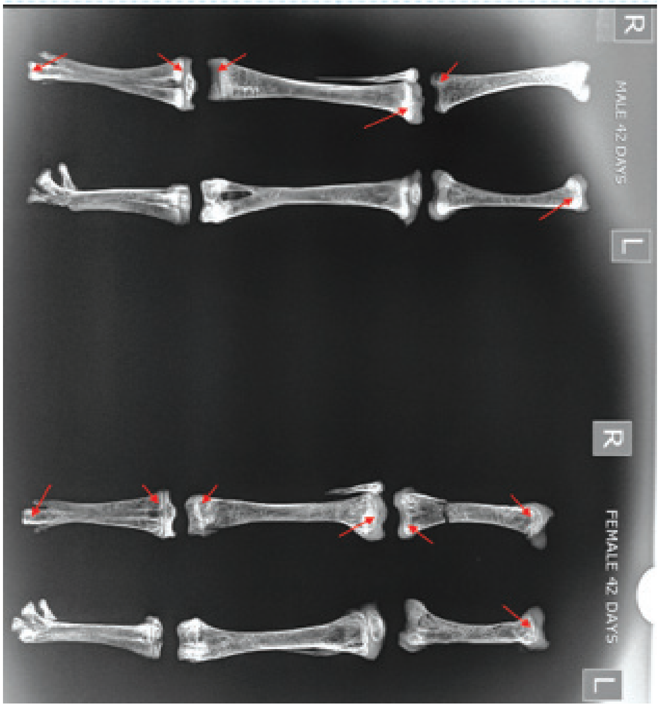

Fig. 3. Radiograph of left and right leg bones of 42 days old male and female broiler chickens showing growth cartilages (arrow).

studied and variation in management and feeding practice offered (Bogusawska -Tryk *et al.* 2012), and variable bioavailability as well as absorption of these minerals (Rani *et al.* 2011, Vijayakumarand Balakrishnan 2014). Comparatively much higher levels of serum Ca and P levels (as observed in the present study) in chicken (avian) than mammals were reported earlier by Brar *et al.* (2014).

Serum Ca levels observed in the present study were inversely proportional to serum P levels throughout the experimental period. It is in accordance with the finding of Gardiner (1969), who also advocated a similar inverse relationship between serum Ca and P levels in broiler-type chickens. The ratio between serum Ca and P levels in the present investigation varied from 2.05:1 to 2.35:1 in males and from 2.04:1 to 2.32:1 in female birds respectively. Rutt (2008) reported this ratio range to be between 1.4 and 2.1. Similar observations in this regard were made by Rani *et al.* (2011). The Ca:P ratio was more or less constant throughout the experimental period in the present study. Skeletal strength and hardness are dependent on normal blood calcium and phosphorus level. Bone mineralization occurs when plasma Ca and P concentrations are normal. Under stable conditions, the rate of bone resorption is equal to the rate of bone formation so that the mineral content of the skeleton remains unchanged (Goff 2004).

The bone mineral content i.e., percentage of Ca and P in all the representative bones of male birds gradually increased with age (from Day7) of the broiler birds in the present study. Ca% varied from 14.8 to 29.1 in the femur, 14.5 to 26.5 in the tibiotarsus, and from 14.2 to 24.7 in the tarsometatarsus respectively. The respective values for bone P (%) were 7.15 to 14.25, 6.90 to 13.0, and 7.04 to 12.3. The values were the maximum on Day42. The changes were significant ($p \leq 0.05$) usually in higher age groups (Days 28, 35, and 42). The present observation compares well with those of Williams *et al.* (2000), Salamatdoustnobar*et al.* (2011),and Vijayakumarand Balakrishnan (2014) in different broiler birds.Robison *et al.* (2015)had similar observations. The ratio between bone Ca and P percentage appeared more or less constant for all the bones at different ages. This is in consonance with the simultaneous observation of plasma Ca and P levels in the present study. Lower bone mineral content (%) in younger birds indicates the presence of

Table1. Age related changes in serum calcium and phosphorus level (mg/ dl) in post - hatch broiler chicken at different ages.

Days	Calcium		Phosphorus		Calcium phosphorus ratio	
	Male	Female	Male	Female	Male	Female
Day 1	5.78 ±0.69*	5.6 ±0.55*	2.82 ± 0.33 ^{NS}	2.61 ±0.25 ^{NS}	2.05:1	2.14:1
Day 7	7.215±0.71*	6.815 ±0.57*	3.06± 0.34 ^{NS}	2.93 ±0.27 ^{NS}	2.35:1	2.32:1
Day 14	7.675 ±0.73 ^{NS}	6.91 ±0.59 ^{NS}	3.49±0.36 ^{NS}	3.285 ±0.29 ^{NS}	2.20:1	2.10:1
Day 21	8.270 ±0.76 ^{NS}	7.26 ±0.61 ^{NS}	3.705±0.37 ^{NS}	3.435±0.32 ^{NS}	2.23:1	2.11:1
Day 28	8.885 ± 0.79^{NS}	7.635 ±0.63^{NS}	3.865± 0.39 ^{NS}	3.730 ±0.35 ^{NS}	2.30:1	2.04:1
Day35	10.50 ±0.81*	9.03 ±0.65*	4.585±0.41 ^{NS}	4.250±0.37 ^{NS}	2.29:1	2.12:1
Day 42	11.78 ±0.85*	10.500 ±0.68*	5.730 ± 0.43*	5.145 ±0.41^{NS}	2.05:1	2.04:1

* Values in a column vary significantly ($p \leq 0.05$) and NS indicates non-significant ($p \geq 0.05$) changes between two successive values in a column.

Table2. Age related changes in bone calcium and phosphorus level (%) in post-hatch male broiler chicken at different ages.

Day	Femur			Tibiotarsus			Tarsometatarsus		
	Ca (%)	P(%)	Ratio	Ca (%)	P(%)	Ratio	Ca (%)	P(%)	Ratio
1	14.82 ±0.69*	7.15 ±0.28NS	2.06:1	14.50±0.69*	6.90±0.29 NS	2.1:1	14.20±0.69 NS	7.02±0.32 NS	2.02:1
7	16.11 ±0.72*	7.75±0.31 NS	2.08:1	15.90±0.71*	7.83±0.31 NS	2.03:1	14.62±0.71 NS	7.25±0.37 NS	2.01:1
14	17.30 ± 0.74*	8.50±0.35 NS	2.03:1	16.83±0.75 NS	8.20±0.33 NS	2.05:1	15.23±0.75 NS	7.45±0.38 NS	2.04:1
21	18.62 ±0.76 *	9.15±0.37 NS	2.03:1	17.61±0.77 NS	8.60±0.39 NS	2.05:1	16.11±0.78 NS	7.95±0.40 NS	2.03:1
28	20.90 ±0.78*	9.80±0.38 NS	2.13:1	18.23±0.79*	8.90±0.41*	2.05:1	16.50±0.81NS	8.10±0.43NS	2.04:1
35	24.22 ±0.79*	11.95±0.41*	2.02:1	22.40±0.81*	11.00±0.43*	2.04:1	21.80±0.84*	10.6±0.45*	2.06:1
42	29.12 ±0.81*	14.25±0.44*	2.04:1	26.52±0.83*	13.0±0.47*	2.04:1	24.73±0.89*	12.3±0.49*	2.01:1

* Values in a column vary significantly ($p \leq 0.05$) and NS indicates non-significant ($p \geq 0.05$) changes between two successive values in a column.

comparatively more porous and less mineralized cortical bone that gradually became more compact and highly mineralized in later stages. Williams *et al.* (2000) opined alike. Plasma Ca and P profile is complementary to bone mineral (Ca and P) content. Bone mineralization takes place only when plasma Ca and P concentrations are normal (Goff 2004). Ca and P concentrations in serum and bones can reflect the changes in Ca and P homeostasis, and their contents within the normal range are important for normal physiological function and optimal bone mineralization (Proszkowiec-Weglarz and Angel 2013). Continued modelling and remodelling of mature skeleton provides a pool for maturing osteons and plays a vital role in maintaining a proper balance between the mineral content of plasma and bone, thus affecting proper mineralization and structural integrity of the bones (Fawcett 1994).

CONCLUSION

The radiographic study confirmed the presence of both proximal as well as distal growth plates in all these three bones from day 1 till day 42, the latter one being more authentic. In the femur, the presence of growth plates was reported for the first time. The secondary centre of ossification in broiler chicken appeared in the epiphyseal ends of the tibiotarsus and tarsometatarsus (but not in the femur) on day 21. The average serum Ca and P levels in plasma and bone (%) in broiler birds gradually increased with age. The ratio between serum Ca and P level (Ca: P) and bone Ca and P percentage remained almost constant during the growing period of the broiler chickens to maintain a proper balance for adequate mineralization of bones.

ACKNOWLEDGEMENT

The authors are very much grateful to the Dean, C.V.Sc. and A.H., OUAT, Bhubaneswar for providing the necessary facilities to carry out the research work.

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Cite this article as: Behera M, Sathapathy S, Mandal AK (2022) Radiographical and biochemical studies of leg bones during post-hatch development of broiler chickens.ExplorAnim Med Res 12(2): 246-251. DOI: 10.52635/eamr/12.2.246-251.