



## OSSIFICATION OF TRACHEA IN KUTTANAD DUCKS (*Anas platyrhynchos domesticus*)\*

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In birds, the trachea is a long tube-like structure composed of complete rings of hyaline cartilage united by narrow membranous ligaments. The long trachea in birds makes the inspired air to get fully saturated with water vapour and raises it to body temperature before reaching the lung. Besides this the trachea will also cause sound modulation. Ossification of hyaline cartilages is a feature of most of the birds. Tracheal rings ossify in birds at later stages of life. Literature on ossification of tracheal rings in waterfowl is scanty; hence this work was carried out in Kuttanad ducks during post natal period.

Ossification of trachea in Kuttanad ducks was studied using 78 female Kuttanad birds from day-old to 24 weeks of age. The material was collected from six birds in each group at fortnightly interval. Toluidine Blue-Alizarin Red S Staining of Cartilage and Bone protocol by Alphonse (1965) was followed.

The specimens were subjected to toluidine blue-alizarin red S staining after formalin, acetic acid and alcohol (FAA) fixation having the ratio of three components as 1:1:8 for approximately 40 minutes. Further specimens were stained in 0.06 per cent toluidine blue in 70 per cent ethyl alcohol for 48 h at room temperature. Twenty volumes of stain solution to the estimated volume of the specimen were used. Soft tissues were destained in 35 per cent ethyl alcohol for 20 h; 5 per cent for 28 h and 70 per cent for 8 h respectively. The specimens were counterstained in a freshly prepared 1 per cent aqueous solution of Potassium Hydroxide (KOH) to which was added two to three drops of 0.1 per cent alizarin red S per 100 ml of solution. The specimens were transferred into the fresh 1 per cent KOH-

alizarin mixture daily for three days, or until the bones and soft tissues had reached the desired intensity of red colour. The specimens were rinsed in water, placed in a 1:1 mixture of glycerol and ethyl alcohol for 1-2h and then transferred into fresh glycerol-alcohol for final clearing and storage.

In Kuttanad ducks, the trachea was a singular, straight hyaline cartilaginous tube extended ventrally all along the neck in continuation to the larynx. The trachea was covered only by skin and was ventral to the esophagus. Ibe *et al.* (2008) reported similar observations in West African guinea fowl. The supporting frame of the trachea was formed by the complete cartilaginous rings which showed varying degrees of ossification with age. Mennega (1964) in chicken and Bradley and Grahame (1960) in domestic fowl observed the same.

Trachea started showing small foci of ossification by eighth week at the cranio-ventral end, viz. on the first 12-14 rings, on the ventral aspect (Fig. 1a and 1b). By tenth week, all the tracheal rings were ossified ventrally, with lesser degree of ossification at the dorsal aspect (Fig. 2a and 2b). After tenth week, the process of ossification progressed with advancing age and by 24 weeks, the trachea was highly ossified, but on the dorsal side lesser degree of ossification was noticed (Fig. 3). This was partially in accordance with the findings of Garside (1968), who observed that in domestic fowl the ossification of the tracheal rings may be initiated as early as 15 weeks of age progressing upto 104 weeks of age. Hogg (1982) also found that in domestic fowl the first indication of mineralisation was encountered by 98 days post-hatching. In Kuttanad ducks,



**Fig.1a** Trachea of Kuttanad duck showing signs of ossification. 8<sup>th</sup> Week (ventral view)



**Fig.1b** Trachea of Kuttanad duck showing less degree of ossification. 8<sup>th</sup> Week (dorsal view)



**Fig.2a** Highly ossified trachea of Kuttanad duck. 14<sup>th</sup> Week (ventral view)



**Fig.2b** Ossification of trachea in Kuttanad duck. 14<sup>th</sup> Week (dorsal view)



**Fig.3** Fully ossified trachea in Kuttanad duck. 24<sup>th</sup> Week

the ossification started at the cranial end ventrally and progressed slowly towards the caudal. However in contrary to this, Hogg (1982), in domestic fowl stated that the process began at the caudal end of the series of tracheal rings and spread cranially. The first ring was not involved until 126 days. Ossification of the cartilages with age particularly on the ventral aspect of the trachea might reduce the damage to deeper tissues on pressing and may also keep the trachea firm without any damage when the bird is held by neck.

## Summary

A developmental study of the ossification of trachea in Kuttanad ducks was undertaken using 78 female Kuttanad birds from day-old to 24 weeks of age. The material was collected from six birds in each group at fortnightly interval. Cartilage and bone were differentiated in whole-mount preparations with toluidine blue-alizarin red S staining after formalin, acetic acid and alcohol (FAA) fixation. Trachea started showing small foci of ossification by eighth week. By tenth week, all the tracheal rings were ossified ventrally. The process of ossification progressed with advancing age and by 24 weeks, the trachea was highly ossified, but with a lesser degree of ossification on the dorsal side.

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