

Lingual structure of the Chabro chicken *Gallus gallus domesticus* L.: A developmental study

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In birds, morphological structures of floor of the mouth cavity play a vital role in food intake, nutrition and ingestion that forms the basis for clinical pathology and pharmacology. Hence, complete anatomical knowledge on the mouth cavity, floor in particular, is essential. In order to clarify certain morphological aspects of development of floor of mouth cavity, here, we studied the timing of the development of the tongue in the mouth cavity of Chabro chicks. Chick embryos were procured from the 5th to 21st days of incubation. Results revealed that the tongue occupied the cranial 2/3rd part of the floor of the mouth cavity and a laryngeal mound was situated caudal to the tongue. The tongue was triangular in shape and was differentiated into tip, body, and root on the 10th day of incubation. All the biometrical parameters increased with the advancement of incubation. The length of the tongue increased about 8.5 times from 6 to 21 days of incubation. Between the 10th to 21st day of incubation, the ratio between the length and width of the tip increased from 1.02 to 1.86 and the body and root decreased from 0.88 to 0.77 and 1.23 to 0.9, respectively. The conical papillae of the tongue developed on the papillary crest from the 12th day onwards and caudally directed pointed cornified papillae on the mound from the 16th day of incubation. These papillae were fully developed by the 21st day of incubation and arranged in primary and small secondary rows. The tongue was completely developed before hatching and ready to collect food.

Keywords: Junglefowl, Oral cavity, Tongue

Anatomical understanding of floor of the mouth cavity of birds is highly essential to analyse the morphological structures that may influence the food intake, nutrition and ingestion and beside this it also forms the basis for clinical pathology and pharmacology. Numerous studies on the beak and tongue architecture indicate that the different types of food and feeding activities influences the diversified morphological structure of the tongue in birds^{1,2}. In birds a significant variability of tongue is noticed in the form of size, shape, and type of lingual papillae which is characterized in between the species and largely related to the type of food and the mechanism of food intake³⁻⁵. To clarify certain morphological aspects of development of floor of mouth cavity containing tongue of Chabro chicken, here, we investigated developing chick between 5th day of incubation and hatching, the period when the tongue anlagen undergoes considerable morphogenetic changes.

Materials and Methods

Procurement of samples

In the present study, 105 incubated Chabro chicken eggs were collected from the Government Poultry farm, DUVASU, Mathura, Uttar Pradesh, India after approval of CPCSEA. The eggs were from the 5th to 21st day of incubation. Six eggs per day from 5 to 21 days were procured for this study. The obtained eggs were first identified for the presence of embryo by candling method and then cracked. The chick embryos were first transferred to a warm saline solution. Then the excess saline solution was removed and embryos were fixed into Neutral buffered formalin. The fixed embryos were used to study the different morphological development.

Collection of samples

The beak of the embryo was opened with the help of a sharp scissor and blade. The developing tongue was situated at the floor of the mouth cavity. The tongue along with the laryngeal mound and glottis was separated from the floor of the mouth cavity with the help of forceps and blade and placed under the stereo-zoom microscope for observation. All gross biometrical parameters viz. The length, width, and

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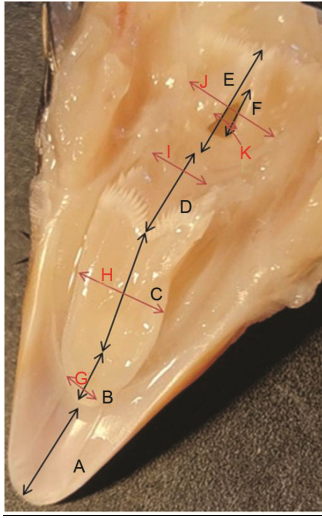


Fig. 1 — (A) Distance of beak and tip of tongue; (B-F) length of tip, body, root, laryngeal mound, and glottis, respectively; (G-K) width of tip, body, root, laryngeal mound, and glottis, respectively

thickness of the different regions of the tongue were measured with the help of a digital Vernier calliper (Fig. 1). The length of the apex/tip of the tongue was measured from cranila most part of the tip to lingual frenulum, body from lingual frenulum to papillary crest and root from papillary crest to the starting of elevation of laryngeal mound

Statistical analysis

The biometrical parameters recorded were subjected to suitable statistical analysis (Duncan test to compare means and Pearson correlation) using SPSS-20 software.

Results and Discussion

There was no clear line of demarcation between the oral and pharyngeal cavities in embryonic Chabro chicks due to absence of soft palate and hence they had a common oropharyngeal cavity. This cavity extended from the beak to the oesophagus as in adult Muscovy duck⁶. The floor was occupied by tongue cranially and laryngeal mound caudally. The lips and teeth were absent as in adult birds of different species⁷. The tongue was triangular in shape in Chabro chick embryo. On 6th day of incubation the tongue became triangular in shape with rounded slightly pointed anterior end. On 8th day of incubation it got elongated in outline with rounded tip. By 14 days of incubation, the tongue became triangular in shape with narrow pointed apex. In many birds, the shape of the tongue reflected the bottom part of the beak. In avian which belongs to the class of Galliformes (chicken, quail, partridge, pheasant etc.),

the shape of the tongue conforms to the lower beak and it has a knife-like or triangular shape⁸. In granivorous birds such as chickens or partridges, the tongue was short, triangular, and pointed⁹. An elongated and oval tongue was a characteristic of water birds such as duck and goose¹⁰. The tongue was narrowed in pigeon and lancet shaped in fowl¹¹. These morphological differences in the tongue of various avian species might be associated with the morphology of beak, dietary specialization, food type and adaptation to their eco-environmental conditions.

At 5th days of incubation the fusion between two lateral swellings was observed near the tip of the tongue. The lateral swellings, the body was far away and root was embedded between them caudally (Fig. 2A). At this stage the length of the tongue was 0.81 ± 0.02 mm and width was 0.36 ± 0.012 mm. Bellairs & Osmond¹² described that in birds the tongue developed as in mammals from two swellings and a median swelling, tuberculum impar on the floor of the pharynx at 4th day of incubation. The two lateral swellings extended towards the midline and fused with the medial swelling forming rostral part of the tongue. The root was formed by gradual proliferation of the hypopharyngeal swelling, copula formed from basihyal and basibranchial cartilages.

At 6th day of incubation the demarcation between body and root was indistinct. The posterior end i.e., body was raised and a “V” shaped slightly elevated structure present between body and root that might be the future junction between two lateral swellings with that of root of the tongue (Fig. 2B). The average length and width of the tongue was 1.17 ± 0.04 mm and 0.52 ± 0.023 mm at this stage. The length of tip was 0.612 ± 0.12 and the posterior part (body and root) was 0.48 ± 0.03 mm. A slightly raised area, laryngeal mound was also noticed posterior to the tongue. The glottis, opening of the trachea lied posterior to the tongue located midline on the floor of pharynx over the laryngeal mound¹².

At 8th to 9th days of incubation the tongue was attached to the bottom part of the beak by a short lingual frenulum¹³. The length of tip, body and root of the tongue was mentioned in Table 1. The width of tongue at the anterior and posterior end was measured 0.645 ± 0.90 mm and 0.96 ± 0.02 mm, respectively. Tongue was located about 1.075 ± 0.24 mm away from anterior part of beak. Just like mammals, the mesoderm of the left and right fourth pharyngeal arch

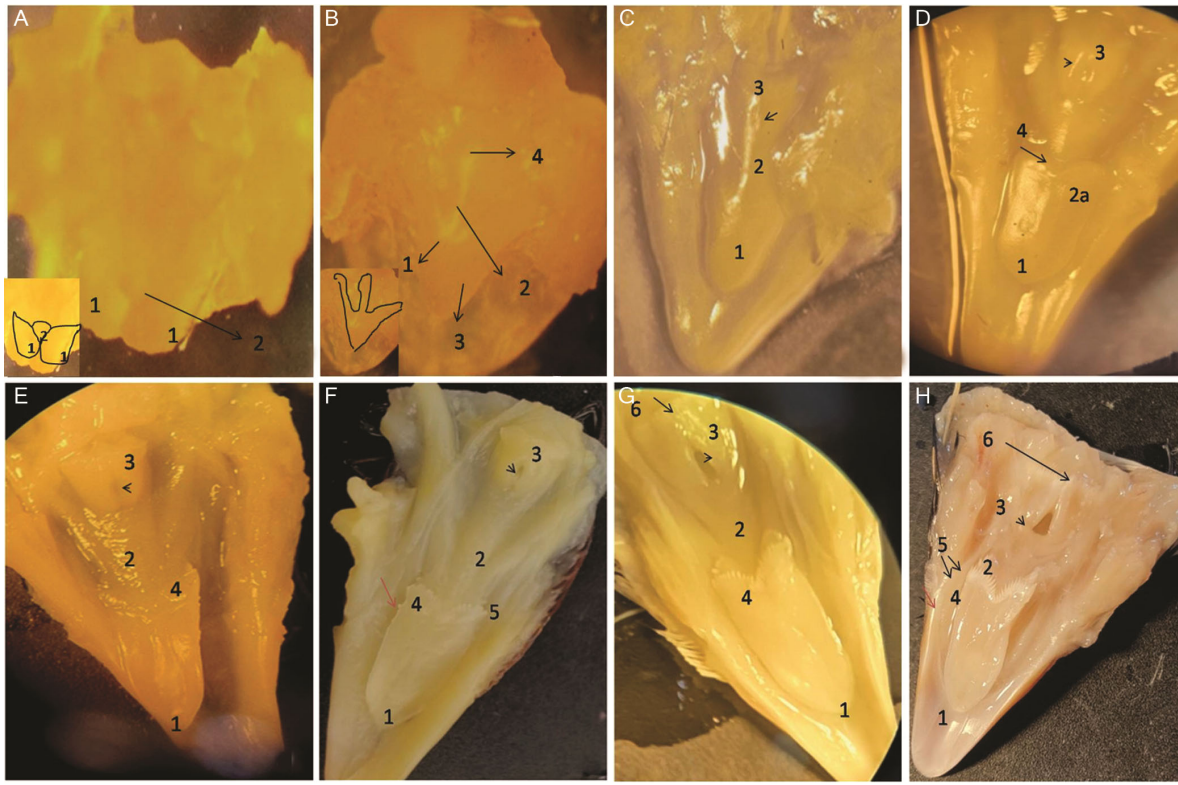


Fig. 2 — (A-H) Floor of the mouth cavity of 5, 6, 8, 10, 14, 16, 18 and 21 days chick embryo, respectively. [(A & B): 1. two lateral swellings, 2. tuberculum impar, 3. developing tongue, and 4. laryngeal mound; (C & D): 1. tip of the tongue, 2. undifferentiated body and root of the tongue (2A Body and 2B Root) 3. laryngeal mound, glottis (arrow head), and 4. papillary crest at the junction of body and root of the tongue; (E & F): 1. tip of the tongue, 2. Root of the tongue, 3. Laryngeal mound, glottis (arrow head) 4. papillary crest at the junction of body and root of the tongue, and 5. Conical papillae, developing small papillae on the lateral border of the body (red arrow); and (G & H): 1. tip of the tongue, 2. Undifferentiated body and root of the tongue, 3. laryngeal mound, glottis (arrow head), 4. Papillary crest at the junction of body and root of the tongue, and 5. conical papillae in two rows, developing small papillae on the lateral border of the body (red arrow)]

Table 1 — Comparative values (Mean ± SE) of linear parameters of tongue during different incubation days

Parameters (mm)	Incubation Day (ID)						
	8	10-11	12-13	14-15	16-17	18-19	20-21
Length of tongue (TON.L)	1.92±0.25 ^b	4.196±0.16 ^c	6.25±0.05 ^d	7.488±0.29 ^c	9.53±0.15 ^f	9.75±0.34 ^f	9.96±0.25 ^f
Tip length (TL)	1.04±0.35 ^a	1.25±0.08 ^a	2.52±0.20 ^b	2.522±0.14 ^b	2.803±0.19 ^{bc}	3.68±0.08 ^{cd}	4.25±0.46 ^d
Body length (BL)	0.53±.21 ^a	1.389±0.10 ^b	1.386±0.07 ^b	1.56±0.15 ^b	2.250±0.22 ^{bc}	2.76±0.25 ^c	2.83±0.40 ^c
Root length (RL)	-	1.328±.087 ^b	2.34±0.17 ^c	2.388±0.06 ^c	2.4±0.61 ^c	2.41±0.18 ^c	3.13±0.18 ^c
laryngeal mound length (LML)	-	1.258±0.13 ^a	1.77±0.09 ^{ab}	1.802±0.24 ^{ab}	2.36±0.20 ^b	3.13±0.18 ^c	4.05±0.51 ^d
length of glottis (GL)	-	0.464±0.04 ^a	0.81±0.09 ^{ab}	0.82±0.12 ^{ab}	1.22±0.17 ^{bc}	1.44±0.17 ^c	1.62±0.17 ^d
Tip width (TW)	0.645±0.90 ^a	1.126±0.09 ^a	1.18±0.13 ^a	1.693±0.17 ^{ab}	1.75±0.21 ^{ab}	2.28±0.31 ^c	2.68±0.31 ^d
Body width (BW)	0.96±0.02	1.58±0.09 ^a	2.23±0.12 ^b	2.380±0.10 ^b	2.667±0.13 ^c	3.10±0.17 ^d	3.63±0.07 ^e
Root width (RW)	-	1.084±0.10 ^a	1.16±0.13 ^a	1.63±0.08 ^{ab}	1.81±0.16 ^b	2.02±0.14 ^b	2.71±0.24 ^c
Laryngeal mound ^{NS} width (LMW)	-	1.13±0.081	1.38±0.10	1.620±0.11	2.08±0.13	3.14±0.26	3.39±0.26
Glottis width (GW)	-	0.206±.028 ^a	0.21±0.01 ^a	0.280.06 ^{ab}	0.44±0.10 ^{ab}	0.53±0.03 ^{bc}	0.60±0.11 ^c
No. of papillae	-	-	-	22.40±0.98	28.66±0.66	30.66±0.66	30.89±0.56

[n= 06, ^{NS} non significant; and ^{ab,c}superscript showed the significant different between incubation days]

gives rise to two swellings lateral to the laryngo-tracheal groove which later on developed as laryngeal glottis¹⁴. The glottis, opening of the trachea lied posterior to the tongue located midline on the floor of pharynx over the laryngeal mound¹² (Fig. 2C). In adult fowl⁹ and African pied crow¹⁵ the

laryngeal mound prominently projected in the caudal part of the floor of the pharyngeal cavity reaching up to the first tracheal ring and entrance to the oesophagus. It consisted of two adjoining, raised, quadrilateral plates in long-legged buzzard and in turkey¹⁶.

At 10th -11th day of incubation all the three parts of the tongue *viz.* apex, the body and the root were well demarcated (Fig. 2D). The tip was pointed and body was raised. The region between two lateral swellings at the midline was raised and had a faint groove laterally. The lateral surface of the caudal part of the body showed swellings indicating the development of lingual papillae. Caudally wide and shallow 'V' shaped raised area, papillary crest was observed at the junction of body and root as observed in goose; quail and in partridge^{4,17,18}. The crest was broader towards the lateral margin. The shape of the groove was an arc shaped in birds of 7 days, "V" shaped in birds of 11 and 18 days and serpentine pattern in birds of 25 and 32 days. The lingual body was separated from the lingual root by a transverse papillary crest, which was "V" shaped in squabs but "U"-shaped in adult pigeons¹⁹. The papillary crest facilitated the movement of each bolus of food towards the oesophagus²⁰. The root was narrow, constricted and raised than the level of body which became wider towards the laryngeal mound. The length and width of apex, body and root were mentioned in Table 1. The distance of tongue from the anterior end of beak was 2.034±0.07 mm and the beak was 2.054±0.093 mm wide at the level of tip of the tongue. The length and width of laryngeal mound and glottis were depicted in Table 1.

At 12-13th days of incubation a single row of small conical papillae were reported at the papillary crest. The average length and width of the tongue, tip, body and root of the tongue and laryngeal mound and glottis were mentioned in Table 1. The distance of tip of the tongue from beak was calculated 2.72±0.19 mm and width of beak at this region was 2.76±0.12 mm. The thickness of tip (TTIP) was 0.40±0.07 mm and body (TB) was 0.60±0.07 mm. By 14-15th day of incubation, the tongue was triangular in shape with narrow pointed spatula shaped apex (Fig. 2E). The median part of the body was convex. On the caudo-lateral border of the body in front of last conical papillae 2-3 raised evaginated structures were noticed. The conical papillae were arranged in a single row. The size of the papillae was shorter at middle which increased laterally. The tips of the conical papillae were directed towards the pharynx. The surface of the papillae was smooth without any secondary projection^{3,18}. The lingual papillae fulfilled diverse functions during food intake, transport, and swallowing. These papillae played major role in directing food to the esophagus and prevented the regurgitation of small and large food pellets²¹. The occurrence of only mechanical

papillae on the tongue is a characteristic feature in birds^{3-5,22-25}. Those papillae have the shape of cones arranged individually in one or two rows or grow from common crest^{3,5,13,26}. As much as 22.40±0.980 conical papillae were recorded on papillary crest. The papillae on the papillary crest were triangular spoonful shaped in young and mature rock pigeon and blunt tubercles shaped in adult²⁷. The average length of tongue was 7.488±0.293 mm and distance of tongue from beak was 2.934±0.206 mm. The length and width of the tip and body of the tongue and length and width of the root, laryngeal mound and glottis were mentioned in Table 1. The thickness of the tongue at the tip was 20.458±0.050 mm and at body was 0.68±0.09 mm, respectively.

On 16-17th day of incubation one large and three small buds of conical papillae were noticed on the lateral surface of the body (Fig. 2F). The length of tongue increased to 9.75±0.397 mm. Average length and width of tip, body and root of the tongue and laryngeal mound and glottis were given in Table 1. The tongue was 0.573±0.029 mm and 0.997±0.050 mm thick at the tip and body region, respectively. Distance of tongue from anterior end of lower jaw was 3.330±0.472 mm and width of jaw at the level of tip was 4.937±0.315 mm. About 28.667±0.667 papillae arranged in a single row were observed on the caudal part of laryngeal mound. The papillae present at the centre of laryngeal mound were longer than lateral side. A single row of pharyngeal papillae occurred behind the laryngeal mound in guinea fowl⁸ and raven and magpie species²⁶. The caudally directed pointed cornified papillae on the mound might be helpful in the ingestion of solid food particles and pellets and in raking movement of the larynx during swallowing^{27,28}. In cage and aviary birds, Evans²⁹ described a few filiform papillae on the laryngeal prominence, whereas, in ostrich the papillae were not seen on the larynx³⁰. The difference might be attributed to environmental and feeding variations.

At 18-19th days of incubation number of conical papillae between body and root increased from 28.667±0.667 to 30.667±0.667. Another row of caudally directed 8-10 lingual papillae were noticed behind the conical papillae. These papillae were smaller than the anterior row of papillae. Unlike the rostral row, the caudal row of papillae was absent medially and situated only near the lateral margin of the tongue. The conical lingual papillae presented between the body and the root of the tongue were arranged in form of U-letter in pigeon and in form of wide V-letter in cattle egret³¹. Whereas, 4 to 5

layers of big conical papillae on the area where the root and body converge was reported in Baghdad indigenous peacock³². The geese's tongue filiform papillae are particularly long, and exhibited on whole of the lateral surface of the tongue³³. The conical papillae on border between the lingual body and root responsible for the last stage of food transport, while the papillae on the sides of root stabilize the path of food transport³⁴. The papillae on laryngeal mound became more prominent with advancement of incubation (Fig. 2G). The average length and width of the tongue, tip, body and root of the tongue and laryngeal mound and glottis was recorded and given in Table 1. The distance of tip of the tongue from beak was calculated 1.25 ± 0.06 mm and width of beak at this region was 4.55 ± 0.35 mm. The thickness of the tip and body of the tongue measured 0.64 ± 0.08 mm and 1.107 ± 0.08 mm, respectively.

At 20-21st days of incubation, tip became more pointed and width of the root increased. Although, median groove was not observed in chicken⁹, the dorsal surface of the tongue showed a faint median groove anteriorly in the present study as in goose⁴, and duck³⁵ from 21 days. Megeid *et al.*³¹ in 1 day old *Khaki Campbell Duck* observed a shallow median groove divided the dorsal surface of the body of the tongue into two symmetrical halves. The median groove presented on surface of the tongue indicated path of food transport³⁴. Transverse groove was present between root and laryngeal mound. Papillae on laryngeal mound were arranged in two rows (Fig. 2H) as in adult Egyptian nightjar³⁶. Whereas, the bulky laryngeal mound has a circular glottic fissure, carrying a single row of papillae at the rear edge Eurasian Collared Dove³⁷. In present study, the size

and number of the papillae on caudal row were less. Posteriorly 4-5 small papillae were present in second row. In Egyptian laughing dove Papillae were not observed on the elevated, triangular laryngeal mound except for the glottic opening, which was bounded by two lateral elevated borders that presented a row of small papillae³⁸. During entire embryonic period taste buds were not observed on the surface of tongue. Whereas, the taste buds were reported on the tongue of Eurasian collared dove³⁷. There was a fundamental difference between avian and mammalian taste systems in terms of anatomy, distribution of taste buds, and the nature and molecular structure of taste receptors³⁹. It has been reported that taste bud cells are not derived from neural crests but from mesenchymal cells with high migratory properties in chickens. In contrast, taste bud cells in mammals are from epithelial origin⁴⁰.

The length of tongue measured 9.96 ± 0.25 mm. Average length and width of tip, body and root measured and mentioned in Table 1. The tongue was 0.79 ± 0.03 mm and 1.16 ± 0.09 mm thick at the tip and body region, respectively. Distance of tongue from anterior end of lower jaw was 3.34 ± 0.31 and width of jaw at the level of tip was 3.72 ± 0.48 mm. Statistical analysis revealed that significant changes were occurred in the length and width of the tongue and its parts *viz.* Tip, body and root with advancement of incubation period of chick embryo. All the biometrical parameters were highly significantly (1% level of significance) correlated with each other except length of the body and tip, length of root and laryngeal mound, length of the body and root width which were significantly (5% level of significance) correlated with each other (Table 2). Some parameters

Table 2 — Correlation coefficient between different parameters of tongue and incubation day (ID)

ID	TON. L	TL	BL	RL	LML	GL	TW	BW	RW	LMW	GW	TTIP	TB	
ID	1													
TON. L	0.966**	1												
TL	0.911**	0.893**	1											
BL	0.737**	0.666**	0.452*	1										
RL	0.701**	0.811**	0.651**	0.221	1									
LML	0.836**	0.783**	0.757**	0.672**	0.473*	1								
GL	0.563**	0.563**	0.518**	0.358	0.091	0.783**	1							
TW	0.708**	0.700**	0.635**	0.690**	0.297	0.699**	0.513**	1						
BW	0.835**	0.800**	0.638**	0.766**	0.382	0.839**	0.726**	0.733**	1					
RW	0.680**	0.598**	0.626**	0.447*	0.175	0.447*	0.202	0.511**	0.481*	1				
LMW	0.146	0.071	0.116	0.27	-0.071	0.198	0.119	0.234	0.167	0.067	1			
GW	0.654**	0.586**	0.671**	0.365	0.177	0.586**	0.432*	0.534**	0.482*	0.619**	0.24	1		
TTIP	0.604**	0.623**	0.266	0.589**	0.036	0.547*	0.549**	0.511*	0.760**	-0.074	0.524*	0.124	1	
TB	0.774**	0.740**	0.629**	.463*	0.161	0.622**	0.442	0.396	0.597**	0.085	0.602**	0.287	0.681**	1

[TON. L, Length of tongue; TL, Tip length; BL, body length; RL, root length; LML, laryngeal mound length; GL, length of glottis; TW, tip width; BW, body width; RW, root width; LMW, Laryngeal mound^{NS} width; GW, Glottis width; TTIP, Thickness of tip; and TB, Thickness of body. Significant at *0.05 level and ** highly significant at 0.01 level]

were non- significantly correlated with each other as indicated in Table 2.

Conclusion

The present investigation has shown that the morphological sequence of tongue development in chickens is similar to that of other birds. Differentiation of three parts of tongue viz. root, body and apex occurred from 10th day of incubation. Conical and cornified papillae showed their presence from 12th and 16th days of incubation, respectively. These papillae were fully formed just before hatching at 21st day of incubation and ready to perform their function of transport of food material. The morphology of the tongue in the Chabro chicken is fully developed before hatching and ready to collect food by pecking and grazing.

Conflict of interest

Authors declare no competing interests.

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