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Regional variations in the height and width of villi of small intestine during pre-hatch development in Turkey (*Meleagris gallopavo*)

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Abstract
A study was undertaken to investigate the regional variations in the height and width of villi of the small intestine during the pre-hatch development in turkey (*Meleagris gallopavo*). Sixty embryos of Beltsville White turkey each from third, fourth, sixth, ninth, twelfth, fifteenth, eighteenth, twenty-first, twenty-fourth and twenty-seventh day of incubation and six day-old poults were studied. Routine histological staining was carried out and the micrometrical data was analyzed statistically. Though the foregut, midgut and hindgut of primitive gut was evident by fourth day of incubation, longitudinal foldings of the intestinal epithelium indicating future villi was evident only by fifteenth day embryo. The folds later developed into individual villi which increased in length and width with advancement of age. When the length of villi decreased from duodenum to ileum, the width of villi increased towards ileum.

Keywords: development, embryo, turkey, villi

1. Introduction
Turkey (*Meleagris gallopavo*) belongs to the order *Galliformes*, along with chicken and is native to North America. A thorough knowledge about the growth and feed utilization of the bird is important due to the rising global demand of turkey and its products. Growth and development of birds depend upon digestion, absorption and assimilation of nutrients. The small intestine consisting of duodenum, jejunum and ileum promotes improved digestion, by formation of specialized structures called villi and microvilli in the tunica mucosa, which increase in the surface area for absorption (Turk, 1982) [1]. Due to the tremendous effect of structural and functional differences on the growth performance of the bird (McLelland, 1975) [2] and the early maturing nature of the alimentary tract (Moran, 1985) [3], studies on pre-hatch development of avian gastrointestinal tract has become imperative in poultry production, for effective management of flock and economical feeding strategies.

2. Materials and Methods
The present study was conducted on 66 fertile eggs of Beltsville White turkey with viable embryos, collected from the University Poultry and Duck Farm, Mannuthy, Thrissur. The eggs were then set in incubator at ideal temperature and humidity conditions. Sixty embryos each were selected from the group, on third, fourth, sixth, ninth, twelfth, fifteenth, eighteenth, twenty-first, twenty-fourth and twenty-seventh day of incubation. Six day-old poults were also studied.

The embryos upto twelfth day of incubation were fixed as such and from fifteenth day of incubation onwards, tissue pieces of duodenum, jejunum and ileum were fixed in 10 per cent neutral buffered formalin. Further, the fixed tissues were processed and paraffin sections of 5 µm thickness were prepared. The sections were stained by Haematoxylin and Eosin (H&E) staining technique for routine histological studies and Periodic Acid Schiff's (PAS) method for detection of brush border (Luna, 1968) [4]. The micrometrical data on the height and width of the villi of the segments in different age groups were recorded and analysed statistically (Snedecor and Cochran, 1994) [5].

3. Results and Discussion
The development of foregut, midgut and hindgut was evident by fourth day of incubation in the present study. The primitive gut wall lined by pseudostratified columnar epithelium by

Fourth day of incubation, increased in its thickness by sixth day of incubation. Undifferentiated mesenchymal cells formed the outer layer of the gut wall. But in chicken embryo, Romanoff (1960) [6] observed simple columnar lining epithelium of the gut during the third day of incubation, which then changed to pseudostratified epithelium by fourth day of incubation.

Further differentiation of the gut wall occurred by ninth day of incubation, wherein the tunica mucosa represented by pseudostratified epithelium was under laid by propria-submucosa.

The broad, irregular longitudinal foldings of the intestinal epithelium indicating future villi was evident only by fifteenth day embryo during the present study. The mesenchymal connective tissue formed the core of the in foldings. Romanoff (1960) [6] observed development of folds during eighth or ninth day of incubation in chick. The difference noticed may be attributed to the relative variation during development of the different species and the length of the incubation period.

By day-old poult stage, the tunica mucosa of the small intestine formed large number of foldings into the lumen, *viz.* the villi, which varied in number, shape and size according to the region.

In agreement to the observations of Hodges (1974) [7] in chicken, there existed no much difference in the histological structure of the three segments of the small intestine, *viz.* duodenum, jejunum and ileum in the present study during the period of incubation.

3.1 Duodenum

The mucosa of the duodenum started to present the foldings by fifteenth day of incubation (Fig. 1), which differentiated further during the later stages of incubation. During eighteenth day, the mucosal folds started dividing into individual villi (Fig. 2), probably attributing to the need for increasing the absorptive surface area with the advancement of age. This was in agreement with the findings of Bohórquez *et al*. (2011) [8] in turkey, where the longitudinal folds was evident in the small intestine by fifteenth day of incubation which developed into mature single villi during the later stages of incubation.

Better differentiation of epithelium with developing brush border was evident from eighteenth day of incubation onwards (Fig. 2).

The mean length of the duodenal villi showed an increase of 7.020 times from fifteenth day of incubation to day-old stage (Table 1). The mean width of the villi showed significant ($p< 0.001$) and progressive increase from eighteenth day of incubation to day-old stage (Table 2). By day-old stage, the duodenal villi were blunt at the apex and narrow at the basal region (Fig. 3).

3.2 Jejunum and Ileum

The villi of both jejunum (Fig. 4) and ileum (Fig. 5) presented a blunt apical region and a wide basal region and were shorter compared to duodenum by twenty-first day of incubation. This finding was similar to the observations of Nazrin *et al*. (2012) [9].

The mean length of villi increased significantly and progressively in jejunum ($p< 0.001$) and ileum ($p< 0.001$), from fifteenth day of incubation to day-old stage (Table 2). The width of jejunal and ileal villi also showed significant increase (Table 2). Similar to the findings in duodenum, the width of the longitudinal folds in jejunum and ileum was greater during fifteenth day of incubation compared to eighteenth day of incubation. The decrease in length of villi from duodenum to ileum during the present was in agreement with the reports of Turk (1982) [1]. In confirmation to the reports of Nazrin *et al*. (2012) [9], the width of ileum was found to be highest compared to duodenum and jejunum in the present study

Table 1: Length of villi of segments of small intestine during pre-hatch period in turkey (Mean±S.E) (n=6)

Age of embryo (days)	Duodenum	Jejunum	Ileum	F- value	p- value
15	$66.033^{eB} \pm 1.135$	$66.067^{eB} \pm 0.559$	$67.750^{dA} \pm 0.820$	4.045*	0.036
18	$84.050^{eB} \pm 2.452$	$67.050^{eB} \pm 0.523$	$69.083^{dA} \pm 1.0321$	31.750**	<0.001
21	$156.467^{dC} \pm 6.780$	$91.317^{dB} \pm 3.658$	$73.317^{dA} \pm 1.999$	90.625**	<0.001
24	$227.050^{cB} \pm 7.154$	$157.267^{cB} \pm 4.859$	$144.867^{cA} \pm 3.672$	66.717**	0.007
27	$374.717^{bC} \pm 7.766$	$267.550^{bB} \pm 10.018$	$195.133^{bA} \pm 6.181$	123.717**	<0.001
28 (Day-old poult)	$463.533^{aC} \pm 12.087$	$331.467^{aB} \pm 6.309$	$270.450^{aA} \pm 6.314$	129.434**	<0.001
F- value	498.272**	425.199**	423.854**		
p- value	<0.001	<0.001	<0.001		

** Significant at 0.01 level, * Significant at 0.05 level

Means bearing same small letter as superscript within a column do not differ significantly

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Table 2: Width of villi of segments of small intestine during pre-hatch period in turkey (Mean±S.E) (n=6)

Age of embryo (days)	Duodenum	Jejunum	Ileum	F- value	p- value
15	$40.733^{eC} \pm 0.436$	$44.517^{bB} \pm 0.399$	$65.617^{aA} \pm 0.727$	613.597**	<0.001
18	$39.467^{eC} \pm 0.777$	$35.433^{cB} \pm 0.471$	$25.350^{eA} \pm 0.852$	166.618**	<0.001
21	$44.033^{dC} \pm 0.233$	$35.433^{cB} \pm 0.983$	$26.633^{eA} \pm 0.774$	459.676**	<0.001
24	$46.883^{cB} \pm 0.954$	$37.483^{cB} \pm 0.764$	$36.883^{dA} \pm 1.343$	46.085**	0.007
27	$54.150^{bB} \pm 1.307$	$44.050^{bB} \pm 1.551$	$45.767^{cA} \pm 1.287$	15.178**	<0.001
28 (Day-old poult)	$82.150^{aC} \pm 0.876$	$53.767^{aB} \pm 1.299$	$49.733^{bA} \pm 1.193$	241.244**	<0.001
F- value	363.863**	59.844**	303.807**		
p- value	<0.001	<0.001	<0.001		

** Significant at 0.01 level

Means bearing same small letter as superscript within a column do not differ significantly

Means bearing same capital letter as superscript within a row do not differ significantly

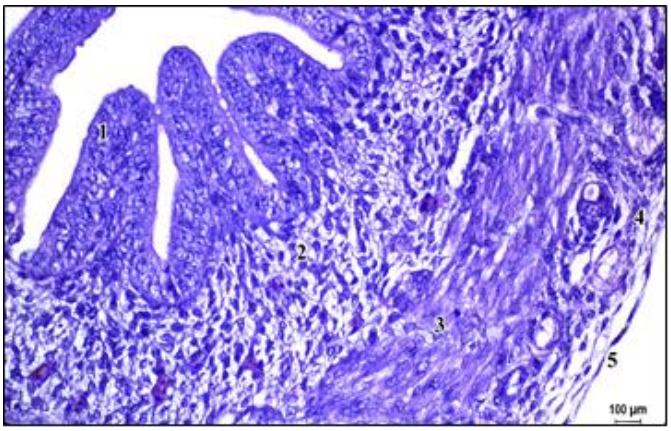

Fig 1: Cross section of duodenum showing the different layers (15th day of incubation). H & E x 400 1. Epithelium, 2. Propria – submucosa, 3. Circular muscle layer, 4. Longitudinal muscle layer, 5. Tunica serosa

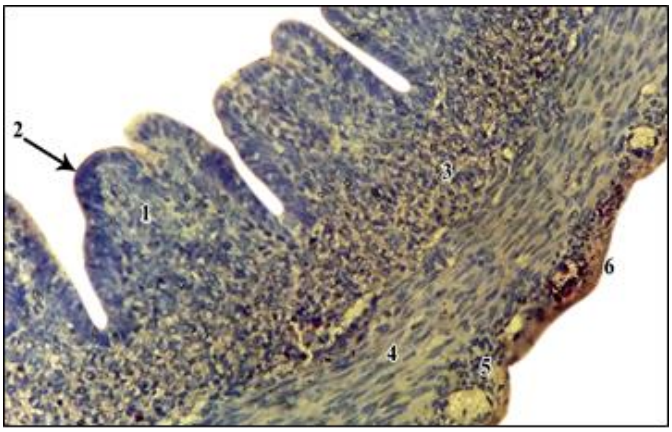

Fig 2: Cross section of duodenum showing dividing villi and developing brush border (18th day of incubation). H & E x400 1. Dividing villi, 2. Brush border, 3. Propria – submucosa, 4. Circular muscle layer, 5. Longitudinal muscle layer, 6. Tunica serosa

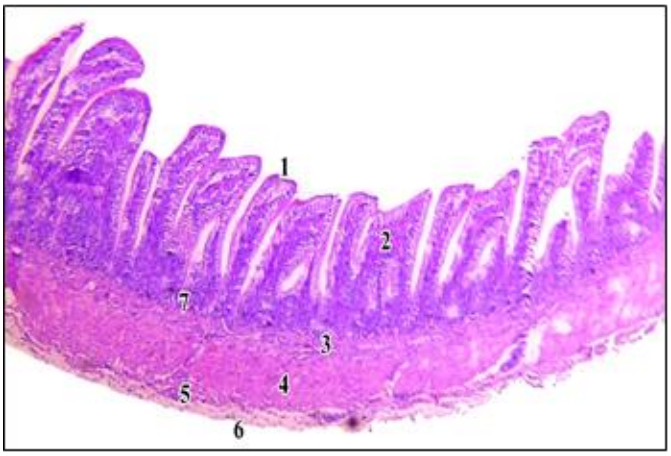

Fig 3: Cross section of duodenum showing the villi (day-old poult). H & E x100 1. Villi, 2. Lamina propria, 3. Muscularis mucosa, 4. Circular muscle layer, 5. Longitudinal muscle layer, 6. Tunica serosa, 7. Crypts of Lieberkuhn

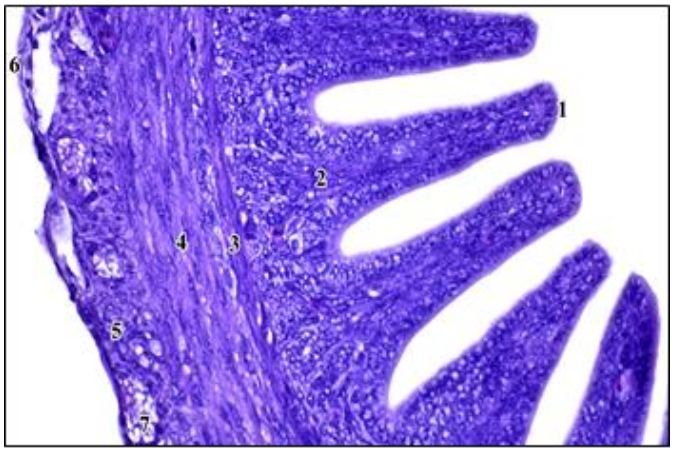

Fig 4: Cross section of jejunum showing the villi (21st day of incubation). H & E x400 1. Epithelium, 2. Lamina propria, 3. Muscularis mucosa, 4. Circular muscle layer, 5. Longitudinal muscle layer, 6. Tunica serosa, 7. Nerve bundle

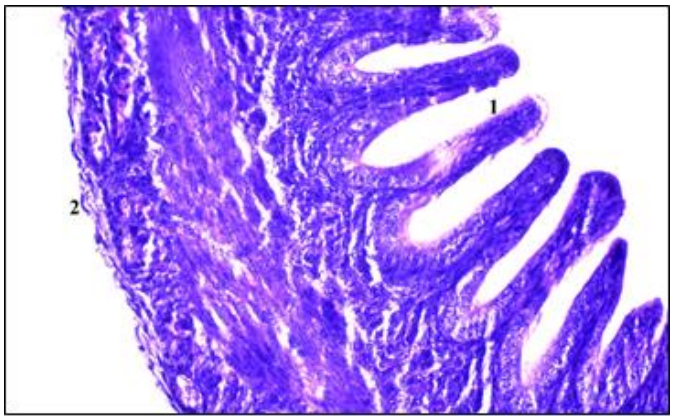

Fig 5: Cross section of ileum showing the villi (21st day of incubation).H & E x400 Villi, 2. Tunica serosa

4. Conclusion

The longitudinal folds which appeared during the fifteenth day of incubation in the present study divided and developed into individual villi during the later stages of development. The length and width of villi increased significantly with advancement of the age. The villi became shorter and broader towards ileum in the present study.

5. Acknowledgement

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