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Histology and histochemistry of the small intestine of the post-hatch Kadaknath fowl

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The intestinal epithelium is a complex system of multiple cell types undergoing continual renewal and change with well-orchestrated patterns of gene expression during the processes of development and differentiation. This system has a major role in determining developmental potential of the hatched chick (Bayer *et al.* 1975, Uni *et al.* 1995, 1998). Chick growth and development are dependent upon nutrient digestion and absorption, which is a direct result of the functional and morphological development of the small intestine (Baranylova and Holman 1976, Sell *et al.* 1991, Akiba and Murakami 1995, Yamauchi *et al.* 1996, Uni 1999). Because the intestine is the nutrient supply organ, the sooner it achieves its functional capacity the earlier the young chick can use dietary nutrients and grow according to its genetic potential (Uni *et al.* 2003, Uni and Ferket 2004). Hence, an urge to elucidate the above concept, and paucity of coherent literature on histology and histochemistry of the organ in the Kadaknath fowl, led to pursue the present study.

The present study was carried out on 6 Kadaknath birds of each age group, i.e. day 1, 7, 14, 28, 56 and 112. The birds were obtained from Instructional Poultry Farm (IPF) of G.B. Pant University of Agriculture and Technology, Pantnagar. They were sacrificed by decapitation under light anaesthesia and the abdominal cavity was immediately opened. After evisceration small intestine was freed from the mesentery, and segments from duodenum, jejunum and ileum were collected and fixed in 10% neutral buffered formalin for 48 h and processed for routine paraffin technique of light microscopy. Paraffin sections of 6 m thick were obtained and stained by routine Harris haematoxylin and eosin method for general histoarchitectural studies, Masson's trichrome stain for collagen fibres, Verhoeff's stain for elastic fibres and Gomori's silver stain for reticular fibres (Bancroft and Stevans 1977).

For histochemical studies, the segments from duodenum, jejunum and ileum were fixed in 10% neutral buffered formalin for 48 h, Bouins fluid for 12 h and Helly's fluid for 48 h. Thereafter, these tissues were processed routinely and 6 m thick paraffin sections were obtained. Sections from each of the three segments were stained with PAS technique for carbohydrate (McManus 1946), Alcian Blue stain for acid and strongly sulphated mucopolysaccharides (Luna 1972), Masson-Hamperl method for argentaffin cells (Singh 1964) and modified Giemsa method for chromaffin cell granules (Giemsa 1902). The aforesaid stained sections were examined under the Olympus microscope (B×40), Japan, and photomicrography was performed.

Histological studies: The mucosa of entire small intestine showed villi of variable shapes and sizes according to the age. In day 1 chicks, the duodenal villi were finger shaped with blunt apex. The shape of the villi varied from finger shaped to foliate and slender shaped at day 7. Each had a wider base with a tapering apex in 112 day-old birds (Fig. 1) which is in accordance with the report of Hilton (1902), who noted that each villus had a square thin base and a distal triangular part with a pointed apex in the duodenum of the duck. There was absence of central lacteals in the villi, each villus core being occupied by a capillary bed. Similar reports were made by Bell and Freeman (1971). This correlated with the poorly developed lymphatic system of the fowl and with the biochemical evidence for lipid absorption into the portal blood.

Between the villi were well marked crypts of Lieberkuhn. In day-old chicks a single gland was observed per crypt with a relatively small number of cells per gland. During the initial post-hatch days the glands number increased. Subsequently the rate of growth declined and by day 14, three to four glands were observed per crypt (Fig. 2). These findings were corroborated by Uni *et al.* (2000) in young chicks.

The villi and glands were lined by a simple columnar epithelium. The epithelium comprised the chief or main cells, the goblet cells and the argentaffin cells. The chief cells varied in shape and size, depending upon their situation and the degree of contraction of the intestinal villi. The oval nucleus was situated in the basal half of the cell, but was usually closer to the middle of the cell than to basal pole (Fig. 3). The chief cells of the glands of Lieberkuhn differed from those of the villi. The nucleus was large and was situated close to the basal

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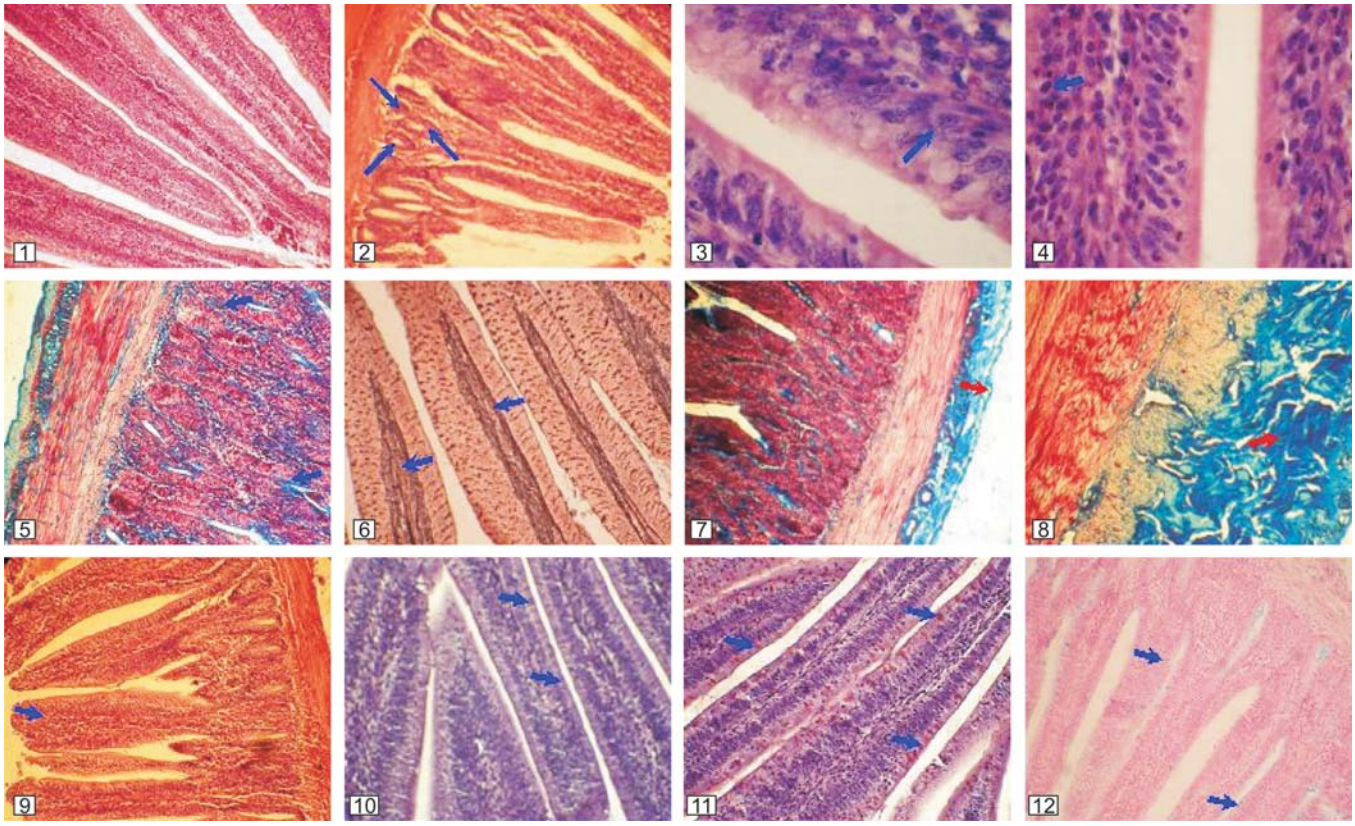
Table 1. Mean height and nucleus size (µm) of villi epithelial cell in small intestine of Kadaknath fowl at different age

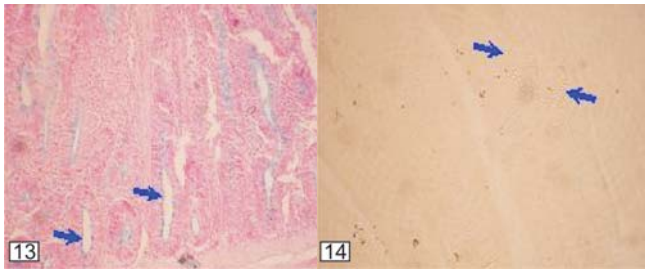
Age (days)	Duodenum		Jejunum		Ileum	
	Cell height	N.S.	Cell height	N.S.	Cell height	N.S.
01	6.67±0.42	2.50±1.88	6.25±0.72	0.52±0.10	3.75±0.00	0.52±0.10
07	7.08±0.42	1.04±0.21	6.67±0.42	0.42±0.10	5.83±0.42	0.31±0.06
14	8.33±0.42	0.83±0.21	7.92±0.42	0.83±0.21	7.08±0.42	0.42±0.10
28	6.67±0.41	1.04±0.21	9.58±0.42	1.04±0.21	7.92±0.42	0.73±0.10
56	9.17±0.42	1.67±0.21	9.17±0.83	1.46±0.21	7.92±0.42	1.87±0.36
112	11.25±0.72	3.13±0.36	11.67±0.55	2.71±0.55	10.42±0.42	2.71±0.75

membrane. A striated border was absent and the cytoplasm had a much stronger affinity for dyes, such as haematoxylin and acid fuchsin, than that of the cells of the villi. Hodges (1974) noted that the chief cells appeared tall, narrow and columnar in shape in the villi and glands of the duodenum. The chief cells and their nuclei measured 11.25±0.72 mm in height and 3.13±0.36 mm in width, respectively in the duodenal villi of 112 day old birds (Table 1), whereas in fowl it measured up to 50 mm in height by 8–10 mm in maximum width (Hodges 1974).

The goblet cells in the duodenal villi of the Kadaknath fowl were always in the form of a neatly shaped goblet. There was



Figs 1–12. Photomicrograph showing: **1**. leaf-like villi in the dudenum of 112-day-old Kadaknath fowl (H&E ×200). **2**. three to four glands per crypt in the duodenal mucoea of 14-day-old chick (H&E × 200). **3**. chief cell of duodenal villi having basal nucleus in 112-day-old Kadaknath fowl (H&E × 1000). **4**. distribution of globular leucocytes in the villous epithelium of duodenal mucoea in 112-day-old fowl (H&E × 1000). **5**. distribution of collagen fibres in the core of the villi and interglandular connective tissue of duodenal mucoea in 112-day-old bird (Masson's trichrome stain × 200). **6**. sparse distribution of reticular fibres in the lamina propria of duodenal mucoea in 7-day-old chick (Gomoris stain × 200). **7**. moderate density of colagen fibres in the tunica serosa layer of duodenum in 7-day-old chick (Masson's trichrome stain × 200). **8**. greater density of collagen fibres in the tunica serosa layer of duodenum in 112-day-old bird (Masson's trichrome stain × 200). **9**. short, club shaped jejunal villi in 28-day-old chick (H&E × 200). **10**. very weak PAS positive reaction in the goblet cells of duodenum of 7-day-old chick (PAS × 200). **11**. very strong PAS positive reaction in the goblet cells of ileum of 14-day-old chick (PAS × 200). **12**. strong reaction of acid mucin in the villous epithelium of ileum of 14-day-old chick (Alcian-blue: pH 1.0 × 200).



Figs 13–14. Photomicrograph showing: **13**. moderate reaction of acid mucin in the glandular epithelium of duodenum of 112-day-old chick (Akian-blue: pH 1.0 × 200). **14**. Photomicrograph of ileal grouping of argentaffin cell in 14-day-old chick (Masson-Hamperl stain × 200).

no clearly defined striated border present apically. They were much more frequent in the glands than on the sides of the villi. Chodnik (1947) noted goblet cells of the domestic fowl to differ from those of mammals in that they always presented the form of a neatly shaped goblet.

Argentaffin cells were present both in the villi and the glands and were most numerous in the glands. Frequency of argentaffin cells was more in 56 and 112 day-old birds. Bell and Freeman (1971) noted high concentration of argentaffin cells in the upper duodenum although, unlike mammals where they were located deep in glands, they occur in the surface epithelium and in the upper part of the glands correlating to present observation.

Globule leucocytes (GL) were observed in the basal half of the intestinal epithelium, lying between epithelial cells (Fig. 4) as reported by Toner (1965). They were rounded or oval in outline and of variable size, with small irregular nuclei and dense cytoplasmic inclusions.

In the lamina propria, the collagen fibres were observed in the core of the villi and interglandular connective tissue with increased density from day 1 to 112 old birds (Fig. 5). Probably it is to provide more structural support to the glandular tissue for physiological adaptation with the advancement of age. The reticular fibres were relatively less conspicuous at day 1 to 7 (Fig. 6); the density of which increased in the birds aged 56 and 112 days. The tracts of smooth muscle fibres were well developed. The reticular tissue of the lamina propria was extensively infiltrated with large number of lymphoid cells, mainly large and small lymphocytes and plasma cells, together with a few eosinophilic leucocytes. Identical observations were reported by Clara (1925), Patzelt (1936) and Rosenberg (1941) in adult birds. Lymph nodules were observed in the lamina propria of the duodenal mucosa in nearly all groups of Kadaknath fowls but they were very few or absent in young chicks. On the contrary Marshall (1960) identified isolated follicles and Peyer's patches throughout the length of the small intestine. With age elastic fibres were evident within the wall of the large blood vessels.

The muscularis mucosae consisted of a layer of longitudinal smooth muscle fibres which extended into the corium of each villus. Farner *et al.* (1972) opined that the muscularis mucosae could be a compact functionally effective layer. The submucosa was so poorly developed as to be almost non-existent in all of the age groups. Hodges (1974) reported identical observations in fowls in contrast with the report of Calhoun (1954) and Rosenberg (1941) who stated that the submucosa was apparent only in a few places and was a very thin layer. The submucosal glands or Brunner's glands were lacking from the duodenum and their mucus secreting role was carried out by numerous goblet cells present between the chief cells of the surface epithelium and in the superficial parts of the simple glands as stated by Aitken (1958).

The tunica muscularis consisted of a well developed inner circular muscle layer and a weakly developed outer longitudinal muscle layer. Blood vessels and nerves were found in association with the tunica serosa layer. The collagen fibres were scanty and sparse with low to moderate density in 1-to 28-day-old chicks (Fig. 7). However, there was greater density of collagen fibres in the serosa layer of 112 days old birds (Fig. 8). The elastic fibres were most pronounced in 112 day old birds, as was reported by Calhoun (1954) in fowl.

The jejunal mucosa of day 1 chicks showed thin, cylindrical villi. The jejunal villi were short and club shaped at 28, 56 and 112 days of age (Fig. 9). Bayer *et al.* (1975) however reported two morphological types, broad finger-shaped and narrow plate like villi in day 1 chicks. The presence of shorter, club shaped and fewer villi in later groups in the present study is in confirmation to the findings of Bradley and Grahame (1960) who reported jejunal and ileal villi to be shorter than those of duodenum. The less number of villi in later groups probably was on account of attaining maximum length of jejunum among the parts of the small intestine. Further, the shorter and club shaped villi observed during the present study may be due to the inhibitory effect of duodenal melatonin on the epithelial growth in the jejunum. A similar observation was also made by Randall *et al.* (2002) in rats and pigeons. The chief cells of a jejunal villi measured 11.67±0.55 mm in height and its nucleus measured 2.71±0.55 mm in width in 112 day old birds. The depth of crypts of Lieberkuhn decreased considerably and there was decrease in the density of argentaffin cells. These observations corroborated findings of Hodges (1974) in the jejunum of fowl.

The ileal villi were long, cylindrical and devoid of secondary villi at day 7 onwards. The absence of secondary villi in later groups may be on account of increase in the size of these villi with the advancement of age. These findings are in line with the report of Lim and Low (1977) but contrary to the observations of Bradley and Grahame (1960) who reported shorter ileal villi in chicks. The greater density of collagen fibres at day 56 and 112 in the interglandular tissue and intermuscular connective tissue in the present study appears to facilitate the movement of mucosa which is extensively folded to increase the absorptive area and moreover due to difference in the physiodynamic forces of different tissues.

Histochemical studies: The PAS reaction was observed in the glands of Lieberkuhn and goblet cells from day 1 to 112 of age. The duodenum of the 7 day old chicks revealed a very weak PAS reaction (Fig. 10). The intestinal goblet cells showed very strong PAS reaction in 14 days old birds, the intensity of which increased towards ileum (Fig. 11). Further, a strong PAS reaction was also observed in the glands of Lieberkuhn of the duodenum of 14 day old birds. Strongly sulphated epithelial mucin was demonstrated in the intestinal goblet cells of the proximal, middle and distal segments of the small intestine in the birds of all the groups under study. The ileum of the 112 day old bird revealed a moderate PAS reaction. Verma *et al.* (1999) observed that the intensity of colour reaction to PAS positive material increased with the advancement of age in post-hatch fowl.

Alcian-Blue stain revealed the strong reaction of acid mucins in the villous as well as glandular epithelium of the ileum of 14 day old birds (Fig. 12). Further, the ileal villous epithelium of 56 day old birds showed a mild alcian-blue reaction. The glandular epithelium of the duodenum of 112 day old birds also showed a moderate alcian-blue reaction (Fig. 13).

Argentaffin cells in less number were dispersed in the villous and more in the gland epithelium of all the three intestinal segments. They varied in shape, being broadly based upon the basement membrane and narrowing towards the apex and did not appear to make contact with the duct lumen. Rarely the argentaffin cells were arranged in small groups of two to three contiguous cells (Fig. 14). Aitken (1958) also reported the presence of argentaffin cells at all the levels of the intestine including the coprodaeum. Modified Giemsa stain for argentaffin granules revealed varied occurrence of granules per cell. In a few only a small number of granules were present and were mainly located in the basal cytoplasm close to the basement membrane. In the latter case the cell might had extended towards the lumen of the gland or surface of the intestine in the form of a slender process contained a few discrete granules or more rarely, as a thicker process filled with densely packed argentaffin material in which the individual granules could rarely be distinguished. Identical reports were recorded by Aitken (1958) in the intestine of younger birds.

SUMMARY

The mucosa of entire small intestine of Kadaknath fowl showed villi of variable shapes and sizes according to the age. The surface of the villi and crypts of the various segments were lined with simple columnar epithelium comprising chief or main epithelial cells, the goblet cells and the argentaffin cells. The epithelial cells of the glands were smaller than those of the villi. The goblet cells were observed nearer the tip of the villus as the age of the bird increased. The Brunner's glands were lacking. Argentaffin cells were found in small numbers at the bases of the crypts and rather infrequently on the villi. The connective tissue of the lamina propria had very few large collagen fibres but possessed a network of fine reticular fibres which were associated with the reticular networks of the blood vessles and muscle fibres. The tracts of smooth muscle fibers were well developed and together with the blood and lymph vessels, comprised a large proportion of corium of villus. The submucosa was so poorly developed as to be almost non-existent in most part of the small intestine. Among various segments a high density of collagen was seen in duodenum and ileum especially in the serosa layer, interglandular connective tissue and intermuscular connective tissue. The collagen fibres were scanty in day-old and 7-day-old bird whereas in 28, 56 and 112-old bird there was greater intensity of these fibres. Elastic fibers were not found in the corium of young birds, whereas thick branching and anastomosing elastic fibres were associated with the coats of the large blood vessels in the serosa layer. The PAS and Alcian Blue staining reaction revealed strongly sulphated epithelial mucins and neutral mucins respectively with mild to strong intensity from one day to 112 days old Kadaknath fowl.

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