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HISTOCHEMICAL STUDIES ON SMALL INTESTINE OF UTTARA FOWL

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Abstract: The objective of this study was to investigate the histomorphological development of the small intestine of Uttara fowl and to examine the changes in the number of goblet cells, argentaffin cell granules and lymphatic nodules by using histochemical techniques. The goblet cells were numerous towards the distal part of the intestine whereas the argentaffin cells and lymphocytes were more in number in the duodenal part. The goblet cells and crypts of Lieberkühn have acid and strongly sulphated mucopolysaccharide secretions. The brush borders as well as the goblet cells of the ileum showed high amount of polysaccharide material. Distribution of non-specific alkaline phosphatase (ALP) enzyme was correlated with age by catalytic histochemistry method. The ALP activity in post hatch chicks gradually increased from proximal to distal end of small intestine within the same age group. The activity also increased with the increasing age of the birds.

Keywords: goblet cells, argentaffin cells, lymphocytes, Small intestine, histochemistry, Uttara fowl.

Introduction

Uttara fowl is generally reared under backyard system in Kumaon division of Uttarakhand state. It is of local importance in the region as it gives nutritional as well as economic security to the rearing families. At hatching, the digestive system of chick is anatomically immature and its functional capacity is not fully developed. The gastrointestinal tract undergoes rapid structural changes during the post hatch period with increasing age. To understand the capacity of the small intestine to absorb nutrients and protective functions, it is important to examine the histomorphological changes occurring in the intestine during development.

MATERIALS AND METHODS

To investigate the structural organization of the small intestine of the Uttara breed of fowl, a total of 24 birds were procured from Instructional Poultry Farm of G.B.P.U.A&T, Pantnagar.

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The birds were divided into four age groups viz day old, 7, 28, and 112 days old birds with 6 birds in each group. The birds were sacrificed and small intestine was removed immediately and 3-4 mm size tissue samples, from various region were collected. After collecting the samples were fixed in 10% neutral buffered formalin, Bouin's fluid, Helley's fluid and Orth's fluid for further processing. The histochemical studies were conducted by using following techniques:

1- General carbohydrates were demonstrated by using the Periodic acid Schiff (PAS) technique (Mc Manus, 1946). In this procedure, a positive reaction is indicated by the appearance of magenta colouration resulting from the reaction between aldehydes liberated from 0.5% periodic acid and the decolourized solution (leucofuchsin) of Schiff's reagent.

2- Acid and strongly sulphated mucopolysaccharides were demonstrated by using the Alcian blue method according to (Luna, 1972). By this method, acid mucins exhibit blue stainabilities.

3-Masson Fontana stain for argentaffin cells and Modified Giemsa stain for chromaffin cell granules technique (Bancroft and Stevens, 1996). Granules of these cells stain black with silver salts.

4-Gomori's technique for Alkaline phosphatase (Gomori, 1951). In this procedure, Alkaline phosphatase activity was observed as a fine granular blackish-brown reaction product along the brush border of the villous epithelium.

Thereafter, the stained tissue sections were examined under the Nikon Microscope ECLIPSE 80i and photomicrography was performed.

RESULTS AND DISCUSSION

A villus is a projection of the intestinal mucosa. The epithelium lining the surface of villi and glands was simple columnar. This observation was similar to the observation of Aitken (1958) and Nasrin *et al.* (2012) in chicken. The intestinal epithelium comprised of multiple cell types i.e. the chief or main epithelial cells, the goblet cells and the enterochromaffin cells with a striated border (brush border). Few lymphocytes were also observed exterior to the enterocytes or base of the columnar epithelial cells, at certain locations they form aggregation of lymphatic tissue. Lymphocytes are small more or less spherical in shape with darkly stained rounded nuclei (Fig. 1). There was a transient increase in the number of goblet cells in the villi of all intestinal segments from 0 to 112 days of age. Goblet cells are responsible for secretion of mucin that is used for the mucinous lining of the intestinal epithelium. Thus, higher density of goblet cells may result in an increase in the secretion of mucin. Changes in

mucin content or the composition of the mucosal surface may decrease nutrient absorption or increase energy requirement for gut maintenance (Langhout *et al*., 1999). The goblet cells are positive to the stains specific for mucus. The strong magenta colored PAS reaction was observed in the lamina propria, glands of Lieberkuhn, goblet cells of villi and apical plasma membranes of the columnar epithelial cells from 0 to 112 days of age. Hamdi *et al.* (2012) in common quail *(Coturnix coturnix)* and Hamdi *et al.* (2013) in the black-winged kite, (*Elanus caeruleus*)*,* also observed a strong magenta colouration in the goblet cells of both the villi and the crypts of Lieberkühn as well as the apical plasma membranes of the columnar epithelial (absorptive) cells of the small intestine by PAS method. The duodenum of the 7 day old chicks revealed a very weak PAS reaction (Fig. 2). The goblet cells of the villus epithelium and glands of Lieberkuhn of the jejunum both showed strong PAS reaction in 28 day old birds as compared to the duodenum (Fig. 3). The intestinal goblet cells showed very strong PAS reaction in 28 days old birds, the intensity of which increased towards ileum (Fig. 4). Further, a strong magenta colored PAS reaction was also observed in the glands of Lieberkuhn of the duodenum of 28 day old birds. El-Banhawy *et al*. (1993) studied that the brush borders as well as the goblet cells of the ileum have a high amount of polysaccharides material in both piscivorous bird, the black headed gull *Larusridi bundus*, as well as those of granivorous bird, the palm dove *Strepto peliasene galensis.* Verma *et al.* (1999) observed that the intensity of colour reaction to PAS positive material in the basal lamina and goblet cells of the glandular and surface epithelium of intestinal mucosa was increased with the advancement of age in posthatch fowl thus correlating the present observations.

The goblet cells and the crypts of Lieberkühn have acid and strongly sulphated mucopolysaccharide secretions, these findings were in agreement with El-Banhawy *et al*. (1993); El-Sayyad (1995) in birds and Hamdi *et al.* (2012) in quail. The distribution of acid mucin was very scanty in the duodenal epithelium (Fig. 5) and other segments of small intestine of 0 to 7 day old birds. Alcian-Blue stain revealed the strong colour reaction of acid mucin was seen in the goblet cells of villus epithelium and glandular epithelium of all intestinal segments at 28 and 112 days of age (Fig. 6). Differences in the density of acidic and strongly sulphated goblet cells of all segments of the small intestine were observed after hatch. However, the number of goblet cells showed an increasing trend from duodenum to ileum in the age group 0 to 7 days also and this trend was more marked in the age group of 28 and 112 days old bird. Contrary to the present study Uni *et al*. (2003) reported that the proximal, middle and distal segments of the small intestine contained similar proportion of

goblet cells producing acidic and neutral mucins in the broiler GIT after hatch and until day 7. A gradient of goblet cell density was observed increasing along the duodenal to ileal axis as increase of age.

Argentaffin cells were dispersed, the cells very few in number in the surface epithelium of villus area and were more in number in the glandular epithelium of all the three intestinal segments. Frequency of argentaffin cells increased in 28 and 112 day old birds. These cells are invariably located in the epithelium and never in the connective tissue and usually close to the basement membrane (Fig. 7). Aitken (1958) also reported the presence of, argentaffin cells at all levels of the intestine including the coprodeum. Bell and Freeman (1971) in fowl and Kalita and Singh (2010) in Kadaknath fowl reported high concentration of argentaffin cells in the upper duodenum, they occur in the surface epithelium and in the glands correlating the present observation. The shape of these cells varied from bottle shaped with narrow neck directed towards the intestinal lumen, to spindle-shaped cells lying between the epithelial cells. The large lightly stained vesicular nucleus was located in the central part of the cell. The cytoplasm was filled with considerable number of granules which were uniformly stained with haematoxylin and eosin. Aitken (1958) reported that only a small number of granules were present in a few cells, these cells were most numerous in the duodenum in a narrow zone at its origin, a feature particularly marked in younger birds in which this zone was very narrow and succeeded by one in which lymphatic tissue was particularly abundant and gland tubules in consequence were widely separated from one another.

Modified Giemsa stain for argentaffin granules revealed varied occurrence of granules per cell. In few cells 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 were 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 granual couldn’t be distinguished. Aitken (1958) reported similar observations about the argentaffin cell granules in the intestine of younger birds. Simard & van Campenhout (1932) also described the development and distribution of argentaffin cells in the chicken and have suggested that at one stage in development (269hr.) argentaffin cells migrate from the epithelium into the lamina propria.

Stained sections by catalytic method showed alkaline phosphatase activity in all the age groups. However, it varied from mild to strong in different parts of the intestine in different

age groups. It was intense in the surface and glandular epithelium and increased from base to apex of the villi. At all ages, alkaline phosphatase reaction was observed along the brush border of the tips, sides and bases of the villous epithelium which extended into the apical cytoplasm (Fig. 8). Alkaline phosphatase reaction was weak in the crypts. Similar findings were reported in Kadaknath fowl (Kalita *et al.,* 2011). A strong ALP activity was observed at the base of the villus epithelium in the ileum of 120-day-old Guineafowl (Singh *et al*., 2011). Moog (1950) also demonstrated alkaline phosphatase in the striated cuticular border in the epithelial cells of the chick duodenum. Grey and LeCount (1970) studied the distribution of alkaline phosphatase in the villi of the chick duodenum at the age of 1-4 weeks and observed that alkaline phosphatase was low or absent in the crypts and highest at the villi tips.

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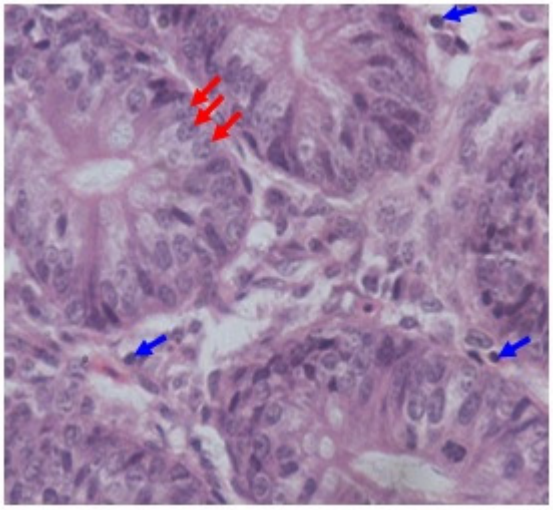

Fig. 1: Photomicrograph of glands of Lieberkuhn showing lymphocytes (blue arrow) and chief cells with large nucleus (red arrow) close to the basal membrane of duodenal villi in 7 day old chick (H&E x 1000)

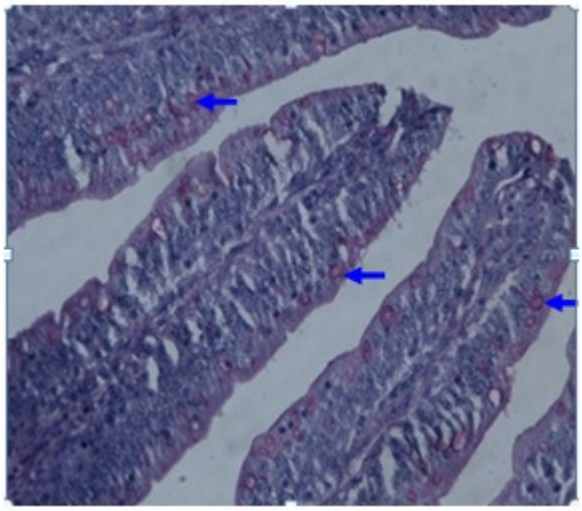

Fig. 2: Photomicrograph showing weak PAS positive reaction in the goblet cells of duodenum of 7 day old chick (PAS x 400)

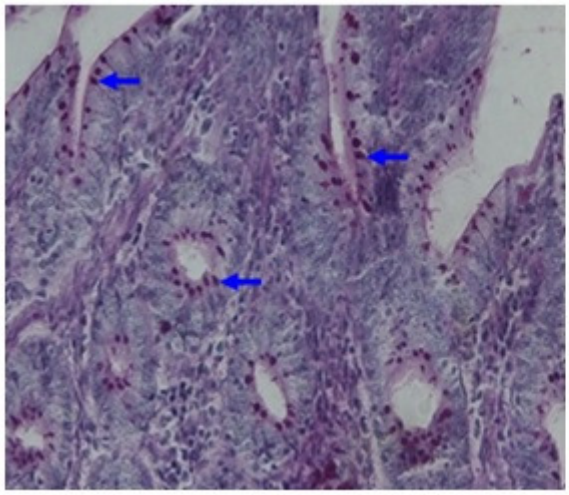

Fig. 3: Photomicrograph showing strong PAS positive reaction in the goblet cells of villus epithelium and glands of Lieberkuhn of jejunum of 28 day old bird (PAS x 400)

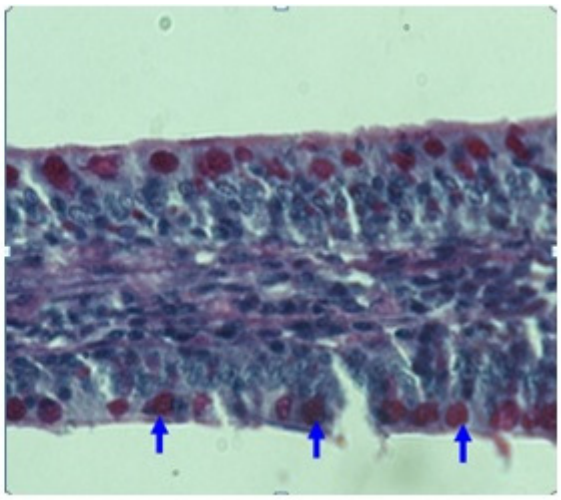

Fig. 4: Photomicrograph showing very strong PAS positive reaction in the goblet cells of ileum of 28 day old bird (PAS x 1000)

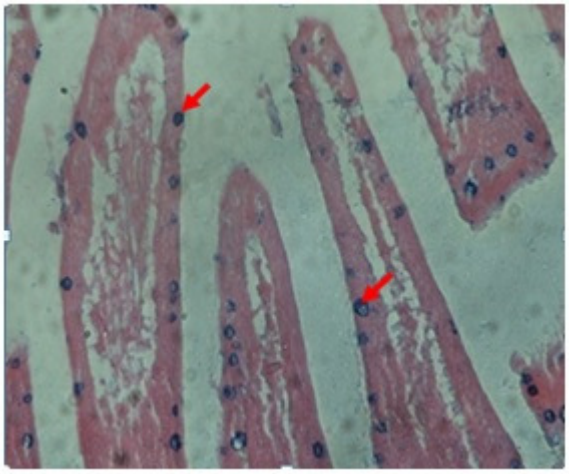

Fig. 5: Photomicrograph showing mild reaction of acid mucin in the goblet cells of villus epithelium of duodenum of day old chick (Alcian-blue: pH 1.0 x 400)

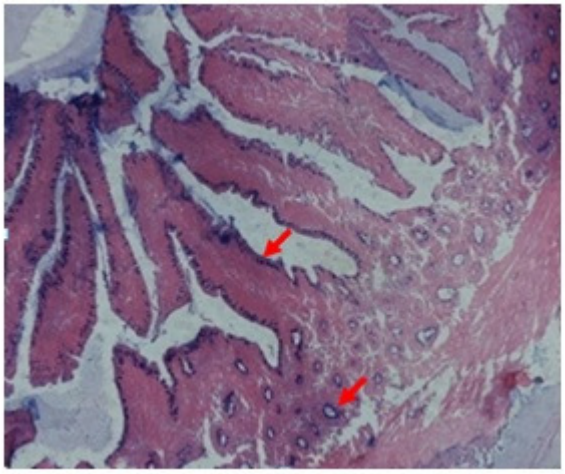

Fig. 6: Photomicrograph showing strong reaction of acid mucin in the goblet cells of villus epithelium and glands of Lieberkuhn of jejunum of 112 day old bird (Alcian-blue: pH 1.0 x 100)

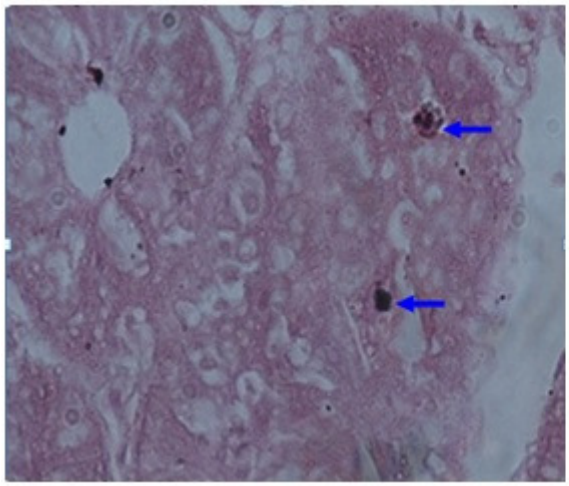

Fig. 7: Photomicrograph showing argentaffin cells granules (arrow) in the basal cytoplasm of gland epithelium of the duodenal mucosa in 28 day old bird (Masson Fontana stain x 1000)

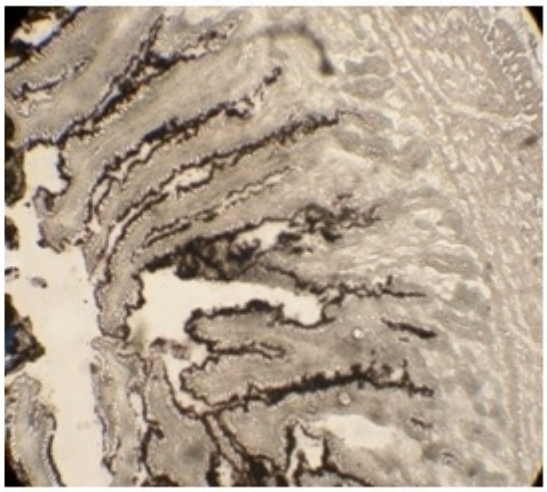

Fig. 8: Photomicrograph showing strong alkaline phosphatase activity in the brush border of the villus epithelium in ileum of 112 day old bird (Gomori's method x 200)