Haryana Vet. (June, 2018) **57 (1)**, 67-69

Research Article

AGE RELATED HISTOMORPHOCHEMICAL STUDIES ON SMALL INTESTINE OF CHICKENS

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ABSTRACT

The intestines of thirty six broiler chickens of 07, 14, 21, 28, 35 and 42 days of age were used for present study. The tissues were collected from different parts of duodenum, jejunum, ileum and their respective junctions. Histologically, the wall of small intestine was having four tunics i.e. mucosa, submucosa, muscularis and serosa. The villi of various shapes and size were lined by simple columnar epithelium having chief, goblet and endocrine cells. The goblet cells were increased from duodenum to ileum whereas endocrine cells decreased from duodenum to jejunum and increased in ileum. At the bases of the villi, intestinal glands were present. Histochemically, the goblet cells of surface epithelium and glands showed different affinities for acidic mucopolysaccharides, mucins, glycogen, weakly sulfated acidic mucopolysaccharides, hyaluronic acid and sialomucins however, the neutral mucopolysaccharides were present in negligible amount. Lamina muscularis mucosae and submucosa were not well-developed. Tunica muscularis was consisted of an inner circular and an outer longitudinal layer of smooth muscle fibres. Tunica serosa was having loose irregular connective tissue, fine blood capillaries and isolated nerve bundles followed by flat mesothelial cell layer. Generally the constituents of different layers of small intestine presented developmental changes with the advancement of age.

Key words: Small intestine, endocrine cell, chief cell, lymphoid tissue, broiler chicken

India is currently producing 3.725 million tons of chicken meat and 70 billion of hen eggs/year (Chatterjee, 2016). The contribution of poultry sector to India's GDP is approximately 1%. 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 (Uni *et al.*, 1999). The entire length of intestinal mucosa studded with villi of variable shapes and size were lined by simple tall columnar cells with goblet and enterochromaffin cells (Nasrin *et al*., 2012; Kachave *et al.,* 2009; Rana *et al.,* 2015). The duodenum had the greatest absorptive surface along the intestine as indicated by the higher and denser villi as compared with the jejunum and ileum (Baranylova and Holman, 1976). The age related developmental changes were lacking in birds hence, the present study has been planned in the broiler chickens.

MATERIALS AND METHODS

The present study was conducted on 36 broiler chickens of 07-42 days of age. These birds were divided into 6 groups having 6 birds in each group. The tissues collected from different parts of duodenum, jejunum and ileum along with their respective junctions were fixed in 10% neutral buffered formalin solution for 48 hours. The tissues were processed for routine paraffin technique and sections of 5-6 µ were cut and stained with routine Harri's haematoxylin and eosin stain, McManus' method, PAS-Alcian blue, Alcian blue, colloidal iron, Mayer's mucicarmine, Weigert's method, Gomori's method (Luna, 1968), Fontana method (Humason, 1972) and Crossman's trichrome stain (Crossman, 1937) to demonstrate the different constituents of the tissues.

RESULTS AND DISCUSSION

The duodeno-gizzard junction was characterized by the presence of the duodenal villi and koilin layer (Figs. 1, 2) as reported in fowl (Hodges, 1974). The duodenal villi were of varying shapes and size, some of the villi were exceptionally large, conical shaped with broader bases (Figs. 3, 4) as described in fowl (Nasrin *et al.,* 2012; Kadhim *et al.,* 2014). The villi of duodeno-jejunal junction were almost similar to those of the duodenum. In jejunum, most of the villi were cylindrical with uniform width and blunt tips. However, a wave like pattern has been observed in broilers (Yamauchi and Isshiki, 1991). Jejuno-ileum junction was characterized by a drastic reduction in the size of the villi as reported in chicken (Kachave *et al*., 2009) and Uttara fowl (Rana *et al.*, 2015). The ileum showed the presence of the villi which were comparatively smaller than the previous segment as reported in chicken (Kachave *et al.*, 2009). However, it was tongue-like in broilers (Yamauchi and Isshiki, 1991). The height of the villi decreased from duodenum to ileum as reported in Uttara fowl (Rana *et al.*, 2015). The height of the villi increased with the advancement of age as compared to previous age group for the same segment. The epithelium of the villi was simple columnar having three types of cells i.e. chief, goblet and argentaffin cells as described in fowl

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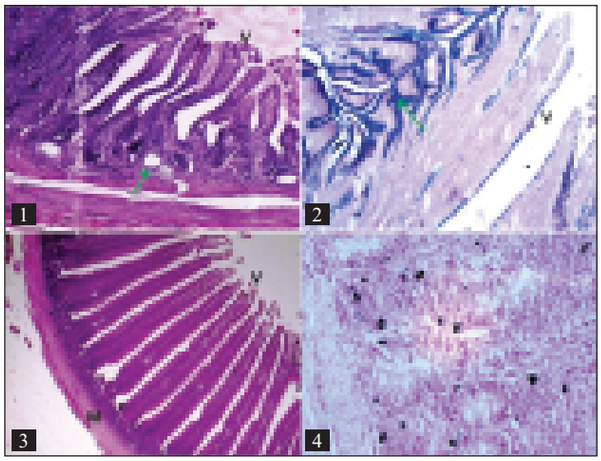

Fig. 1-4. Photomicrograph of duodenal-gizzard junction of 21-days old chicken showing the villi (V) and presence of Brunner's glands (↑). H. & E. x 100 2. Photomicrograph of duodenal-gizzard junction of 42-days old chicken showing presence of acidic mucopolysaccharides (blue colour) in the koilin layer (↑) and in the goblet cells of duodenum villi (V). PAS-AB x 100 3. Photomicrograph of duodenum of 14-days old chicken showing presence of duodenal villi (V) and tunica muscularis (M). H. & E. x 100 4. Photomicrograph of duodenum of 35-days old chicken showing presence of endocrine cells (black color) in the intestinal gland and lamina propria mucosae. Fontana method x 400

(Hodges, 1974 and Kachave, 2009). The chief or columnar cells had oval to elongated nuclei showing condensation of fine chromatin material irregularly present throughout the nucleoplasm. The argentaffin cells were more in the epithelial villi of duodenum than jejunum and ileum as reported in ostrich (Bezuidenhout and Aswegen, 1990). The number of argentaffin cells was increased with the advancement of age. The goblet cells were interspersed in between the epithelium cells and their number was more in ileum than the duodenum and jejunum as described in African pied crow (Igwebuike and Eze, 2010; Godwin *et al.,* 2016). These cells increased with the advancement of age. The nuclei of goblet cells were also narrow elongated in shape and pushed towards the basal portion. The goblet cells showed strong activity for acidic mucopolysaccharides by PAS-AB (Figs. 2, 6). However, the neutral mucopolysaccharides were very less, as observed in broiler (Ozaydin and Celik, 2012). These cells also showed strong affinity for Alcian blue method indicating the presence of weakly sulfated mucosubstances, sialomucins and hyaluronic acid whereas colloidal iron method demonstrated the presence of acidic mucopolysaccharides. A moderate to strong activity for Mayer's mucicarmine stain and McManus' PAS demonstrated the presence of mucin and glycogen, respectively. These activities increased with higher age group. The Paneth cells were not observed as reported in fowl (Hodges, 1974) and ostrich (Bezuidenhout and Aswegen, 1990).

Lamina propria mucosae were having loose irregular connective tissue. A few connective tissue cells in the core of the villi were observed. The intestinal glands (Figs. 5, 6) were oval to elongated in shape and were continued at the pits or the crypts of the villi, which were similar to those of fowl (Hodges, 1974 and Kachave *et al.*, 2009). Number and size of glandular units increased with age indicating that secretory activities increased with age to combat the increased rate of digestion. It also contained some diffuse lymphoid tissue and solitary lymphatic nodules which increased along with the age in the core of the villi. The intestinal glands were also having simple columnar type of epithelium along with all the three types of cells as present in the surface epithelium as observed in fowl (Kachave *et al.*, 2009). Only isolated collagen was present and the reticular fibres formed the basement membrane and surrounded the periphery of intestinal glands. No lacteals were found in the core of the villi as reported in fowl (Kachave *et al.*, 2009). The argentaffin cells were more in number in intestinal glands (Fig. 4) as compared to villus epithelium and were more in duodenal than jejunum and ileum but ileum had more argentaffin cells in comparison to jejunum. However, it decreased from duodenum to ileum in fowl (Hodges, 1974). A few argentaffin cells were present in the connective tissue of lamina propria. The

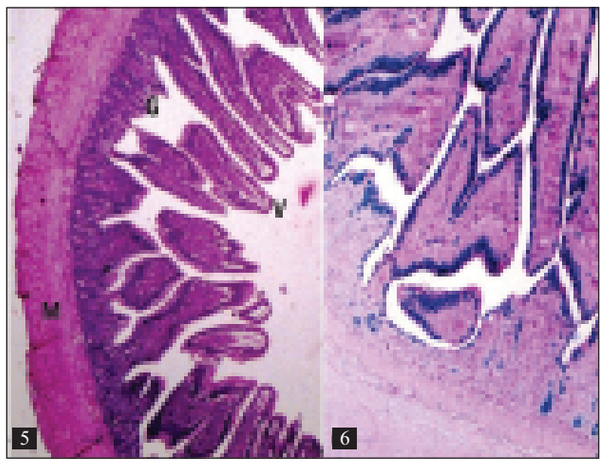

Fig. 5-6. Photomicrograph of ileum of 35-days old chicken showing presence of villi (V), intestinal glands (G) and tunica muscularis (M). H. & E. x 100 6. Photomicrograph of ileum of 42-days old chicken showing presence of strong reaction of acidic mucopolysaccharides in the goblet cells of villi and intestinal glands. PAS-AB x 100

goblet cells showed the presence of different mucopolysaccharides with more intensity than those of epithelial cells (Figs. 5, 6). Intestinal glands and connective tissue cells generally increased along with the higher age group in birds.

Lamina muscularis mucosae was very thin and having longitudinal oriented layer of smooth muscles however it was well developed in *Elanus caeruleu* (Hamdi *et al.,* 2013) and striated scope owls (Al-Saffar and Al-Samawy 2016). But, it was not reported in the fowl (Kachave, 2009) and African pied crow (Igwebuike and Eze, 2010). Thickness increased with higher age group birds.

Tunica submucosa was very thin, having few small sized blood capillaries and loose irregular connective tissue which was in agreement with study in fowl (Hodges, 1974), striated scope owls (Al-Saffar and Al-Samawy, 2016) and African pied crow (Igwebuike and Eze, 2010). Submucosal glands or Brunner's glands were observed only at the duodeno-gizzard junction of 21 days old birds.

Tunica muscularis was thicker. Its smooth muscles were arranged as an inner circular and an outer longitudinal layer which was similar to the earlier observations in fowl (Hodges; 1974). However, it was reported three layered in fowl (Kachave *et al.*, 2009). Thickness of this layer increased with the advancement of age. In between these two muscle layers, small nerve bundles representing myenteric plexus was also observed. Tunica serosa was thicker and also having loose irregular connective tissue, blood vessels of varying shapes and size, nerve fibers, fatty tissue and flat mesothelial cell layer. Thickness of serosa increased with higher age group birds.

REFERENCES

Al-Saffar, F.J. and Al-Samawy, E.R.M. (2016). Histomorphological and histochemical study of the small intestine of the striated scope owls (*Otus scors brucei*). *Singapore J. Chem. Biol*. **5**: 1-10.

Baranylova, E. and Holman, J. (1976). Morphological changes in the intestinal wall in fed and fasted chickens in the first week after hatching. *Acta Veterinaria Brno*. **45**: 151-158.

Bezuidenhout, A.J. and Van Aswegen, G. (1990). A light microscopic and immunocytochemical study of the gastrointestinal tract of the ostrich (*Struthio camelus L.*). *Onderstepoort J. Vet. Res.* **57**: 37-48.

Chatterjee, R.N. (2016). Vision 2050. *Diretorate of Poultry Research*, ICAR, India.

Clarke, R. (1967). On the constancy of the number of villi in the duodenum of the postembryonic domestic fowl. *J. Embryol. Exp. Morph.* **17**: 131-138.

Crossman, G.A. (1937). A modification of Mallory's connective tissue stain with a discussion of principles involved. *Anat. Rec.* **68**: 33-38.

Godwin, O.C., Abiaezute, N.C. and Adigwe, A. (2016). Evaluation of the morphological adaptations of the small intestine of the African pied crow (*Corvus albus*). *J. Basic Appl. Zool.* **75**: 54-60.

Hamdi, H., El-Ghareeb, A.W., Zaher, M. and AbuAmod, F. (2013). Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: II-*Elanus caeruleus*. *Int. J. Sci. Eng. Res.* **4**: 1355-1364.

Hodges, R.D. (1974). *The Histology of the Fowl*. Academic Press. London.

Humason, G.L. (1972). *Animal Tissue Techniques.* (3rd edn.), W.B. Freeman and Co., San Francisco, U.S.A.

Igwebuike, U.M. and Eze, U.U. (2010). Morphological characteristics of the small intestine of the African pied crow (*Corvus albus*). *Anim. Res. Int.* **7**: 1121-1124.

Kachave, C.D., Bhosle, N.S., Mamde, C.S. and Lambate, S.B. (2009). Histological observations on small intestine in broiler and layer of poultry birds. *Royal Vet. J. India* **5**: 5-9.

Kadhim, K.K., Bakar, Z.A., Noordin, M.M., Babjee, M.A. and Saad, M.Z. (2014). Light and scanning electron microscopy of the small Intestine of young Malaysian village chicken and commercial broiler. *Pertanika J. Trop. Agric. Sci.* **37**: 51-64.

Luna, L.G. (1968). *Manual of Histologic Staining Methods of Armed Forces Institute of Pathology*. (3rd edn.), McGraw-Hill Book Co., New York.

Nasrin, M., Siddiqi, M.N.H., Masum, M.A. and Wares, M.A. (2012). Gross and histological studies of digestive tract of broilers during postnatal growth and development. *J. Bangladesh Agric. Univ.* **10**: 69-77.

Rana, J., Dhote, B.S., Mrigesh, M. and Ambwani, T.K. (2015). Histomorphometry of small Intestine in post-hatch Uttara fowl. *Indian J. Vet. Anat.* **27**: 1-3.

Ozaydin, T. and Celik, I. (2012). Histological, histochemical and immunohistolochemical investigation on the developing small intestines of broiler embryos. *J. Anim. Vet. Adv*. **16**: 2936-2944.

Uni, Z., Noy, Y. and Sklan, D. (1999). Posthatch development of small intestinal function in the poult. *Poult. Sci.* **78**: 215-222.

Yamauchi, K.E. and Isshiki, Y. (1991). Scanning electron microscopic observations on the intestinal villi in growing White Leghorn and broiler chickens from 1 to 30 days of age. *Br. Poult. Sci.* **32**: 67-78.