Indian Journal of Animal Sciences **85** (4): 379–381, April 2015/Short communication

Innervations pattern of the intestinal wall with reference to gut endocrine cells in post-hatch broiler chickens

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Received: 17 September 2014; Accepted: 5 November 2014

Key words: Broiler chicken, Endocrine cells, Gut wall, Innervations

The esophagus, crop, proventriculus, gizzard, part of small intestine, pancreas and gall bladder are innervated by the vagus nerve. The rest of the intestine is innervated by a unique nerve of Remak (Argenzio 2004, Mandal *et al.* 2013). Rawdon and Andrew (1999) gave an overview on gut endocrine cells in birds. A variety of gut hormones affect digestion, mainly by controlling motility of gut and associated structures, and by regulating acid production of stomach and bicarbonate secretion of pancreas (Sturkie and Whittow 2000). The present study was undertaken to record the systematic intrinsic innervation patterns of the gut wall in post-hatch broiler chickens.

The present investigation was carried out on broiler chickens. The day-old chicks (50) were reared up to day 43 in the experimental pen. Standard management practices were followed uniformly for all birds. Histological and fluorescence microscopic studies were conducted on different intestinal segments of broiler birds at different ages (on days 1, 8, 15, 22, 29, 36 and 43). After sacrifice, each bird was carefully dissected to collect the entire intact gastrointestinal tract (GIT).

Histological study: For histological study, small tubular pieces of different gut segments from duodenum up to cloaca were collected. After fixation in 10% neutral buffered formalin, the tissue pieces were routinely processed for obtaining 5 – 7 μm thick serial paraffin sections. These tissue sections were subjected to the following staining techniques: (a) Harris' haematoxylin and eosin (H & E) staining for general histomorphological study, (b) Bielschowsky method for nerve fiber demonstration, (c) Cresyl fast violet staining method for nerve plexuses and ganglia, (d) lead haematoxylin method for endocrine cells, (e) ferric ferricyanide reduction test, and (f) Singh's modification of the Masson-Hamperl argentaffin technique for argentaffin cells (Bancroft and Stevens 1996).

Fluorescence microscopic study: For the fluorescence microscopic study, paraffin sections and cryosections of the said gut segments were used. Cryosections were subjected to paraformaldehyde vapour fixation at 60°C for 3 h in a desiccator as per Bancroft and Stevens (1996). The sections were finally observed under microscope with fluorescence attachment using A and I filters.

A number of endocrine cells were localized, mostly in the crypts and few in the villi of different intestinal segments (Figs 1–3). Out of different gut endocrine cell types (morphological), pyramidal, piriform, oval and spherical cells showed weak to intense yellow fluorescence after formaldehyde treatment (Fig. 4). These cells were abundant in duodenum, jejunum and colo - rectum. These cells were more concentrated in the intestinal crypts than in the villi. The nerve fibers and terminals appeared black thread – like structures (Figs 5–7) by Bielschowsky method.

In duodenum, the nerve fibers entered the submucosa layer after innervating the muscular tunic. From submucosa, the neuronal components extended into the mucosal layer to surround the crypts. Even few nerve fibers reached the core of the villi. Innervations pattern in jejunum and ileum was almost similar. The nerve fibers were localized in tunica muscularis, submucosa and mucosal layer of both these gut segments. These fibers were also seen to be extended from submucosa to mucosa, where they surrounded the crypts like those observed in duodenum (Figs 5, 6).However, the neuronal elements were better localized in tunica muscularis and submucosa layer in caecum. Few nerve fibers were also identified in the mucosa layer. Colo - rectum showed wider distribution of neuronal elements than the previous gut segments. The nerve fibers were observed around the blood vascular wall in serosa layer, in muscular tunic specifically between inner and outer smooth muscle layer and in the submucosa including those seen along the outer surface of the blood vessels. Many nerve fibers were protruding from serosa layer into the tunica muscularis (Fig.7). In cloaca, the neuronal elements were most prominent in tunica muscularis and submucosa layer. The external tunic of the blood vessels in serosa or adventitia layer in cloaca also revealed the presence of nerve fibers like those of colo-rectum.

The nerve fibers entering the external tunic of the blood vessels in serosa or submucosa layer of the intestinal wall

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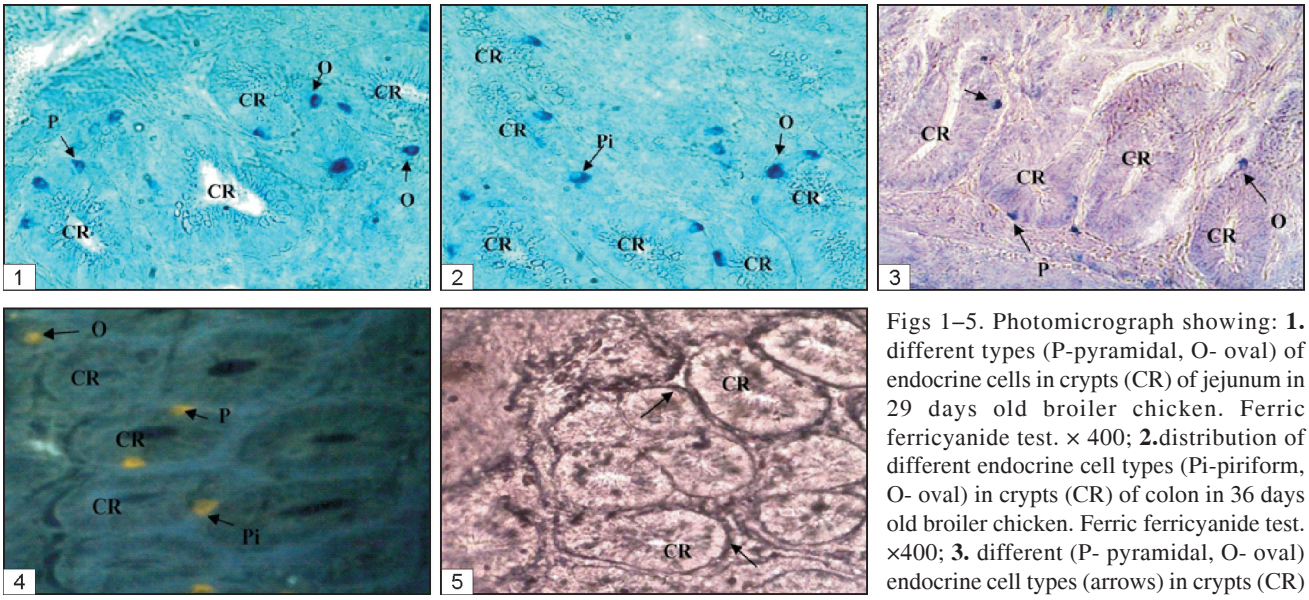

Figs 1–5. Photomicrograph showing: **1.** different types (P-pyramidal, O- oval) of endocrine cells in crypts (CR) of jejunum in 29 days old broiler chicken. Ferric ferricyanide test. × 400; **2.**distribution of different endocrine cell types (Pi-piriform, O- oval) in crypts (CR) of colon in 36 days old broiler chicken. Ferric ferricyanide test. ×400; **3.** different (P- pyramidal, O- oval) endocrine cell types (arrows) in crypts (CR) of jejunum in 8 days old broiler chicken. Lead haematoxylin stain. ×400; **4.** yellow fluorescence emitting cells (P- pyramidal, Pi-piriform, O- oval types) in the crypts(CR) of jejunum in 22 days old broiler chicken. FIF. × 400; **5.** nerve fibers (arrows) around the crypts (CR) of jejunum in 29 days old broiler chicken. Bielschowsky method. × 400.

were also identified distinctly (Fig.8).

Ganglia and plexuses: In all the intestinal segments, cresyl violet positive multipolar neuronal cell bodies with vesicular, spherical or oval, eccentric nuclei were identified in the myenteric plexuses (Fig.9) between the outer and inner smooth muscle layers as well as in the submucosal plexuses. The Nissl substance was marginally distributed in the cell body.

The elements of submucosal plexuses showed light green fluorescence when observed under fluorescent microscope in formaldehyde fixed tissues. They were seen in the form of fibers and beaded chains. Similar neuronal elements were evident in the myenteric plexuses as well as around the peripheral layer of the vascular wall (Fig.8).

The gut endocrine cells were found more concentrated in the intestinal crypts than in the villi in the broiler chickens. These findings compare well with those of Hiramatsu *et al.* (2005), Mendes *et al.* (2009) and Pirone *et al.* (2011) in different species of birds. Many of these cells were argentaffin in nature as earlier revealed by Singh's method. Simultaneous immunohistochemical staining using serotonin (5 – HT) antiserum by these workers confirmed that the yellow fluorescence emitting cells were serotonin secreting cells. Bioactive amines like 5 - HT react with paraformaldehyde or formaldehyde to form beta carboline (Bancroft and Stevens 1996). This compound produces the yellow fluorescence under fluorescence microscope. So, it is apparent that these fluorescent cells in our study were identical to the serotonin secreting cells, which is evident not only from formaldehyde induced fluorescence (FIF) technique, but also from the distribution of these cells in different gut segments as detailed in the available literature (Watanabe *et al*. 1987, Rawdon and Andrew 1994).

Presence of some fluorescent nerve fibers around the vascular wall is actually an indication of catecholamine fibers (Aisa *et al.* 1997). Observation of Ikeda *et al*. (1971), i.e. catecholamine containing nerve fibers characterized by the green and yellow fluorescence on the vascular wall, in the myenteric and submucosal plexuses in different gut segments, also confirms our finding.

The linking of myenteric plexus with submucosal plexus by nerve fibers in tunica muscularis layer of the gut wall seems to be related with the complicated reflex arch responsible for the intestinal motility (Ikeda *et al.* 1971).

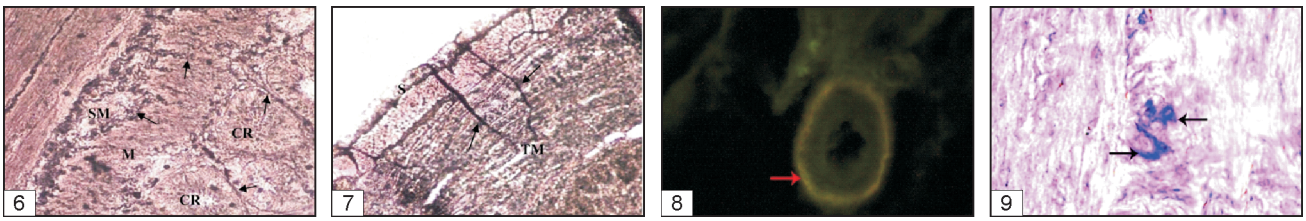

Fig.6–9. Photomicrograph showing: **6.** nerve fibers (arrows) in submucosa (SM), its extension into mucosa (M) and around the crypts (CR) of jejunum in 43 days old broiler chicken. Bielschowsky method. × 400; **7.** Photomicrograph showing nerve fibers (arrows) extending from serosa (S) to tunica muscularis (TM) of colon in 43 days old broiler chicken. Bielschowsky method. × 100; **8**. Micrograph showing yellowish green, catecholamine nerve fibers (arrow) around a vascular wall of cloaca in 29 days old broiler chicken. FIF. × 400; **9.** ganglionic neurons (arrow) in myenteric plexus of colon in 36 days old broiler chicken. Cresyl violet stain. × 400.

From the present study, it is evident that neuronal elements in the gut wall appeared to be more prominent in older birds which can be attributed to their more active participation in gut functions suited to the needs of their body growth. Extension of some nerve fibers from the submucosa to mucosa (up to crypts or villi) established an intimate association of nervous and endocrine system of the gut.

SUMMARY

The present study was conducted on post-hatch broiler chickens to elucidate the intrinsic innervation patterns of their intestinal wall. Histological and fluorescence microscopic studies were carried out on different segments of intestine of broiler birds at different ages. The innervations were more prominent in the colon and cloaca. Nerve fibers even extended from the submucosa layer into mucosa to terminate around the intestinal crypts and the villi. Few catecholamine containing nerve fibers were observed around the vascular wall in serosa as well as submucosa layer of the gut wall. The gut endocrine cells were mostly populated in the crypts as well as in the villi epithelium in different segments of intestine. This sort of association between neural elements and the enteroendocrine cells seems to reflect on their anatomical and physiological correlation, specifically with respect to gut hormone secretion.

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