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Comparative Studies of Mucosa and Immunoglobulin (Ig)-Containing Plasma Cells in the Gastrointestinal Tract of Broiler and Native Chickens of Bangladesh

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The aims of this study was to find out the histological differences of the mucosa and comparative analysis of Ig-containing plasma cells among the different segments of the gastrointestinal tract of broiler and native chickens of Bangladesh. The conventional histological study revealed that the lining epithelium of the proximal segments (esophagus, crop, and proventriculus) were thicker in the broiler. The esophageal glands were more in the broiler than the native chickens. The villi of the duodenum, jejunum and ileum were slender and longer in the broiler in comparison to the native chickens. The number of goblet cells in the duodenum, jejunum, and ileum was more in the native chickens than the broiler. The indirect immunohistochemistry revealed that very few immunoglobulin (Ig)-containing plasma cells were present in the epithelium and lamina propria of esophagus, crop, and proventriculus of the broiler and native chickens. The frequency of the population of these cells were abundantly located in the lamina propria, around the intestinal gland and in the core of the villi from duodenum to ileum of broiler and native chickens. The intraepithelial IgA-containing epithelium was observed only in the epithelium of the native chickens. Segmental variation of Ig-containing plasma cells was noticed in these two strains of chickens. The IgA-, IgG-, and IgM-containing plasma cells were significantly more in the most of the segments of the small intestine of the native chickens. This suggested that besides the existence of histological variation in the gastrointestinal tract of broiler and native chickens, the Ig-containing plasma cells were significantly more in the different segments of the digestive tract of native chickens possible due to their scavenging.

Key words: broiler, gastrointestinal tract, immunoglobulin cells, mucosa, native chickens

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Introduction

It is well known that the mucosal immune system play an important role in the defense against microorganisms because of the presence of migrating immunocompetent cells (ICs), such as globule leukocytes (GLs), lymphocytes, macrophages and plasma cells in this system (Husband and Gowans, 1978; McDonald and Spencer, 1994). When mucosa was expose to foreign antigens, the continuous epithelial layer and covering mucous layer function as the first line of defense. In addition to the nonspecific defense system, the mucosa-associated lymphoid tissues (MALT) supply lymphocytes and plasma cells that contribute to the local immune system (McDermott and Bienenstock, 1979; Befus *et al.,* 1980; Arai *et al.,* 1988). This mucosal immune system undergoes changes with aging (Khan *et al.,* 1996a); influenced by sex hormones (Milicevic and Milicevic, 1993; Khan *et al.,* 1996b), and antioxidant such as selenium and vitamin E (Khan *et al.,* 2008).

The digestive tract of chickens was the major site of antigenic challenge in the body, being continuously exposed to antigens and commensal bacteria (Mowat and Viney, 1997). To deal with these challenges the mucosa of the digestive system of chickens are populated by a significant proportion of T lymphocytes, B lymphocytes, plasma cells, macrophages, dendritic cells and non-professional antigen presenting cells (APCs) (McDonald and Spencer, 1994). These cells principally developed and mobilize from "Gut Associated Lymphoid Tissue (GALT)", which may include esophageal tonsils, Meckel's diverticulum, Peyer's patches, cecal tonsils, and bursa of Fabricius (Arai *et al.,* 1988). The plasma cells were the cells of the immune system that secrete large amounts of antibodies

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(McGhee *et al.,* 1997). They differentiate from B cells upon stimulation by an antigen and were the principal effector cells involved in humoral immunity. These immunoglobulin (Ig)-containing plasma cells are distributed in the lamina propria of the respiratory tract (Bienenstock *et al.,* 1993), digestive tract (Jeurissen *et al.,* 1989), genital tract (Khan *et al.,* 1997), lymphoid organs (Khan *et al.,* 1998), and Harderian gland (Khan *et al.,* 2007). In the chickens, particularly in the digestive tract, the Ig-containing plasma cells have been reported in the esophageal tonsil, proventriculus and Payer's patches (Arai *et al.,* 1988; Jeurissen *et al.,* 1989).

In Bangladesh, most of the farmers rear Kasilla broiler and native chickens (*Gallus domesticus*). Both of the chicken breeds attain a weight around 1000 gm at 1 month and 6 month of their ages, respectively. The broilers reared in well hygienic condition, in contrast, the native chickens are scavenger in nature (Rahaman *et al.,* 2003) fed by kitchen by-products, seeds and grains, garden left-over, insect, green grasses and all other human refusal that would otherwise go to waste. These two strains of chickens reared in two different conditions totally. Therefore, the present research work was designed to understand the histological variation of the mucosa, and the frequency of Ig-containing plasma cells in the gastrointestinal tract (esophagus to ileum) of broiler and native chickens of Bangladesh.

Materials and Methods

Chickens

A total of ten adult chickens, five from Kasilla broiler (30 days old) and five from native chickens (*Gallus domesticus*) (6 months old) irrespective of sex were purchased from a local market of Bangladesh Agricultural University. The broilers were reared by the commercial poultry farmers with commercial feed (Aftab poultry feed) and water *ad libitum* before selling these chickens in the market, in contrast, the native chickens were fed by kitchen by product, garden left over and green grasses.

Tissues used for the study

The birds were killed by cervical sub-luxation method. After exsanguinations different segment of gastrointestinal tract e.g., esophagus, proventriculus, crop, duodenum, jejunum, and ileum of both the breeds of chickens were used for study purpose which were free from pathological lesions. The cecal tonsil is the lymphoid organ, and the colorectum is the terminal part of the digestive tract which has less importance in the digestion of food, therefore we omitted these two parts of the digestive tract from the present study.

Preparation of samples for histological studies

For histological studies all the samples were cut into pieces and then fixed in the "Bouin's fluid" (Gridley, 1960), dehydrated in a series of ascending grades of alcohol, cleared in several changes of xylene, and infiltrated with different grades of melted paraffin in the oven. The tissues were then embedded in paraffin and finally the sections were cut at 6-μm thickness using sliding microtome (MIC 509, Euromex, Japan). The sections were then stained with Hematoxylin and Eosin staining method (Gridley, 1960).

Antibodies

The antibodies for detecting Igs-containing plasma cells used in this experiment were normal rabbit serum (Biosource, Camarillo, California, USA), goat anti-chicken IgA (Bethyl Lab, USA), goat anti-chicken IgG (Bethyl Lab, USA), goat anti-chicken IgM (Bethyl Lab, USA), and HRP-conjugated rabbit anti-goat IgG (Bethyl Lab, USA).

Immunohistochemical staining method

Indirect immunoperoxidase staining method was done for the study of the distributional pattern and frequency of the Ig-containing plasma cells in the different segments of digestive tract of Kasilla broilers and native chickens. The tissues were fixed in ice-cold PLP (Periodate-lysine-paraformaldehyde), dehydrated in a series of graded alcohol, cleared in xylene, and embedded in paraffin. Paraffin sections, 6-μm thickness, were immunostained by the indirect immunoperoxidase method as described earlier (Khan *et al.,* 1997, 2007).

Histoplanimetry

Only lamina propria and core of the villi of the different segments of gastrointestinal tract were selected for counting of Ig-containing plasma cells, because the frequencies of immune cells were more and homogenously distributed in these regions. The immunopositive cells in the different segments of gastrointestinal tract of broilers and native chickens were counted in 20 fields using ocular micrometer at a magnification of 40, where Ig-containing plasma cells were evenly and diffusely distributed and their relative frequency per 0.1 mm^2 was calculated according to Weibel (1969). The areas containing the nodular aggregates of lymphocytes and plasma cells were excluded from counting. The goblet cells also counted in the similar way in the mucosa of duodenum, jejunum, and ileum of broiler and native chickens.

Statistical Analysis

The number of Ig-containing plasma cells in the lamina propria and core of the villi, and goblets cells in the epithelium of gastrointestinal tract was counted and compared between broiler and native chickens and the data were evaluated by Student's *t*- test (Zar, 1996).

Results

Histological studies of the mucosa of gastrointestinal tract of broiler and native chickens

The mucosa of the digestive tract of chickens was consisted of lamina epithelia, lamina propria, and lamina muscularis. The lining epithelium of the esophagus, crop and proventriculus was lined by stratified squamous epithelium which was thickened in the broilers than the native chickens in the present study (Fig. 1a-b). The mucous type of glandular cells was located in the lamina propria of the esophagus of these chickens. These es-

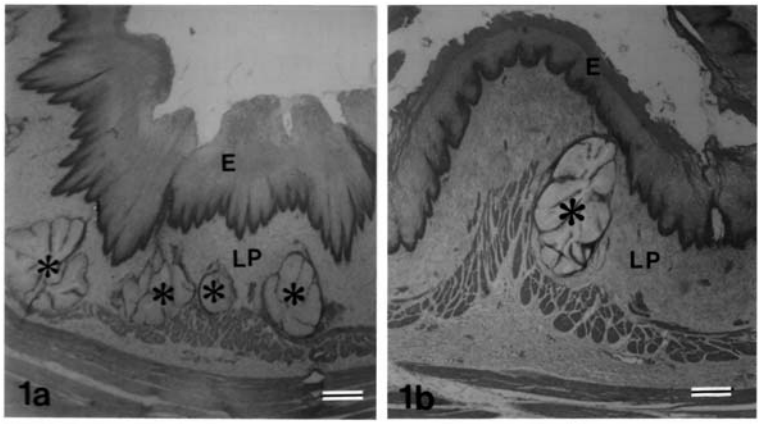

Fig. 1. Histological sections of the esophagus of broiler (1a) and native chickens (1b). The epithelium (E) of the broiler is thicker than the native chickens. The mucous glands (asterisks) located in the lamina propria (LP) of the esophagus is comparatively showing more in the broiler chickens. HE staining. Scale bars represent 180 μm.

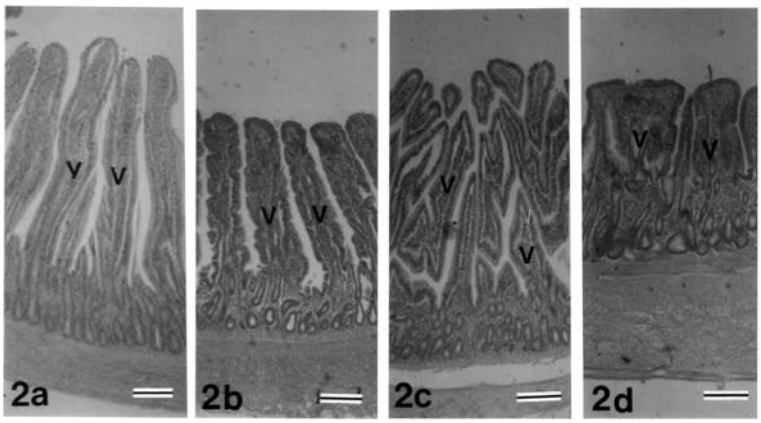

Fig. 2. Histological sections of the jejunum and ileum of broiler (2a, 2c) and native chickens (2b, 2d). The villi (v) of the jejunum of broiler (2a) are longer and the apex is blunt. In comparison, the villi (v) of the jejunum of native chickens (2b) are shorter in their length. The villi (v) of the ileum of broiler (2c) are longer and pointed at their apex, whereas, the villi (v) of the ileum of the native chickens (2d) are shorter and their apex is wider. HE staining. Scale bars represent 180 μm.

Table 1. The segmental comparison of the frequencies of goblet cells in the gastrointestinal tract of broiler and native chickens (Duodenum to ileum).

Chickens	Duodenum	Jejunum	Ileum
Broiler	56.25±12.21	155.25±11.27	1073±36.59
Native	88.75±6.84	300±17.19*	1554.75±116.36*

The present table is showing that the goblet cells are more in their frequencies in the different segments of gastrointestinal tract of native chickens than the broilers.

ophageal glands were more in the broiler than those of native chickens (Fig. 1a-b). In the present study there were 6–7 glandular units around an esophageal crypt of the broiler, and in contrast, around the crypt of the esophagus of native chicken there were 3–5 or less glandular units.

The distal part of the segments of the digestive tract of the chickens was lined by simple columnar epithelium. In broiler, the apical parts of the villi of the duodenum were slightly pointed, and the basal parts were wider. In contrast, the apical part of the villi was blunt and the base was also wider in the duodenum of the native chickens. The number of goblet cells was more in the duodenum of the native chickens (Table 1). The most common sites for the presence of plasma cells were the core of the villi and the lamina propria of the duodenum in both the strains of the chickens.

In the present study, the jejunal villi of the broiler were longer, its apical part was blunt (Fig. 2a). The villi of the jejunum of the native chickens, in contrast, were shorter than broiler (Fig. 2b). The number of goblet cells was more in the jejunum of native chickens than that of the broilers (Table 1). The common sites for the presence of plasma cells in the jejunum were the core of the villi and the lamina propria in these two strains of chickens.

The villi of the ileum were long and slender, and, the goblet cells were more in comparison to the other segments of the digestive tract. In the broiler, most of the villi of the ileum had pointed apical part and wide basal part (Fig. 2c). In contrast, the villi of the native chickens were shorter and its apex was wider (Fig. 2d). The presences of goblet cells were more in the ileum of the native chickens than those of the broilers (Table 1).

Distribution and frequency of immunoglobulin (Ig)-containing plasma cells in the mucosa of different segments of the digestive tract of broiler and native chickens

In the present study, the immunoglobulin (Ig)-containing plasma cells were not stained abundantly in the lamina propria of the esophagus, crop, and proventriculus of the broiler and native chickens; however, few cells of the stratum spinosum of broiler esophagus (Fig. 3a) and few cells of the lamina propria of the esophagus of native

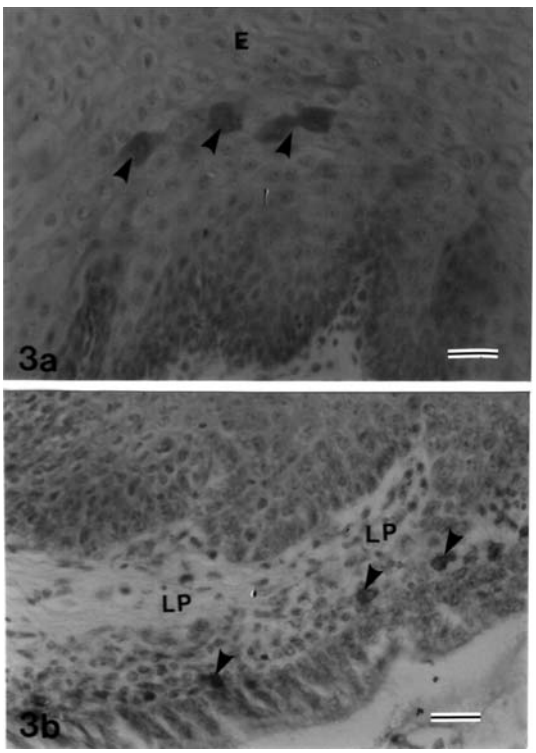

Fig. 3. Immunostained sections of the esophagus of broiler (3a) and native chickens (3b) showing anti IgA-positive cells (arrow heads) in the epithelium (E) of the broiler and in the lamina propria (LP) of the native chickens. Scale bars represent 7.5 μm.

chickens were stained for IgA-containing plasma cells (Fig. 3b).

The population of Ig-containing plasma cells was abundantly located in the lamina propria interglandular space and in the core of the villi of the different segments of intestine of the digestive tract of both the broiler and native chickens in the present study (Fig. 4–5), and their frequency of population were more from duodenum to ileum in both the strains of chickens (Fig, 6–10). In the proventriculus, however, very few Ig-containing plasma cells were located in both the strains of the chickens (Fig. 6–10). In the present study it was found that, IgA-containing plasma cells were more followed by IgM- and IgG-containing plasma cells from proventriculus to ileum in broiler; and in native chickens, the frequency of these cells were more from proventriculus to jejunum (Fig. 6–7). The Ig-containing plasma cells were also intraepithelial in the intestinal glands of the jejunum of native chickens in the present study (Fig. 5b). The glandular cells of this segment of the native chickens were reactive for only anti-IgA (Fig. 5b).

The frequencies of occurrences of Ig-containing plasma cells, in the present study, were varied among the segments of the digestive tract of the broiler and native chickens (Fig. 8–10). In the native chickens, the IgA- and IgM-positive cells were more in the proventriculus, duodenum, and ileum (Fig. 8 and 10); IgG-positive cells were more in the proventriculus and ileum (Fig. 9). In the broiler, however, the IgA- and IgM-positive cells were more in the jejunum (Fig. 8 and 10); and IgG-positive cells were more in the duodenum and jejunum (Fig. 9).

Discussion

The various system of chickens, such as, digestive, respiratory and reproductive system are exposed frequently too many microorganism both externally and internally. Among them the digestive system of broiler and native chickens are more frequently exposed to microorganisms because feed is one of the main source of microorganism in chickens. Moreover, the native chickens are scavenger animal, and in most of the small house hold farms the quality feed are not supplied to the broilers in Bangladesh. The mucosal lining barrier and the immunocompetent cells (plasma cells containing different classes of immunoglobulins) of the mucosa of digestive tract play a defensive role normally. Therefore, the present research focused on these two points only.

In the present study, the mucosal fold of esophagus, crop, and proventriculus was thicker in broiler in comparison to the native chickens, and esophageal glands were more in the broiler than the native chickens. The apical parts of the villi of the duodenum of broiler in the present study were slightly pointed and the basal part of the villi was wider. On the other hand, the base of the villi was wider and the apex of the villi was blunt in the duodenum of the native chickens. The villi of the jejunum and ileum of broiler were longer in comparison to the native chickens in the present study. Reports in this regard were not found in the available literature.

In the present study, the immunoglobulin (Ig)-containing plasma cells were not stained abundantly in the lamina propria of the esophagus and crop of the broiler and native chickens, however, scattered few Ig-containing plasma cells were stained in the epithelium and lamina propria these two segments of broiler and native chickens. The frequencies of the population of Ig-containing plasma cells were abundantly present from proventriculus to the ileum of broiler and native chickens. In the broiler and native chickens the IgA-containing plasma cells were more followed by IgM- and IgG-positive cells. This observation was similar with the statement of McDermott and Bienenstock (1979) in rat.

In the present study segmental variation in the presence of Ig-containing plasma cells was observed. The frequencies of occurrences of IgA-, IgG-, and IgM-containing plasma cells were significantly more from the duodenum to ileum in both the broilers and native chickens in the present study. In the lamina propria of the White leghorn strain A (WLA), Suzan *et al.* (1989) recognized some IgA-, and IgM-containing plasma cells in the different segments of intestines, and all three types of Igs-containing plasma cell in the proventriculus only. In contrast,

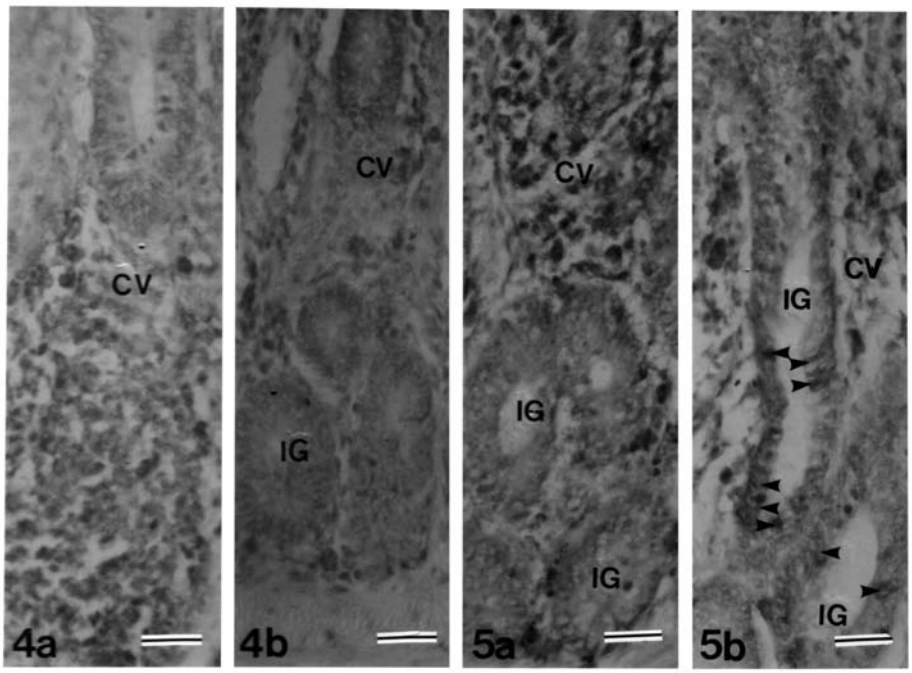

Fig. 4. Immunostained sections of the jejunum of broiler (4a) and native chickens (4b) showing IgG cells in the core of the villi (CV) and around the intestinal gland (IG) of the lamina propria. The IgG-positive cells are more in the jejunum of broiler than the native chickens. Scale bars represent 7.5 μm.

Fig. 5. Immunostained sections of the ileum of broiler (5a) and native chickens (5b) showing IgA cells in the core of the villi (CV) and around the intestinal gland (IG) of broiler and native chickens. In the native chickens the intraepithelial IgA cells (arrow heads) are stained. Scale bars represent 7.5 μm.

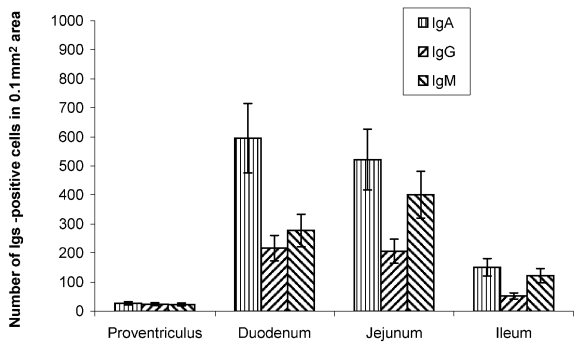

Fig. 6. Frequencies of Ig-containing plasma cells in the different segments of the gastrointestinal tract of broiler. The present graph is showing that the frequency of population of IgA is higher in all the segments of broiler followed by IgM and IgG cells. These Ig-containing plasma cells are more in the duodenum and jejunum followed by ileum and proventriculus. Values are given as the mean$\pm$SEM (n$=$4).

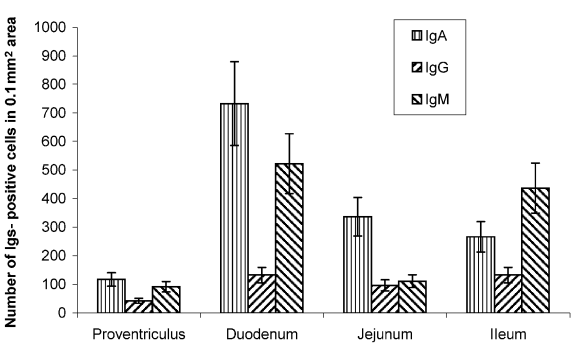

Fig. 7. Frequencies of Ig-containing plasma cells in the different segments of the gastrointestinal tract of native chickens. The present graph is showing that the frequency of IgA-positive cells are higher in the proventriculus, duodenum, and jejunum followed by IgM and IgG cells. The frequencies of occurrence of these Ig-positive cells are more in the duodenum and ileum followed by jejunum and proventriculus. Values are given as the mean$\pm$SEM (n$=$4).

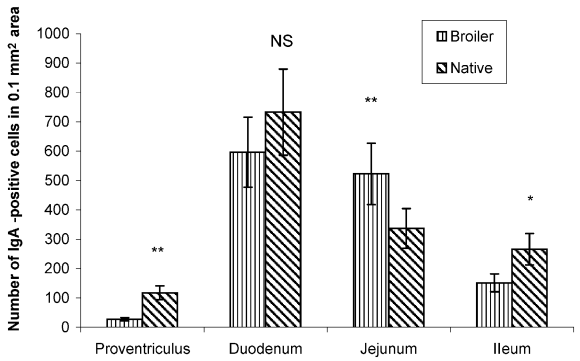

Fig. 8. Comparison of frequencies of IgA-containing plasma cells among different segments of the gastrointestinal tract in between broiler and native chickens. The present graph is showing that the frequency of IgA-positive cells is higher in the proventriculus, duodenum, and ileum of native chickens; in contrast, IgA-positive cells are more in the jejunum of the broiler. Values are given as the mean±SEM (n=4).

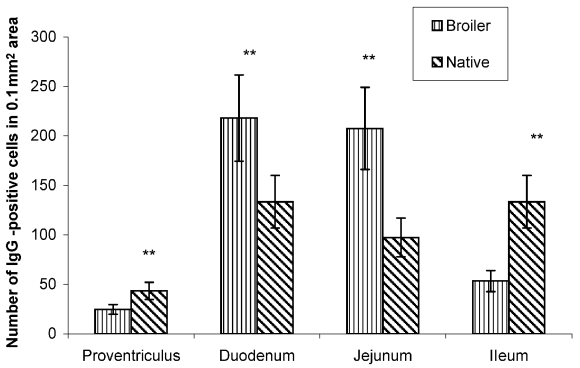

Fig. 9. Comparison of frequencies of IgG-containing plasma cells among different segments of the gastrointestinal tract in between broiler and native chickens. The present graph is showing that the frequency of IgG-positive cells is significantly more in the duodenum and jejunum of broiler, in contrast, in the IgG-positive cells are more in the proventriculus and ileum of native chickens. Values are given as the mean ±SEM (n=4).

the report of the present study was similar with the observation of Husband and Gowans (1978) in rat and Senda *et al.* (1988) in young and old mice. They found more IgA- and IgM-containing plasma cells in the duodenum followed by jejunum and ileum in rat and in the young and old mice. This similarity suggests that the frequency and distribution of Ig-cells are in similar fashion in chicken and mammals although they are different in phylum.

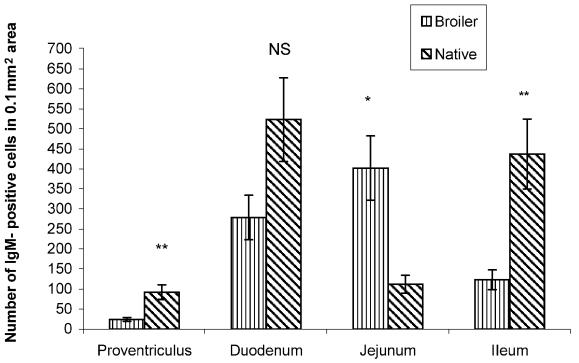

Fig. 10. Comparison of frequencies of IgM-containing plasma cells among different segments of the gastrointestinal tract in between broiler and native chickens. The present graph is showing that the frequency of IgM-positive cells is more in the proventriculus, duodenum, and ileum of native chickens; in contrast, IgM-positive cells are more in the jejunum of the broiler. Values are given as the mean±SEM (n=4).

Acknowledgments

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