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Gross morphology, histomorphology and histomorphometry of the jejunum in the adult river buffalo

Hasanzadeh, S.* **and Monazzah, S.**

Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

***Correspondence:** S. Hasanzadeh, Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. E-mail: s.hasanzadeh@mail.urmia.ac.ir

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Summary

For this study the jejuni of 50 adult (2-4 years), apparently healthy Iranian river buffaloes were collected from the abattoir for gross morphology, histomorphology, and histomorphometry. Our statistical analysis revealed that, there are significant ($P<0.001$) differences in the lengths, external diameters, internal diameters, external circumferences, internal circumferences as well as wall thicknesses of jejunum between all of the animals under study. Our macroscopic investigations also revealed that, in these buffaloes, jejunal Peyer's patches (jejpp) are not grossly visible in the mucosa of this part of gut. The histomorphology of submucosa revealed a pear shaped jejpp through the whole length of the jejuni at mesenteric as well as antimesenteric parts. The distributions of the jejpp in the middle and posterior parts of guts were greater than in the anterior part. The mean thickness of the mucosal glandular region was highly significant ($P<0.001$) between the anterior, middle, and posterior regions. There were no significant differences ($P>0.05$) in lengths as well as thicknesses of jejunal villi between the 3 different regions. The mast cells distributions were highly significant ($P<0.001$) between the superficial and deep regions of jejunal tunicae mucosae in all buffaloes. There were also highly significant ($P<0.001$) differences in the distribution of goblet cells between the superficial and deep regions of jejunal mucosa, and their population was more in the superficial than in the deep region.

Key words: Buffalo, Jejunum, Morphometry, Histomorphometry

Introduction

Domestic buffaloes (*Bubalus bubalis*) have been broadly classified into the swamp and river types. The river buffalo (2n = 50 chromosomes) is the dairy animal in countries extending from India and Pakistan to the Mediterranean countries and Egypt (Jainudeen and Hafez, 2000; Hasanzadeh and Orojee, 2003). Iran is one of the places in the world in which the buffaloes are reared and this animal plays an important role in the food industry and the economy (Hasanzadeh and Orojee, 2003). Iranian buffaloes are mostly distributed in the southwest (Khouzestan), and northwest (Azerbaijan and Gilan) provinces of the country which belong to the river type. Their diploid model number of chromosomes is 2n = 50 (Khavary, 1978).

GALT in the large intestine has been characterized in normal cattle (Liebler *et al*., 1988), sheep (Pabst and Reynolds, 1987), pigs (Biswal and Morrill, 1953; Inoue and Sugi, 1978), dogs (Atkins and Schofied, 1972), humans (Watanabe *et al*., 1983), rats (Bland and Britton, 1984), and river buffaloes (Alboughobish, 2005). The aggregates of lymphatic nodules occurring in the small intestine, seen grossly as elevations in the mucosa, are called Peyer's patches (PP). These patches are most conspicuous in the ileum, appearing in ruminants (Panchal *et al*., 1998), including river buffaloes (Hasanzadeh and Orojee, 2003). About 100-200 clusters, filled with closely packed lymphocytes, can be found throughout the length of the antimesenteric wall of the mouse small intestine (Hiromasa *et al*., 2002). The histogenesis of Peyer's patches initiate at 159 days and are well-organized into oval shaped patches in the

tunica mucosa of ileum at 198 days after gestation in buffalo feti (Roy *et al*., 2007). As the age increases, the number and size of the follicles of Peyer's patches decreases in buffaloes, and the regressions are more significant in 3–4-year-old buffaloes, and at this age only a few dispersed follicles remain (Alboughobish and Porkar, 2000).

Zabobonin (1990) reported that, in human newborns, 21% of Peyer's patches parenchyma is made up of lymph follicles, but at 1-3 years of age it increased to 50% and then decreased to 8.3% in senile persons. Thus in humans, at 1-3 years of age the highest amount of lymph follicles were seen in IPP.

The eosinophils were reported in the mucosal and submucosal layers of ileum of domestic fowl (Christopher and Rodney, 1996), domestic mammals (Dellmann and Johan, 1998) and humans (Lesson *et al*., 1988). Kawanishi and Kiely (1989) reported that in mice, with increase in age, the number of lymphocytes (mainly T-cells) decreased.

The different fields of veterinary and animal sciences are dependent on the anatomy and histology of body organs (Argenzio, 1980), and this basic knowledge is limited in river buffalo body organs. Jejunum is the second part of the small intestine, although belonging to the digestive system, due to its lymphatic tissue aggregation, in the form of gut associated lymphoid tissue (GALT) and mucosal associated lymphoid tissue (MALT), has outstanding immunologic importance (Ian, 1982; Ivan *et al*., 1998). Although, Barnwal and Yadava (1975), Lalitha (1990), and Neelam *et al*. (2000) studied histomorphological structure and age correlated changes in small intestine of Indian buffalo, the comprehensive gross morphometry, histomorphology, and histomorphometry of the jejunum in Iranian river buffalo have not been studied. The present study has been done for the promotion and advancement of knowledge in this field.

Materials and Methods

The jejuni of 50 adult (2-4 years of age), apparently healthy river buffaloes were collected for gross morphologic, histologic and histomorphometric studies. Immediately after slaughter of the animals, the jejuni from the beginning at the sigmoid flexure i.e., end of duodenum till the beginning of the ileo-cecal fold i.e., end of jejunum were taken out. For microscopic studies the specimens were fixed in 10% neutral formalin solution. For sufficient fixation of all parts of each specimen, the two ends were tied and fixative solution injected to the lumen and then the whole of it kept in 10% formalin solution. After completion of tissue fixation, each specimen was divided into three equal parts. Then, each part was again subdivided into ten pieces. From each of them, 5 pieces with a dimension of 0.5 cm^2 taken for the purpose of tissue preparation, and totally 150 specimens from each jejunum were processed through routine paraffin embedding. Transverse sections were cut at 5-7 µm thickness, and stained with haematoxylin and eosin, periodic acid schiff (PAS), and toluidine blue, staining techniques for investigation of general fibro-cellular architecture, goblet cells, and collagen fibers and distribution of mast cells, respectively (Gretchen, 1979). For macromorphometric studies, the specimens were kept on ice and transported to the laboratory.

The gross morphology and morphometry

This study was carried out on fresh and unfixed specimens. For morphometry, ordinary measuring tape, and Vernier's caliper were used. The mean length, outer circumference, inner circumference, external diameter, internal diameter, and thickness of intestinal wall were found in three, i.e. anterior, middle, and posterior regions. For the precise macroscopic observation of the luminal (mucosal) surface of jejuni, they were cut open longitudinally and after washing and spreading on the table, observed for the probable presence of the macroscopic jejunal Peyer's patches (jejpp).

Micromorphology (histology)

In this part the microscopic structures and characteristic features, such as mucosal, submucosal, muscular, serosal layers, Peyer's patches, mucosal villi, mucosal

folds, mucosal (Lieberkuhn) glands, various cell types, i.e., goblet cells, lymphocytes, mast cells, and fibers (elastic and collagen) of the jejunum were studied under light microscope.

The histomorphometry

In this section the measurements of all layers, including tunica serosa, tunica muscularis (external and internal), tunica submucosa, tunica mucosa, and lengths, as well as thicknesses of mucosal villi were carried out by using histomorphometric lens device in all the regions. The assessment of the glands and goblet cells distributions were carried out at 0.25 mm^2 surface units in all areas of the jejunum. The data were subjected to statistical analysis by using SPSS (Statistical Package Society of Science) computer soft ware. $P<0.05$ was considered significant.

Results

Gross morphometry

Statistical analysis revealed that there are significant ($P<0.001$) differences in the lengths, external diameters, internal diameters, external circumferences, internal circumferences as well as thicknesses of the jejunal wall between all of the animals under study. Table 1 represents the mean values of the gross morphometrical parameters.

Micromorphology

In the microscopic investigation, it was confirmed that the tunica mucosa of jejunum is well developed (Fig. 1) and the distributions of jejunal villi are uneven through the whole length of it. It was revealed that the lymph tissue present, both in nodular as well as diffuse forms in the tunica mucosa (Figs. 2 and 3).

The epithelium of jejunum was simple columnar. At the basal part of the epithelium low columnar cells (basal cells) were observed. In-between the columnar cells, goblet cells were also seen. The distribution of these cells was seen in the superficial as well as the deep regions of mucosa, including the mucosal glands (Fig. 4). The jejpp were seen at mesenteric as well as antimesentric regions of the jejunum (Fig. 2).

The mast cells were mostly located in the connective tissue of the interglandular region of tunica mucosa (Fig. 5A), tunica submucosa (Fig. 5B), where mostly distributed at the periphery and inside of the lymph nodules, in the *T. muscularis* (Fig. 5C) and *T. serosa* (Fig. 5D). In all the regions these cells were mostly seen around the blood vessels.

In the different parts of the mucosal layer, the lymphocytes are seen in diffuse form, i.e. at subepithelia, lamina propria, around the mucosal glands, but, in some areas of mucosa and more frequently in submucosa, the lymphatic tissue appeared as solitary nodules (Fig. 2). The jejpp in the cross section was almost pear shaped, but ellipsoid and elongated forms were also seen. Our observation revealed that the morphology of jejpp is quite different in different places of this part of the gut. In some specimens, the accumulation of diffused lymphatic tissue around the Liebercohn's glands was seen, and this was more frequently seen in the intestinal folds (Fig. 3).

Micromorphometry

This study was comprised of different parts of jejuni *viz* anterior (A), middle (M), posterior (P), and in each part mesenteric and antimesenteric regions were studied separately i.e., Am (anterior mesenteric), Aam (anterior antimesenteric), Mm (middle mesenteric), Mam (middle antimesenteric), Pm (posterior mesenteric), and Pam (posterior antimesentric). In these 6 regions comparative statistical analyses were carried out and the results are as follows:

There were no significant differences ($P>0.05$) in the thicknesses of the tunica serosae of the jejuni between the anterior (part 1) and the middle (part 2) regions, but this parameter was highly significant ($P<0.001$) between the anterior (part 1) and posterior (part 3), as well as the middle (part 2) and posterior (part 3) regions in all buffaloes (Table 2).

There were significant differences ($P<0.001$) between the mean thicknesses of the external muscular layer of part 1 and part 3, but this was not significant ($P>0.05$) between parts 2 and 3 or parts 1 and 2 (Table 2).

There were significant differences (P<0.05) in the internal muscular layer between parts 1 and 2, as well as 1 and 3, but this difference was not significant (P>0.05) between parts 2 and 3 (Table 2).

There were no significant differences (P>0.05) in the thicknesses of tunica submucosa between parts 1 and 2 and between parts 1 and 3, but this parameter was significant (P<0.05) between parts 2 and 3 (Table 2).

The mean thickness of the mucosal glandular region was highly significant (P<0.001) between all 3 parts (Table 2).

There were no differences (P>0.05) in the lengths of villi between the 3 different parts (Table 3).

There were no differences (P>0.05) in the thicknesses of jejunal villi between the 3 different parts (Table 2).

The mean distribution of goblet cells at 0.25 mm^2 of surface area; the statistical analyses revealed that, the differences of mean distribution of goblet cells in between superficial and deep regions of mucosa are highly significant (P<0.001) in all buffaloes and in all cases the distribution of these cells was greater in the deep than the superficial regions (Table 3).

The distribution of mast cells at 0.25 mm^2 of surface area:

The distribution of mast cells was higher in tunica submucosa than in the tunica mucosa. Statistical analyses revealed that, in all the buffaloes, significant differences

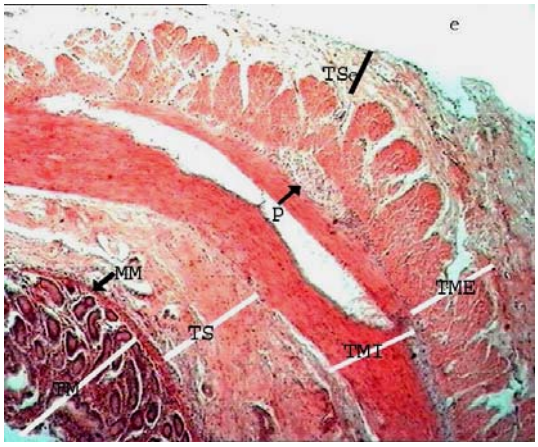

Fig. 1: In this figure different layers including tunica mucosa (TM), muscularis mucosa (MM), tunica submucosa (TS), internal muscular layer (TMI), myentric nerve plexus (P), external muscular layer (TME), and tunica serosa (TSe) are clearly seen, (H&E, ×300)

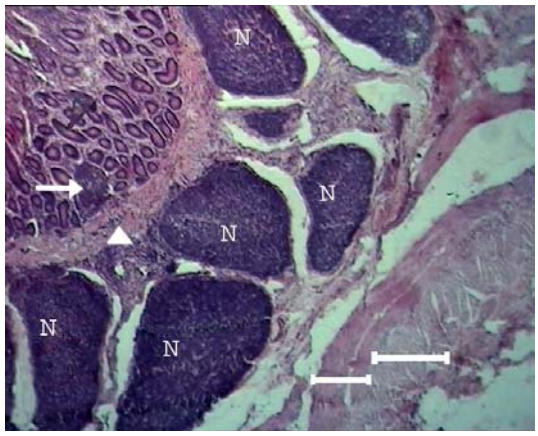

Fig. 2: In this figure the mucosal, submucosal and muscularis layers of jejunum are seen. A lymph nodule is situated between mucosal glands (⟶). Muscularis mucosa (▲). Numerous lymph nodules are seen in submucosa (N). Internal and external layers of tunica muscularis (⟝), (H&E, ×300)

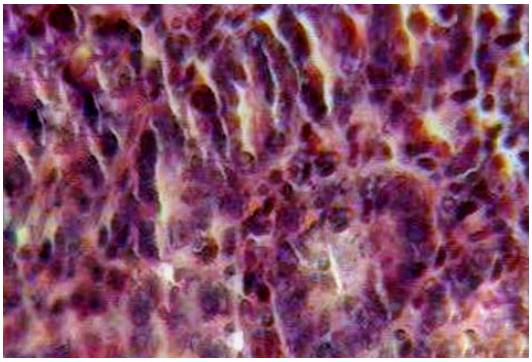

Fig. 3: Cross section from mucosal glands. Around the glands numerous lymphocytes and granulocytes (with red granules) are seen, (H&E, ×300)

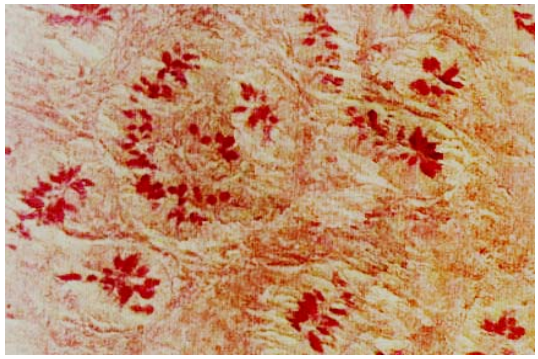

Fig. 4: Cross section from mucosal glands which shows goblet cells as deep red and collagen and reticular fibers are light pink, (PAS, ×250)

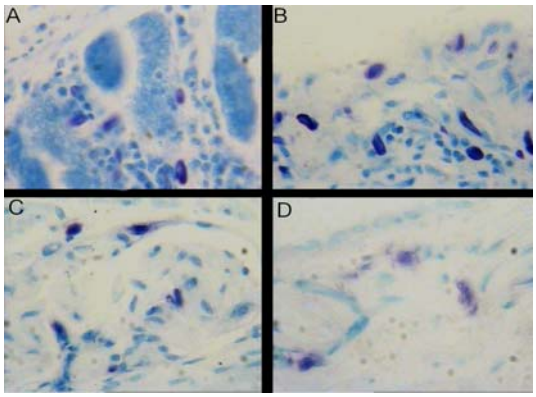

Fig. 5: Mucosal region (A), submucosal region (B), connective tissue in muscular layer (C) and tunica serosa (D) of the jejunum. The mast cells are purple in colour, (toluidine blue, ×400)

(P<0.001) exist in the distribution of mast cells between the superficial and deep regions of tunica mucosa, and their distribution was higher in the superficial than in the deep region (Table 3).

Discussion

Our study revealed that, the mean diameter of jejunum in this animal is 3.80 ± 1.90 cm. The diameter of jejunum in cow, dog, horse, and human is 3-5.5, 5.2, 3.5-4.2 and 2.5-3 cm, respectively (Barone, 1984). According to Getty (1975), the diameter of jejunum and ileum in horse is 6-7 cm. The diameter of the ileum in the river buffalo is 2.69 ± 0.07 cm (Hasanzadeh and Orojee, 2003). According to Nickel *et al*. (1979) the length of ileum in the horse is 70-80 cm. Our study revealed that, the mean length of the jejunum in river buffalo is 18.43 ± 0.79 m. Therefore, the lengths as well as diameters of the small intestines (here jejunum), varies between different animal species. In the ileum the solitary lymphocytes aggregation are referred to as ileal Peyer's patches (ilPP), and in the jejunum as jejunal patches (jejPP). Our study revealed that, the jejPP are randomly distributed through whole length of this part of the gut, at mesenteric as well as antimesentric regions. So it seems that their distribution is not anticipated by any discipline or principal. Hasanzadeh and Orojee (2003) reported that the ilPP are not seen grossly in the inner surface of the ileum of adult river buffaloes. In this study, the jejPP are also not seen grossly in the jejunum of adult river buffalo. Whereas Getty (1975) reported that, in the adult cow, the ileal Peyer's patches are large enough to be seen grossly at the mucosal surface of ileum, their number being 18-40, with a length and thickness of 12-20 mm, and 8-12 mm, respectively. The ileal Peyer's patches of man consist of 10-200 nodules and are grossly seen. At the antimesentric side of the

Table 3: Comparative representations of mast cells and goblet cells distribution (at 0.25 mm^2) tunica mucosa of jejunum (Mean ± SE)

Mast cells	Goblet cells	Jejunal region
125±4.29***	163.93±6.09	Superficial parts of mucosa
108.95±3.91	263.08±9.67***	Deep parts of mucosa

***Indicating differences of values in column are highly significant (P<0.001)

Table 1: Morphometric values of the jejunum in the river buffalo (Mean ± SE)

Length (meters)	External diameter (cm)	Internal diameter (cm)	External circumference (cm)	Internal circumference (cm)	Thickness of wall (cm)
18.43 ± 3.09	3.80 ± 1.90	3.68 ± 1.70	9.51 ± 1.45	8.45 ± 0.79	0.31 ± 0.39

Table 2: Comparative representations of different parameters at different regions of jejunum (Mean ± SE)

Parameters	Jejunal parts		
	Anterior (a)	Middle (b)	Posterior (c)
Tunica submucosa (µm)	266.04±13.32	248.49±16.66^{bc}	289.11±20.61^{bc}
Tunica muscularis interna (µm)	376.12±23.5$^{ab, ac}$	450.59±17.31^{ab}	459.62±21.7^{ac}
Tunica muscularis externa (µm)	323.21±22.97^{ac}	344.02±12.25^{bc}	377.62±16.6$^{ac, bc}$
Tunica serosa (µm)	541.62±37.59^{ac}	662.48±51.4^{bc}	838.48±49.79$^{ac, bc}$
Length of mucosal villi (µm)	863.58±7.73	885.05±33.26	902.95±22.8
Thickness of mucosal villi (µm)	73.46±4.55	75.44±4.28	75.97±4.61
Thickness of mucosal glandular layer (µm)	429.78±24.8$^{ab, ac}$	501.24±26.11$^{ab, bc}$	629.88±34.26$^{ac, bc}$

^{abc}Values with in row with same superscripts differ (P<0.05)

mucosal surface of the small intestine of man, 30 Peyer's patches are seen and most of them belong to the ileum (Junqueira *et al*., 1983), but according to this study, in the adult buffalo they are not seen grossly, whereas present microscopically at some areas of mucosa as well as the submucosa of ileum and jejunum.

One of the main aims of this study was to explore the histomorphometry as well as the macromorphometry of jejunal mucosa and this was taken into consideration in both macroscopic as well as microscopic studies. Our study revealed that, the mucosal folds are in the longitudinal as well as transverse forms in all the 3 parts that were undertaken to study. According to Lesson *et al*. (1988), mucosal folds in human small intestines start about 5 cm from the pyloric region in the duodenum and reach maximum distribution in the jejunum, vanishing at the distal end of the ileum. According to Hasanzadeh and Orojee (2003), the mucosal folds are present through the whole length of the ileum in the river buffalo, but its distribution reaches a maximum level at the middle, while in its distal portions appear in the longitudinal form only. According to the results of our study, in all parts of the jejunum the distribution of mucosal folds is almost uniform.

The results of macroscopic investigations revealed that, there are significant ($P<0.001$) differences in lengths of jejuni between buffaloes. Thus, in a given age group of buffaloes, the lengths of jejuni display considerable variations. These differences may be due to effective parameters on animal biology, such as nutritional, hygienic and managemental measures from one side and the age of the animals on the other side.

The outer circumference of jejunum was significantly ($P<0.001$) different between animals, and its range was 8.27 ± 0.27 cm to 9.43 ± 0.23 cm with a mean of 8.85 ± 1.7 cm. The highest length of jejunum was 20.14 ± 0.79 m, the lowest $16.72 \pm .079$ m, and the mean length was 18.43 ± 3.09 m. Hasanzadeh and Orojee (2003) reported that the mean length and mean outer circumference of ileum in river buffalo is 52.15 ± 1.83 cm and 2.69 ± 0.07 cm, respectively, and Nickel *et al*. (1979), reported that, in the adult horse, the length of the ileum is 70-80 cm. By comparison of the mean outer circumferences of the ileum and jejunum, it is evident that jejunum has a greater outer circumference than the ileum. The jejpp and ilpp are grossly seen on the surface of intestines in adult human and cow. According to the results of this study on jejunum, and previous studies on the ileum of adult water buffaloes, these structures are not grossly visible on the mucosal surface of the ileum as well as jejunum, but according to the report of Alboughobish *et al*. (2000), in buffalo calves they are large enough to be seen grossly.

In this investigation, due to the long length of the jejunum, we divided it into 3 regions viz; region 1 (anterior), region 2 (middle), and region 3 (posterior). In these regions different parameters were taken into consideration.

Statistical analyses revealed that there are significant ($P<0.05$) differences in the thicknesses of tunica serosae between all 3 regions of jejunum.

There were no differences ($P>0.05$) between regions 1 and 2 in the thickness of the external muscular layer, but there were significant ($P<0.05$) differences between regions 1 and 3, and 3 and 2.

These results reveal that, the external muscular layer of jejunum has no uniform thickness along its whole length. This may be due to extrinsic factors, such as nutrition, management and intrinsic factors such as genetic, hormonal status (growth hormone).

There were significant ($P<0.05$) differences between regions 1 and 2 in the thicknesses of the internal muscular layer, but this was highly significant between regions 1 and 3, whereas there were no differences between regions 2 and 3.

Interpretation of these results is quite difficult, but, as the results suggest, in the anterior and middle regions of the jejunum, due to their closeness to each other, quite good uniformity exists between them, but between regions which are far away from each other, differences in the thickness of muscular layers is more evident.

Our statistical analyses revealed that, there are no differences ($P>0.05$) between regions 1 and 2, and 1 and 3 in the thickness of tunica submucosa, but there were

significant ($P<0.05$) differences between regions 2 and 3 in the thickness of this layer. These results reveal that, in comparison to the tunica serosa, the tunica muscularis as well as tunica submucosa have more constancy.

Our results revealed that, between all 3 regions there is a highly significant difference ($P<0.001$) in the thickness of Leiberkuhn's glandular region. This result confirms the fact that the thickness of the glandular region of the tunica mucosa has no constancy and exerts differences in thickness in different areas. According to the physiologic role of this region of the mucosa, this difference seems quite ethical, because it reflects more likely the differences in secretary activity between different regions. Accordingly, wherever the secretion is required in high quantity, indeed, the thickness of the glandular region will be high and vice versa.

Our results revealed that, between the 3 regions of jejunum there are no differences in the lengths or thicknesses of villi. Thus, it seems that in different regions of jejunum the villi lengths as well as the thicknesses have considerable constancy. As their role indicates, the villi distribution should be constant through the whole length of the jejunum, because the main function of these structures is nutrient absorption and this function is achieved uniformly through the whole length of it.

The results of our study on the distribution of goblet cells (at 0.25 mm^2) of jejunal mucosa revealed that there are significant differences ($P<0.001$) within regions (at superficial as well as deep regions) of all buffaloes as well as between regions (between superficial and deep regions). The result of this study revealed that, there is fluctuation in the distribution of goblet cells in different regions and in all areas of study (anterior, middle, and posterior regions) the distributions of these cells were higher in deep than in the superficial regions.

The mean distribution of mast cells (at 0.25 mm^2) of different regions of the jejunal tissue was worked out in this study. The data analyses revealed that, in the jejuni of different buffaloes at deep regions of mucosa, significant differences ($P<0.001$) exist in the mean distribution of mast cells, and this was also true in the case of the superficial region. Our conclusion is that, the mean distribution of mast cells in between superficial and deep regions of the mucosa and between all buffaloes is significantly different. Because the mast cell function is mostly related to the immune system, and the release of different substances such as heparin and histamine and some other chemotactic substances, each of the animals, depending on their immunity and the microfloral load of their intestines may react differently. The alterations in distribution of these cells more likely are due to factors such as physiologic as well as immunologic states of the animals.

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