Gross and histomorphometric differences in the caecum of domesticated cattle and water buffalo

VINOD KUMAR SHUKLA[1], ASHWANI KUMAR[1]*, OPINDER SINGH[2], VANDANA SANGWAN[1], DEVENDRA PATHAK[2]

[1] Department of Veterinary Surgery and Radiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141001, Punjab, India
[2] Department of Veterinary Anatomy, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141001, Punjab, India

SUMMARY

Domesticated cattle and water buffaloes are major dairy animals and considered alike morphologically. Recent studies highlighted differences in the behaviour, vital clinical parameters, the topography of abdominal organs and clinical manifestations of some disease conditions in cattle and buffaloes. This study investigated species differences in the gross and histomorphometry of the caecum and ileocaecal mesentery in domestic cattle and water buffalo. The study was conducted on 8 bovine cadavers (4 crossbred cattle and 4 Murrah water buffaloes that were euthanized due to causes other than gastrointestinal disorders) to evaluate the species-specific gross and histomorphometric differences in the caecum at the apex, and body including ileocaecal mesentery. Histomorphometry was done using H&E, Picrosirius Red and Verhoeff's Elastic stain.
On gross examination, the mean length of the caecal apex (devoid of ileocaecal mesentery) and length of the ileocaecal mesentery were significantly less whereas the length and diameter of the caecal body were non-significantly less in buffaloes as compared to cattle. On histomorphometry, the thickness of the total caecal wall (at apex and body) along with its histological layers and the sub-epithelial connective tissue layer of the ileocaecal mesentery was also significantly less in buffaloes as compared to that in cattle. The collagen fibres were significantly less, quantitatively and qualitatively, in the caecal body and ileocaecal mesentery of buffaloes as compared to that in cattle.
In conclusions, the caecum of domestic water buffalo and cattle show species specific gross and histomorphometric differences, which might have implications concerning the pathophysiology of caecal disorders or their sequel including surgical exploration.

KEY WORDS

Bovine, caecum, gross anatomy, histology, large intestine, micrometry.

INTRODUCTION

The cattle and water buffaloes are major dairy animals that are considered alike as being belonging to the same subfamily (Bovidae). Besides, African buffalo, bison, yak and antelopes are also part of subfamily 'Bovidae'. Studies have highlighted differences in the behaviour, vital clinical parameters, the topography of the reticulum and omasum, relative predisposition to long bone fractures, clinical manifestations of traumatic reticuloperitonitis, gross morphometry of caecum and pericarditis in cattle and buffaloes[4-8].
The caecum is a blind tube which extends backwards and upwards approximately at the level of 4th lumbar vertebra in the right flank region and its blind end commonly protrudes from the supraomental recess. The free end extends into pelvic cavity when fully distended. Caecal dilatation/impaction is commonly encountered in domestic cattle and water buffaloes. The condition of caecal volvulus is reported in cattle due to the free end of cecum being devoid of mesentery in cattle[1]. Right flank caecotomy is recommended in a standing position to decompress the dilated caecum in cattle[1]. The cecum in domestic buffalo is mostly impacted along with the involvement of colon and decompression/evacuation through the right flank is difficult[2,] in clinical scenario due to the limitation in exteriorizing the cecum. This difficulty in exteriorizing the cecum in domestic buffalo in comparison to cattle is hypothesized to be due to the smaller apex of cecum (devoid of mesentery) and less elasticity of caecal tissue in domestic buffaloes in comparison to cattle. There is a paucity of literature on the gross and histomorphometry of caecum in the domestic water buffaloes and its comparison with the caecum of cattle.
Therefore, this study was aimed to investigate the gross anatomy and histomorphometry of the caecum at the apex, body and ileocaecal mesentery in the domestic cattle and water buffaloes.

MATERIALS AND METHODS

Animals: The study was carried out on 8 adult bovine cadavers [4 crossbred cattle (*Bos taurus* and *Bos indicus*) and 4 Murrah water buffaloes (*Bubalus bubalis*)] that were euthanized due to reason(s) unrelated to gastrointestinal ailments. The caecum along with the adjoining structures was identified, isolated and subjected to gross and microscopic study.

Corresponding Author:
Ashwani Kumar (drashwanikumar@rediffmail.com).

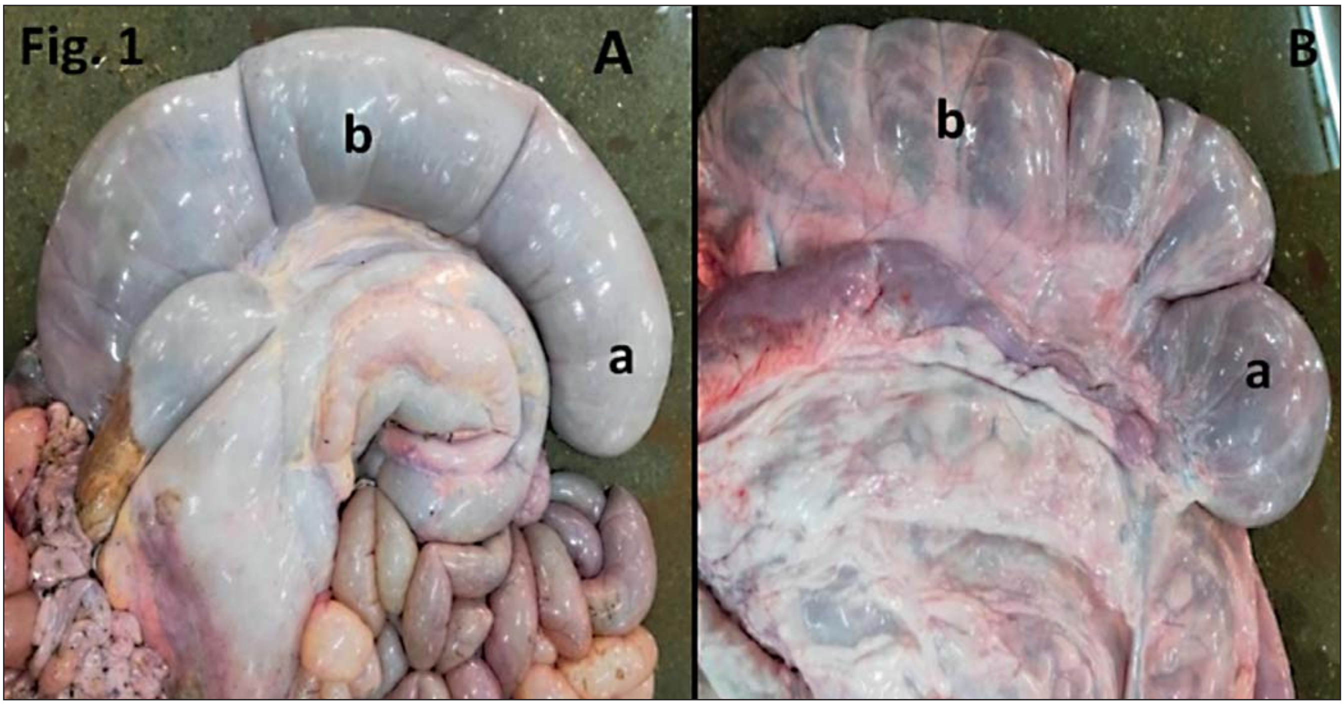

Figure 1 - Photograph showing morphometric examination of a gross specimens of caecum in cattle (**A**) and buffalo (**B**). Apex (**a**), body (**b**).

Gross morphometric study: On isolated specimens of the caecum and adjoining structures in cattle and buffaloes (Fig. 1A and 1B), various measurements (cm) such as the length of caecal apex (devoid of the mesentery), length of the ileocaecal mesentery (with the qualitative status of fat content based on gross appearance), length (tip of the apex upto the entrance of ileum) and mid body diameter of the caecum (in cm) were obtained using inch tape (Table 1).

Histomorphometric Study: The tissue samples collected from the caecal apex, body and ileocaecal mesentery were fixed in 10% neutral buffered formalin for 2 days. The samples were processed for paraffin block preparation by acetone benzene schedule and sections of 4-5μ thickness were obtained on glass slides using rotary microtome[9]. The cut sections were placed on the microscopic slides, de-paraffinized in xylene, rehydrated through descending grades of ethyl alcohol to running water. Sections were stained using Haematoxylin and Eosin (H&E) for histomorphometric study[10], Verhoeff's stain for elastic fibres[11] and Picrosirius Red for collagen fibres[12].

Microphotography and Histometry: Stained microscopic sections were examined and photographed using a light microscope (Nikon 80i) attached with a digital camera. Images were processed and measurements of the whole section from tunica mucosa to tunica serosa depicting total wall thickness and individually of each layer i.e. tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa were obtained using Fiji (Image J) software[13].

Staining for collagen fibres: Collagen fibres were stained with Picrosirius red (PSR) according to published research[14]. Briefly, sections were de-paraffinized and hydrated to water. Weigert's hematoxylin was applied for 7 minutes, and then washed in flowing tap water for 10 minutes before staining with 0.1% PSR in saturated picric acid solution for one hour. Stained slides were rinsed in acidified water and sections were dehydrated in alcohol, cleared in xylene and mounted with DPX.

Quantification of collagen by staining intensity method: Counting the PSR stained area of stained samples was done using Fiji (ImageJ) software[13] as described earlier[15]. A total of 10 representative photomicrographs (at 400 X magnification) were captured in from each tissue (one tissue/animal). "Image-type-RGB stack" was selected and the slider was placed on green colour channel, followed by selecting image, adjusting and thresholding. Then the setting of stack was adjusted by sliders until all the stained areas were selected. Thereafter, select "Analyse-Set Measurements" followed by "Area", "Area fraction", "Limit to threshold" and "Display label". Finally, stained and unstained areas were analysed by selecting "Analyze-Measure" to get the results in tabular form. The results were saved individually for each animal. The data was pooled to each category of the treatment and was analysed statistically.

Table 1 - Various parameters recorded during gross morphometry of caecum.

Parameter	Detailed description
Caecal apex (devoid of the mesentery)	From the point of caecal apex to the start point of the ileocaecal mesentery
Caecal body diameter	Width of caecum at the mid of body
Caecal body total length	From the point of caecal apex to the point of ileum entering into the caecum
Width of the ileocaecal mesentery	From point of attachment to the caecum to the point of attachment to the ileum

Qualitative scoring of Collagen and Elastic Fibers: The qualitative analysis/grading of collagen and elastic fibres was done based on the microscopic presentation of collagen/elastic fibres in the microscopic slides i.e. their amount (nil to abundant) and on their arrangement; (loosely arranged or densely packed) as described in Table 2.

Table 2 - Microscopic qualitative grading of collagen and elastic fibres.

Amount of Collagen Fibers	Qualitative Grade
Nil	-
Very few	+
A significant number (but loosely packed)	++
Abundant (but loosely packed)	+++
Densely packed	*

Statistical Analysis: The objective data on various gross and microscopic observations of the caecum and ileocaecal mesentery in cattle and buffaloes were processed for mean ± S.D. using Microsoft Excel and analysed for the level of significant differences concerning species and site (caecal apex and body) using t-test.

RESULTS

Gross Morphometric Examination: The gross morphometric observations of the healthy caecum and adjoining structures in cattle and buffaloes are depicted in Table 3 and Fig. 2.
The serosal surface of the caecum in cattle and buffaloes was smooth without any sacculations or bands. The mean length of caecal apex in buffaloes was 11.27 ± 1.32 cm, which was significantly (p=0.03) less than that in cattle (22.22 ± 5.63 cm)

Table 3 - Gross morphometric observations of the healthy caecum and adjoining structures in cattle and buffaloes.
[statistical difference between cattle and buffaloes at p<0.01 (**) or p<0.05 (*)]

S. No.	Parameters	Buffalo (Mean ± SD) (Range)	Cattle (Mean ± SD) (Range)
1	Age	4.88 ± 0.25 (4.5-5.0)	4.25 ± 1.19 (2.5-5.0)
2	Length of the caecal apex (cm)	11.27±1.32* (9.52-12.7)	22.22±5.63* (15.24-27.94)
3	Ileo-caecal mesentery length (cm)	4.57±1.21** (3.81-6.35)	12.7±2.92** (10.16-15.24)
4	Caecal body diameter (cm)	10.36±0.76 (9.65-11.43)	15.57±3.93 (10.16-19.2)
5	Total caecal body length (cm)	46.68±4.80 (41.91-50.8)	66.67±14.27 (48.26-76.2)
6	Presence of fat at the ileo-caecal mesentery		
	Absent	3/4=75%	0
	Fair	1/4=25%	0
	Good	0	3/4=75%
	Abundant	0	1/4=25%

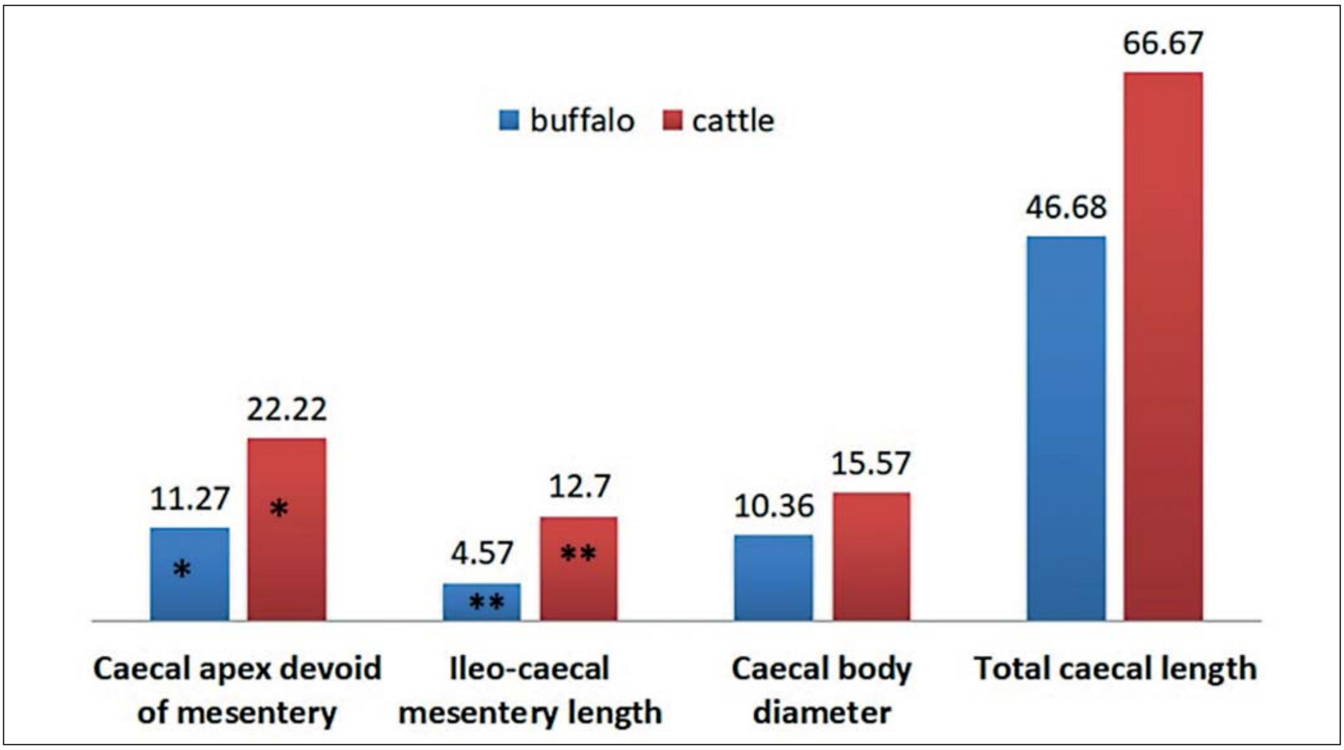

Figure 2 - Bar diagram showing mean ± SD comparative gross morphometric parameters (cm) of healthy caecum and adjoining structures in buffalo and cattle.

Figure 3 - Photograph showing measurement of length of caecal apex devoid of ileo-caecal mesentery in cattle (**A**) and buffalo (**B**).

(Fig. 3A and 3B). The mean length of the ileocaecal mesentery in buffaloes was 4.57 ± 1.21 cm, which was significantly (p=0.007) less than that in cattle (12.7 ± 2.92 cm). The mean length (p=0.075) and diameter (p=0.087) of the caecal body in buffaloes were 46.68 ± 4.80 and 10.36 ± 0.76 cm, respectively, were non-significantly less than that in the cattle (66.67 ± 14.27 and 15.57 ± 3.93 cm). The fat deposition was not observed in 3 out of 4 caecum specimens of buffaloes, whereas it was assessed as good in 3 out of 4 caecal specimens in cattle. The cattle and buffalo caecum were similar in topography extending from ventral end of last rib to right flank region, however there was limited mobility in buffalo caecum due to short ileocaecal mesentery.

Histomorphometric observations on caecal wall in Buffaloes

The caecum at the apex and body in buffaloes was comprised of the four tunics namely, Tunica Mucosa, Tunica Submucosa, Tunica Muscularis and Tunica Serosa (Fig. 4A). The total wall thickness at the caecal apex was significantly (p=0.02) high than at the body in buffaloes. The comparative histomorphometric values of various layers of caecum at the apex and body in buffaloes are depicted in Table 4.

Tunica Mucosa: The tunica mucosa of the buffalo caecum was composed of lamina epithelialis having simple columnar epithelium, lamina propria composed of loose connective tissue and lined by intestinal glands, and lamina muscularis mucosae composed of smooth muscles both at the apex (Fig. 4A) and body of caecum. The tunica mucosa at the apex was significantly (p=0.04) thicker (258.51 ± 16.47μm) as compared to the body (203.36 ± 26.25μm).

Tunica Submucosa: The tunica submucosa was predominantly composed of collagen fibres (Fig. 4B) and few elastic fibres (Fig. 4C) mainly in the tunica intima of blood vessels, lymphatics, neuronal elements, and connective tissue cells both at apex and body. The adipose tissue was scanty in the submucosa of buffalo both at the apex and body of caecum (Fig. 4D). The mean thickness of tunica submucosa at the apex was significantly (p=1.64E-03) thick as compared to that at the body of buffalo caecum.

Tunica musularis: Tunica muscularis was composed of inner circular and outer longitudinal smooth muscle layers however randomly arranged smooth muscle bundles were frequently observed both at the apex and body of the cecum in buffalo (Fig. 4D, 5A). The muscle bundles in tunica muscularis were separated by well-developed connective tissue containing abundant collagen fibres (4D, 5B) and forming fascicles. There was non-significant (p=0.88) difference in the average thickness of tunica muscularis at apex and body of caecum in buffaloes.

Tunica Serosa: The tunica serosa was a thick connective tissue layer both at the apex and body of the caecum in buffalo comprising of abundant collagen fibres, blood vessels, lymphatics and neuronal elements (Fig. 4A, 5A). Occasional adipose tissue was also observed in tunica serosa. The tunica serosa at the apex was significantly (p=0.04) thinner as compared to that at body of caecum in buffaloes.

Table 4 - Histometry (mean ± SD) of various layers of caecal apex and body in buffaloes (H&E stained sections).
[Values with the same superscript differ significantly at p<0.05 (single superscript) and p<0.001 (triple superscript)]

Cecum	Mucosa (μm) (range)	Submucosa (μm) (range)	Muscularis (μm) (range)	Serosa (μm) (range)	Total wall (μm) (range)
Body	203.36 ± 26.25[a] (170.69-233.92)	112.30±4.43[bbb] (108.53-117.10)	833.05 ± 78.86 (720.70-895.69)	176.09 ±5.96[c] (170.10-183.33)	1386.65±73.50[d] (1298.20-1473.90)
Apex	258.51 ± 16.47[a] (235.19-273.98)	243.47 ± 20.88[bbb] (230.35-274.59)	823.45 ± 46.60 (764.69-892.75)	142.57 ±18.97[c] (123.29-166.37)	1464.36±46.05[d] (1406.78-1504.83)

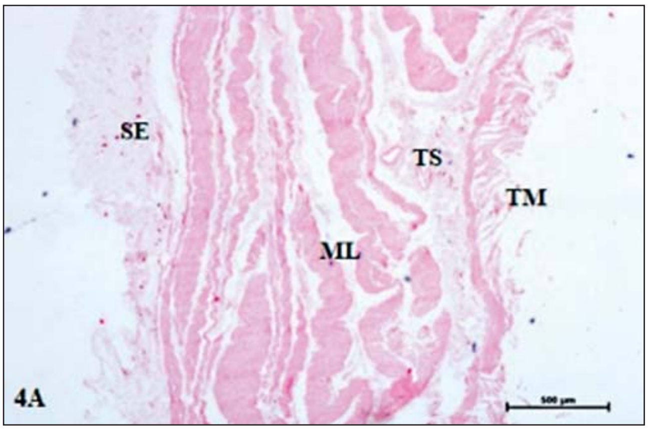

Figure 4A - Section of apex of buffalo caecum showing tunica mucosa (TM), tunica submucosa (TS), tunica muscularis (ML) and tunic serosa (SE) (H&E X 4x).

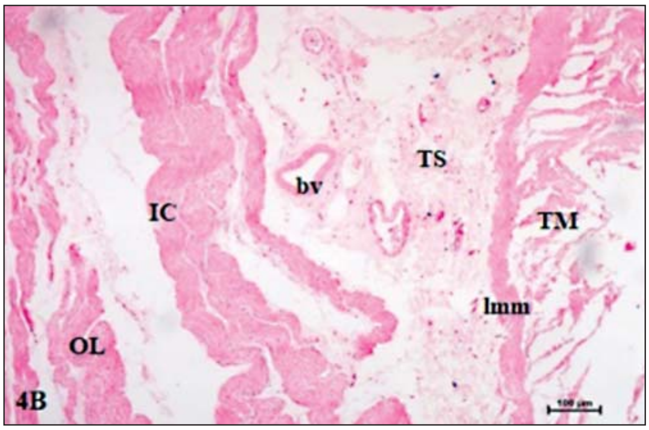

Figure 4B - Section of apex of buffalo caecum showing lamina muscularis mucosae (lmm), tunica submucosa (TM), showing connective tissue fibers and blood vessels (bv) and inner circular (IC) and outer longitudinal (OL) muscle bundles separated by large interfascicular connective tissue (H&E X 10x).

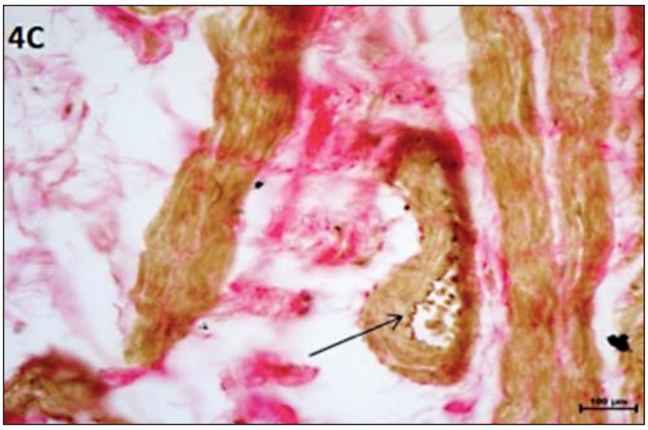

Figure 4C - Section of apex of buffalo caecum showing elastic fibers in blood vessels (arrow) between muscle bundles (Verhoeff Vangieson X 40x).

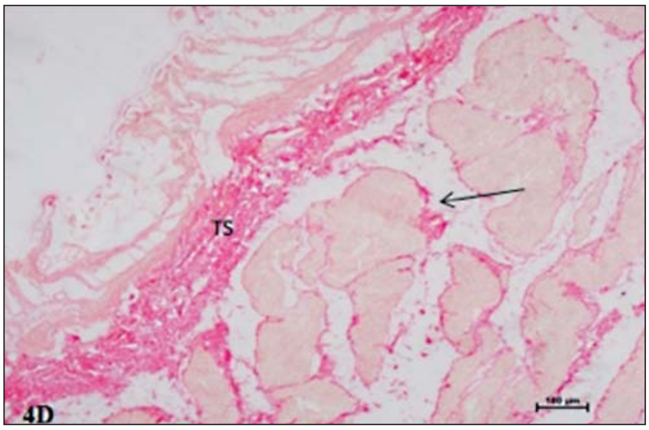

Figure 4D - Section of apex of buffalo caecum showing abundant collagen fibers in tunica submucosa (TS) and muscle fascicles surrounded by collagen fibers (arrow) (Picrosirius Red X 10x).

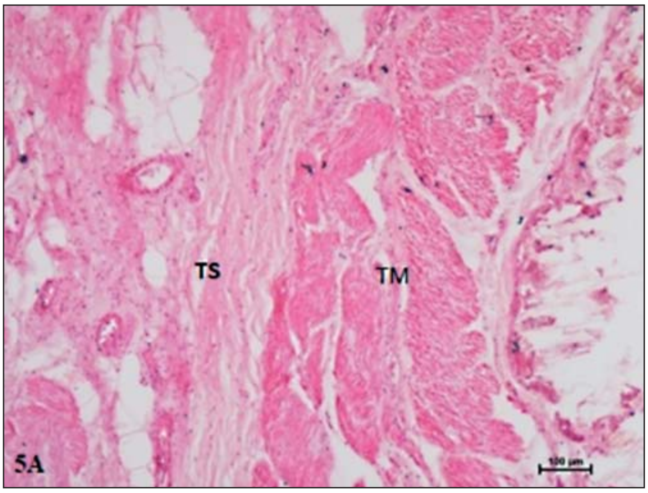

Figure 5A - Section of body of buffalo caecum showing randomly arranged muscle bundles in tunica muscularis (TM) and thick tunica serosa (TS) (H&E X 10x).

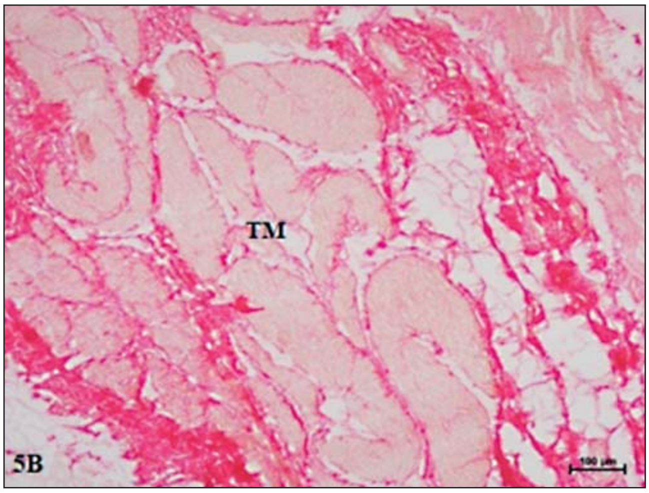

Figure 5B - Section of body of buffalo caecum collagen fibers in tunica muscularis (TM) (Picrosirius Red X 10x).

Histomorphometry of caecal wall in cattle

The caecal wall at the apex and body in the cattle (Fig. 6A, 6B) was similar to that in buffaloes. The total wall thickness at the caecal body was significantly (p=4.47E-03) higher than at the apex in cattle. The comparative histometry of various layers of caecum at the apex and body in cattle are depicted in Table 5.

***Tunica mucosa*:** The tunica mucosa at the apex and body of caecum in cattle was similar to that in buffaloes. There was no significant (p=0.53) difference in the mean thickness of tunica mucosa at the body and apex of caecum in cattle.

Tunica submucosa: Tunica submucosa both at the apex and body of the cecum in cattle was similar to that in buffaloes, except for the distribution of adipose tissue. The distribution of adipose tissue varied in the apex and the body of the caecum. In the region of the apex, adipose tissue was present in the form of islands and enclosed by connective tissue fibres (Fig. 6B, 6C) which were mainly collagen fibres, however, some areas at apex were devoid of adipose tissue and mainly collagen fibres were present (Fig. 6D). In the region of the body of caecum a continuous layer of adipose tissue was observed in the submucosa (Fig. 7A, 7B) and it was enclosed by collagen fibres toward the

Table 5 - Micrometry (mean ± SD) of various layers of caecal apex and body in cattle (H&E stained sections). [Values with the same superscript shows significant difference in each column at p<0.001]

Cecum	Mucosa (μm) (range)	Submucosa (μm) (range)	Muscularis (μm) (range)	Serosa (μm) (range)	Total wall (μm) (range)
Body	632.58±55.92 (573.18-681.75)	1077.02±134.90^{aaa} (888.46-1175.18)	2014.73±21.39^{bbb} (1989.48-2039.37)	165.92±16.46 (146.44-185.47)	3890.70±137.56^{ccc} (3719.64-4006.31)
Apex	666.76±52.10 (590.85-709.00)	567.10±51.62^{aaa} (497.00-619.33)	1096.21±118.38^{bbb} (969.35-1130.59)	184.01 ± 19.66 (158.34-199.61)	2434.08±299.18^{ccc} (1994.12-2634.97)

Figure 6A - Section of apex of caecum in cattle showing tunica mucosa (TM), tunica submucosa (TS), tunica muscularis (ML) and tunic serosa (SE) (H&E X 4x).

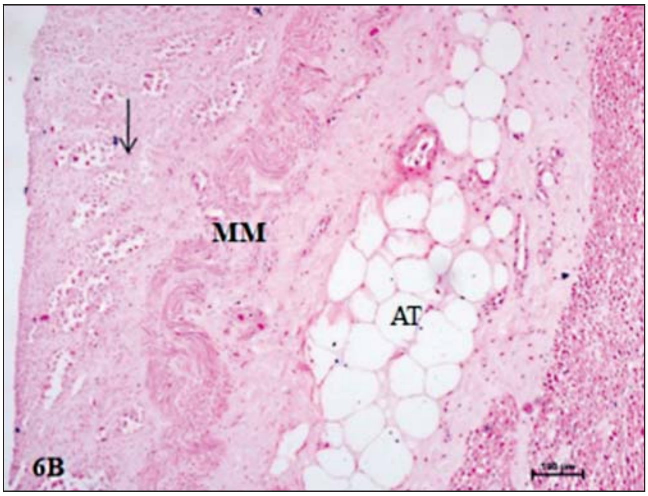

Figure 6B - Section of apex of caecum in cattle showing tunica mucosa comprising of intestinal glands (arrow) in lamina propria, thick lamina muscularis mucosae (MM) and adipose tissue (AT) in submucosa enclosed by connective tissue (H&E X 10x).

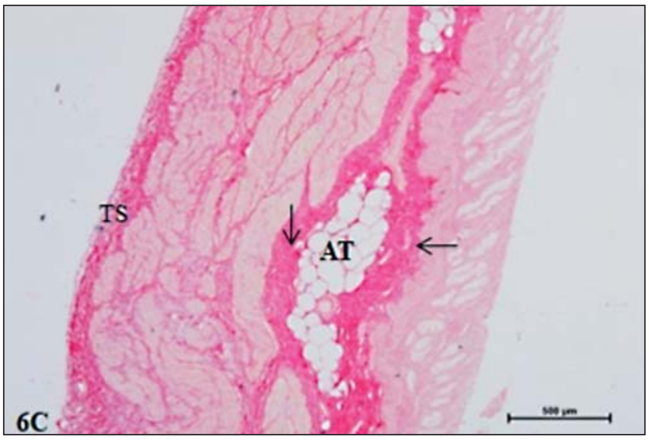

Figure 6C - Section of apex of caecum in cattle showing thick collagen bundles (arrow) surrounding adipose (AT) in submucosa. Tunica serosa (TS) and interfascicular connective tissue in tunica muscularis showing collagen fibers (Picrosirius Red X 4x).

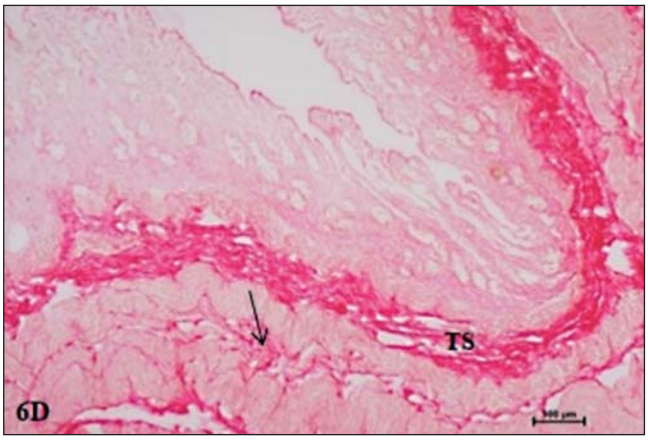

Figure 6D - Section of apex caecum in cattle showing predominant collagen bundles in tunica submucosa (TS) and collagen fibers in interfascicular connective tissue (arrow) in tunica muscularis (Picrosirius Red X 10x).

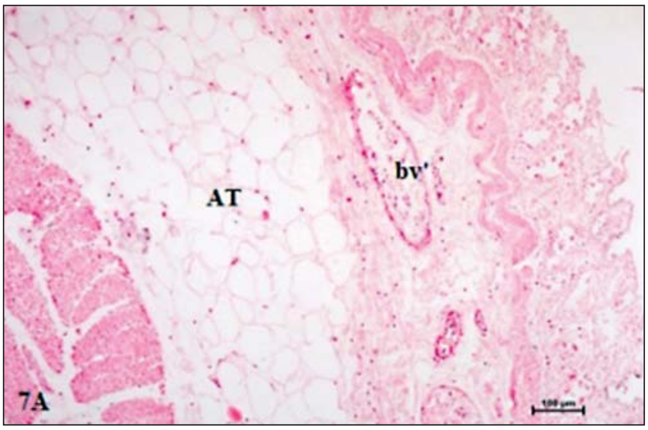

Figure 7A - Section of body of caecum in cattle showing the inner part of tunica submucosa entirely filled with adipose tissue (AT) and outer part having connective tissue fibers with blood vessels (bv) (Verhoeff Vangieson X 40x).

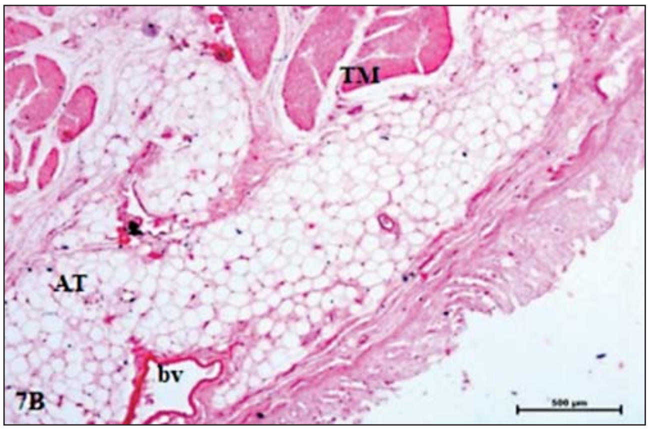

Figure 7B - Section of body of caecum in cattle showing adipose tissue (AT) with blood vessels (bv) extending into the tunica muscularis (TM) with randomly arranged muscle bundles (H&E X 4x).

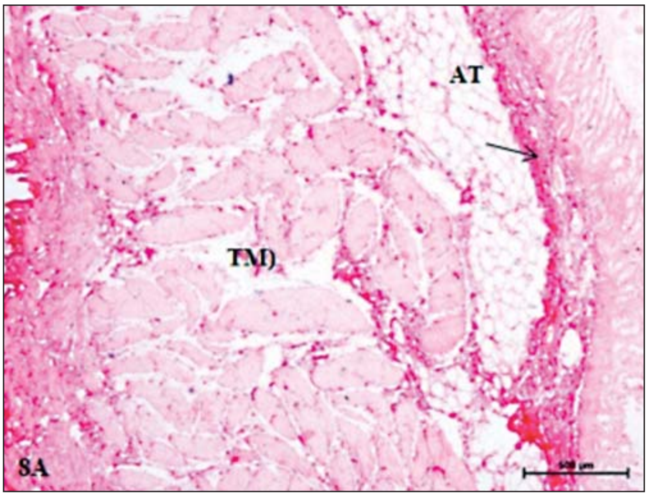

Figure 8A - Section of body of caecum in cattle showing collagen bundles (arrow) close to lamina muscularis mucosae. Inner part of submucosa entirely filled by adipose tissue (AT). Tunica serosa (TS). Tunica muscularis (TM) showing randomly arranged muscle bundles (Picrosirius Red X 4x).

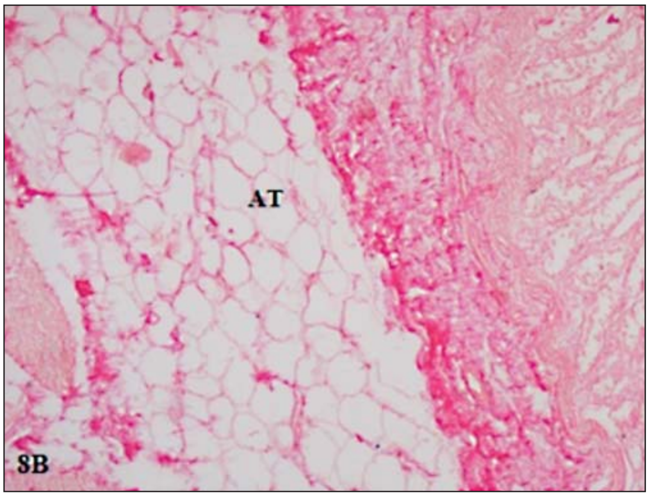

Figure 8B - Section of body of caecum in cattle showing abundant adipose tissue (AT) in submucosa and a few collagen fibers (Picrosirius Red X 10x).

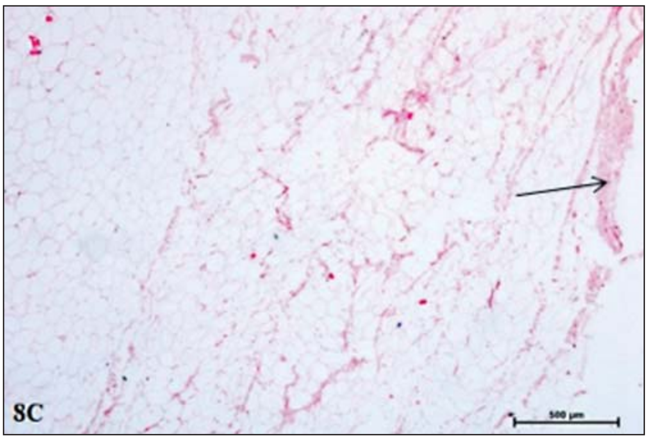

Figure 8C - Section of buffalo mesentery showing thin subepithelial connective tissue (arrow) and abundant adipose tissue (H&E X 4x).

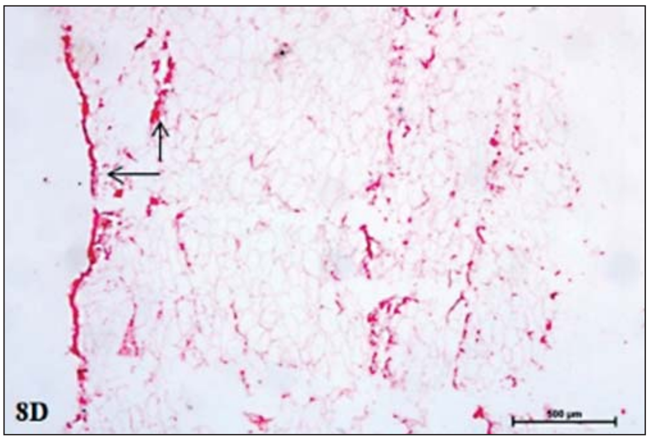

Figure 8D - Section of buffalo mesentery showing collagen fibrils (arrow) in subepithelial connective tissue and in between adipose tissue (Picrosirius Red X 4x).

side of lamina muscularis mucosae (Fig. 8A, 8B). The mean thickness of submucosa at the apex of the caecum was significantly less (p=7.28E-03) than at the body in cattle; however, it was the thickest layer.

Tunica muscularis: Similar to buffaloes, the tunica muscularis in the cattle was arranged in inner circular and outer longitudinal layers at the apex of the caecum. In the body of the caecum randomly arranged muscle fibres were frequently observed (Fig. 7A, 7B). The average thickness of tunica muscularis at the apex of the caecum in cattle was significantly less (p=4.62E-04) as compared to that at the body.

Tunica Serosa: The tunica serosa in cattle was similar to that in buffaloes. The tunica serosa in the cattle was composed of a connective tissue layer lined by mesothelium. The average thickness of tunica serosa at the apex and body of the caecum were non-significantly (p=0.38) different from each other.

Comparative histomorphometry of caecal wall in cattle and buffaloes

The thickness of mucosa (p=1.04E-04), sub-mucosa (p=7.33E-04), muscularis (p=3.14E-05) and total wall (p=1.42E-06) of the caecal body was significantly less in buffaloes as compared to that in cattle (Table 6). However, the serosa of the caecal body wall was non-significantly (p=0.31) thicker in buffaloes than in cattle.

In the caecal apex, all the layers; mucosa (p=2.29E-04), submucosa (p=3.33E-04), muscularis (p=0.01), serosa (p=0.02) and total wall thickness (p=6.69E-03) were significantly less in buffaloes as compared to that in cattle (Table 7).

In comparison to cattle, there was a scanty amount of adipose tissue in buffaloes irrespective of the region of the caecum (apex or body). In cattle, islands of adipose tissue enclosed by thick bundles of collagen fibers were observed in the submucosa of apex region. On the other hand, the distribution of adipose tissue in the submucosa of the caecal body was in the form of a continuous layer which was lined by loosely arranged (compared to caecal apex) collagen fibres towards the side of lamina muscularis (Table 8).

Tunica muscularis in both cattle and buffaloes were composed of both inner circular and outer longitudinal muscle layer, however a random arrangement of muscle fibres was also observed in both apex and body of buffaloes and only body in cattle.

Ileo-caecal Mesentery in buffaloes: The mesentery in buffaloes was lined by mesothelium and was supported by thin sub-epithelial connective tissue (Fig. 8C) which had randomly arranged collagen fibrils (Fig. 8D). The mesentery was composed of adipose cells supported by collagen and few elastic fibres, connective tissue cells and blood vessels (Fig. 9A, 9B). The mean thickness of sub-epithelial connective tissue in buffaloes

Table 6 - Comparative histometry (mean ± SD) of various layers of caecal body wall between cattle and buffaloes (H&E stained).
[Values with the same superscript differ significantly at p<0.001 level of significance]

Species	Mucosa (μm) (range)	Submucosa (μm) (range)	Muscularis (μm) (range)	Serosa (μm) (range)	Total wall (μm) (range)
Cattle	632.58±55.92^{aaa} (573.18-681.75)	1077.02±134.90^{bbb} (888.46-1175.18)	2014.73±21.39^{ccc} (1989.48-2039.37)	165.92±16.46 (146.44-185.47)	3890.70±137.56^{ddd} (3719.64-4006.31)
Buffaloes	203.36±26.25^{aaa} (170.69-233.92)	112.30±4.43^{bbb} (108.53-117.10)	833.05±78.86^{ccc} (720.70-895.69)	176.09±5.96 (170.10-183.33)	1386.65±73.50^{ddd} (1298.20-1473.90)

Table 7 - Histometry (mean ± SD) of various layers of caecal apex wall between cattle and buffaloes (H&E stained).
[Values with the same superscript differ significantly at p<0.05 (single superscript), p<0.01 (double superscript) and p<0.001 (triple superscript) level of significance]

Species	Mucosa (μm) (range)	Submucosa (μm) (range)	Muscularis (μm) (range)	Serosa (μm) (range)	Total wall (μm) (range)
Cattle	666.76 ± 52.10^{aaa} (590.85-709.00)	567.10 ± 51.62^{bbb} (497.00-619.33)	1096.21±118.38^{cc} (969.35-1130.59)	184.01 ± 19.66^{d} (158.34-199.61)	2434.08±299.18^{eee} (1994.12-2634.97)
Buffaloes	258.51 ±16.47^{aaa} (235.19-273.98)	243.47 ± 20.88^{bbb} (230.35-274.59)	823.45 ± 46.60^{cc} (764.69-892.75)	142.57 ±18.97^{d} (123.29-166.37)	1464.36±46.05^{eee} (1406.78-1504.83)

Table 8 - Qualitative scoring of collagen fibres in cattle and buffaloes.

Different layers	Cattle		Buffaloes	
	Apex	Body	Apex	Body
Tunica mucosa	+*	+*	-	-
Tunica submucosa	+++*	++	++	++*
Tunica muscularis	++*	++	+	++*
Tunica serosa	+++*	++	++	++*

* indicate densely packed collagen fibres; + indicate the amount of collagen fibres.

was 147.96±9.03 μm. Randomly distributed connective tissue fibres particularly collagen fibres were abundant without any septa in the mesentery of buffaloes (Fig. 9A).

Ileo-caecal Mesentery in cattle: The ileocaecal mesentery in cattle was similar to that in buffaloes. The sub-epithelial connective tissue was thick and contained abundant collagen fibres (Fig. 10A, 10B). The major constituent of the mesentery was adipose cells, along with the connective tissue cells, collagen fibres and a few elastic fibres and blood vessels (Fig. 10A, 9C). The sub-epithelial connective tissue also sent connective tissue septa into the mesenteric folds (Fig. 9C). The mean thickness of sub-epithelial connective tissue in cattle was 339.55 ± 10.71 μm. The elastic fibres were abundant in the wall of blood cells (Fig. 9D).

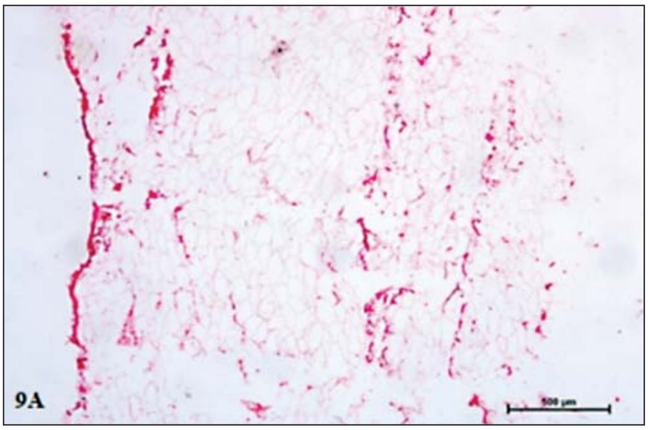

Figure 9A - Section of buffalo mesentery showing thin subepithelial connective tissue with collagen fibers and collagen fibrils in the adipose tissue (Picrosirius Red X 4x).

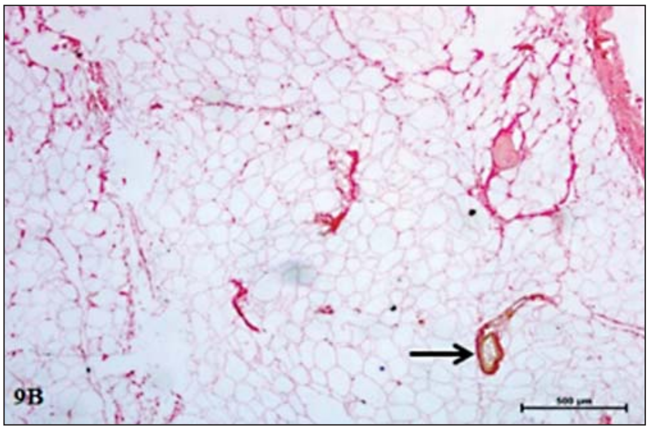

Figure 9B - Section of mesentery of buffalo showing blood vessels (arrow) and collagen fibers in the adipose tissue (Verhoeff Vangieson X 4x).

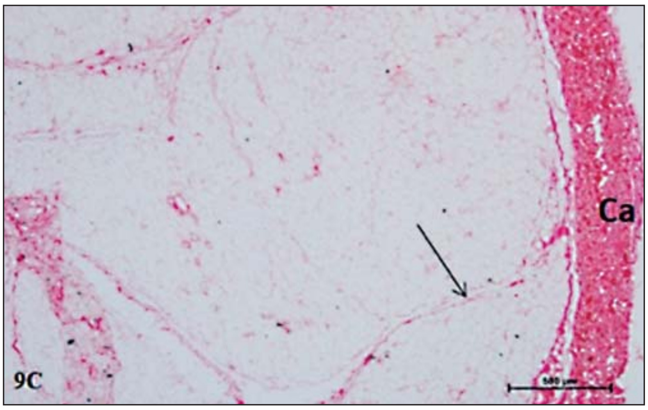

Figure 9C - Section of mesentery of cattle showing collagen fibers (Ca) and collagen fibers (arrow) in adipose tissue (Picrosirius Red X 4x).

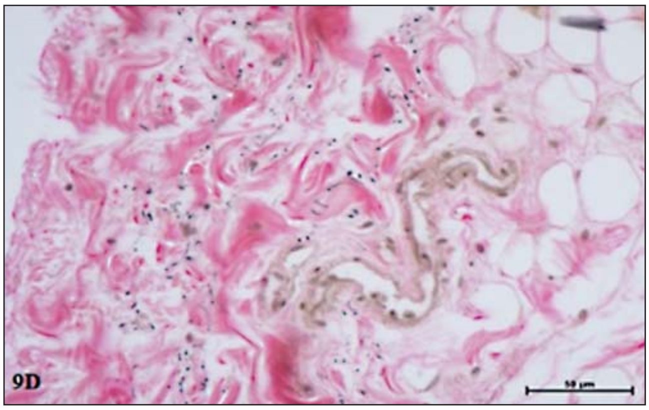

Figure 9D - Section of mesentery of cattle showing elastic fibers in the blood vessels (Verhoeff Vangieson X 40x).

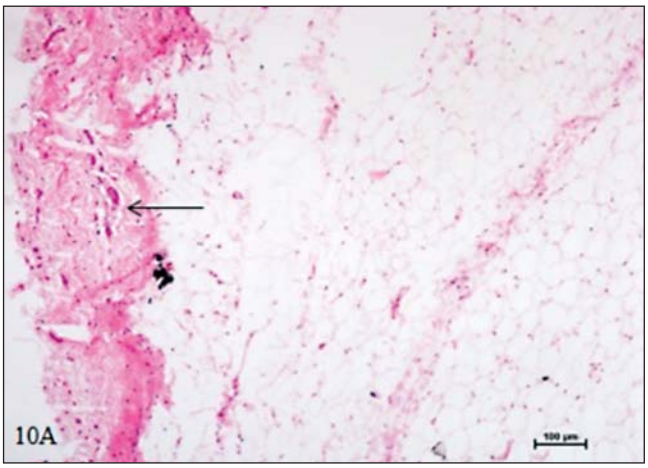

Figure 10A - Section of mesentery of cattle showing thick subepithelial connective tissue (arrow) and abundant fat (H&E X 10x).

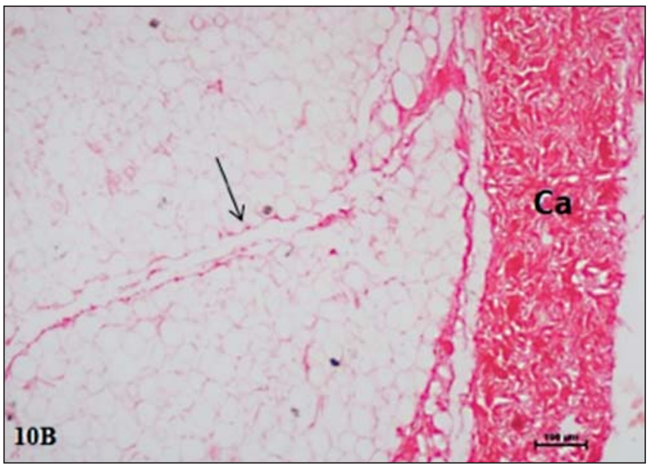

Figure 10B - Section of mesentery of cattle showing abundant collagen fibers (Ca) in subepithelial connective tissue and septa (arrow) extending into adipose tissue (Picrosirius Red X 10x).

Comparison of Ileo-caecal Mesentery in cattle and buffaloes: The sub-epithelial connective layers of the ileocaecal mesentery in buffaloes (147.96 ± 9.03 µm; range 139.78 - 158.98 µm) was significantly (p=2.20E-07) thin as compared to that in cattle (339.55±10.71 µm; range 328.43 - 353.05 µm). The connective tissue septa that were seen in ileo-caecal mesentery of the cattle were found absent in the buffalo.

Histomorphology of collagen fibres in Buffaloes

In an overall qualitative comparison of the caecal apex and body of buffaloes, the collagen fibres were densely packed in the caecal body. Tunica mucosa had a scanty to negligible amount of collagen fibres. The orientation of collagen fibres was wavy in appearance. The tunica submucosa, tunica muscularis and tunica serosa of the caecal body had more densely packed collagen fibres than that at the apex in buffalo (Fig. 4D, 5B).

Histomorphology of collagen fibres in cattle

The collagen was found scanty in tunica mucosa of caecum in the cattle. It was present in the basement membrane of lamina epithelialis. Loosely arranged collagen fibres were seen in lamina propria.

In the tunica sub-mucosa, thick bundles of collagen fibres were densely arranged especially towards lamina muscularis mucosae surrounded the island of adipose tissue. At locations where submucosa was devoid of adipose tissue, compactly arranged collagen fibers were present in caecal apex (Fig. 6C, 6D). In the submucosa of caecal body collagen fibers were loosely arranged especially toward lamina mucularis mucosae around a continuous layer of adipose tissue (Fig. 7A, 7B). Collagen fibers were also observed in the adipose tissue layer around adipose cells, both in caecal apex and body (Fig. 6C, 8A, 8B). The orientation of collagen fibers was wavy in appearance (Fig. 6C, 6D). In the tunica muscularis collagen fibres surrounded the muscle fascicles. A thin collagen layer was also observed inside the fascicles possibly representing the collagen in the perimysium (Fig 6C, 8A). In the tunica serosa layer collagen was densely packed (Fig. 6C) at the apex in comparison to body.

Comparison of collagen fibres in cattle and buffaloes: The collagen fibres in the body (p=0.012) and mesentery (p=0.001) were significantly less in buffaloes as compared to cattle. However, no significant difference (p=0.34) was recorded in the collagen fibres of the apex of the cecum in the two species.

The orientation of collagen fibres was similar in both the species but their arrangement varied (Table 8 and 9). Qualitatively, the amount of collagen fibres was more in cattle than in buffaloes. The amount of collagen fibres in the caecal apex were more and densely packed than at the caecal body in cattle which was contrary to that in buffaloes (Table 8 and 9).

Histomorphological observation of elastic fibres in cattle and buffaloes

Both cattle and buffalo samples showed very less amount elastic tissue fibres in both caecal apex and body. The elastic fibres were present in the tunica intima of blood vessels (Fig. 8C, 8D, 4C).

DISCUSSION

Cattle anatomy is a reference for other bovine species[16]. The gross morphometry of the caecum has been described in cattle, deer[17], giraffe[18,19] and camel[20]. The caecum in cattle is a large mobile blind sac with a caudally directed apex and is the site where digestion of residual carbohydrates by passing fore-stomach takes place[21]. It is also involved in the absorption of volatile fatty acids and transportation of the chime into the colon. The caecum is located on the right side of the abdomen in the supraomental recess extending from the level of 4th lumbar at ileocolic junction and its blind free end protruding from the supraomental recess. Dorsally, the caecum is attached to the proximal loop of the ascending colon by caeco-colic fold, ventrally with ileum by ileo-caecal fold, cranially to the right side of the mesentery and caudally the apex is free which can be felt per-rectal at the pelvic inlet when distended[22].

Despite several studies, the aetiopathogenesis of the caecal dilatation and dislocation remained unclear but is considered similar to as that of abomasal displacement[23] (Meylan 2008). Accumulation of gas in the blind sac of caecum causes its free end (caecal apex) to rotate in the clockwise or anticlock wise direction through the proximal colon that is relatively fixed structure[24] (Smith 1987). Trans-rectal palpation was reported as sufficient to detect distended, displaced, or twisted cecum in about 95% of the sick cattle. Palpation of the caecal apex extending into the pelvic inlet indicates a simple dilatation; whereas, palpation of the caecal body suggest presence of retroflexion in which the caecal apex is directed cranially resulting in partial or complete cessation of defecation[25].

There is a paucity of literature on the gross and histomorphometry of caecum and adjoining structures in buffaloes. As per the author's knowledge, this is the first report of its kind on the gross and micromorphometry of caecum in buffaloes. The study compared the gross and histometry of caecum between cattle and buffaloes. Scanty reports on caecal disorders in buffaloes in comparison to cattle and a difficult exteriorisation of caecal apex during right flank surgery prompted authors to plan comparative gross and histomorphometric studies on caecum in these species.

The knowledge of gross and microscopic anatomy of various regions of the caecum and adjoining organs is important as these may get involved in many surgical disorders such as caecal dilatation with or without torsion or dislocation[1]. In cattle suffering from caecal dilatation, a long free end of caecum i.e. apex

Table 9 - Quantitative analysis of collagen fibres in cattle and buffaloes.
[Significant difference between cattle and buffaloes at p<0.05 (single superscript) and p<0.01 (double superscript)]

Species	Apex	Body	Mesentery
Cattle	24.33 ± 1.96	18.03 ± 1.63*	14.54 ± 1.93**
Buffaloes	22.22 ± 3.47	14.30 ± 1.28*	3.36 ± 0.35**

and longer ileocaecal mesentery may a reason for the caecal apex to become dislocated or torsion. A paucity of literature on the similar sequel of caecal dilatation in buffaloes could be due to the smaller caecal apex devoid of the mesentery and short ileocaecal mesentery. The published literature lacks detailed description of caecal apex devoid of mesentery in various herbivores.

During decompression surgery, dilated caecum is approached from the right flank and caecal apex in cattle is easily exteriorized by gently pushing the cecum from behind toward the body wall with the palm of the hand[23] (Meylan 2008). The accumulated contents in the caecum and colon are drained via a small stab incision made at the apex. There are some reports on partial caecectomy (resection of the caecal apex) for cattle which have had a recurrence of caecal volvulus along with evidence of infarction. However, there is lack of literature concerning such complications in buffaloes suffering from caecal dilatation. So the longer caecal apex devoid of the mesentery in cattle might be facilitating the easy exteriorization of caecum during surgical intervention. Previous studies reveal that in buffaloes, it was difficult to exteriorise dilated caecum from the right flank incision so necessitating suturing of the caecum with skin margins before caecotomy[2,3].

The length and width of caecum in buffaloes were smaller than that in cattle which suggests species variation. Similarly in comparison to cattle, the buffaloes have been reported with shorter intestinal tract[26]. Grossly, the outer appearance of the caecum in cattle and buffaloes was smooth and without sacculations or bands and was similar to camel[20], giraffe[18,19] and deer[17]. Histologically, the caecal wall in cattle and buffaloes was similar to the previous findings in cattle[21] and camel[20], but the thickness in total and individual layers of caecal wall was significantly different in the two species. The fat content in the ileocaecal mesentery was more in cattle than buffaloes that corroborated with the previous findings[27].

In general, the various histological layers and total wall thickness in the caecum at the body and apex in buffaloes was significantly thinner than that of the cattle which might be due to species differences. Previous study also reported that tunica sub-mucosa of cattle to be thicker as compared to sheep and goat[21]. Another unique species-specific difference observed was that the tunica sub-mucosa of the caecal apex was significantly thicker in buffaloes and thinner in cattle in comparison to caecal body.

The lesser collagen fibres of the caecal body and mesentery in buffaloes as compared to cattle indicated the caecum in cattle to be more elastic than that in buffaloes. More elasticity may be the reason for the higher incidence of caecal dilatation induced retroflexion or dislocation in cattle[1] than in buffaloes[2,3].

In the current study, the mean length of the ileocaecal mesentery in buffalo was significantly less than cattle which might be the reason for comparative restricted mobility of the caecum in buffaloes. Another species-specific difference observed was that unlike cattle, ileo-caecal mesentry in buffalo was thin and lack the connective tissue septa.

This study attempted to compare gross and histomorphometry of caecum between cattle and buffaloes; however, the smaller sample size was the limitation. Further investigations on a large sample size to compare other ruminant species are warranted.

Based on the findings of the current study following conclusions were drawn:

- The caecum of buffalo differs grossly and in histometry from that of cattle.
- The caecal apex (devoid of ileocaecal mesentery), body and ileocaecal mesentery were shorter in buffaloes as compared to that in cattle.
- Grossly and histologically, the ileocaecal mesentery has markedly low-fat content in buffaloes as compared to that in cattle. Histologically submucosa of cattle caecum contained more adipose tissue in different parts as compared to buffaloes.
- Histologically, the caecal wall at the body and apex and its various layers (particularly tunica submucosa and muscularis) are thinner in buffaloes as compared to cattle.
- The buffalo caecum has lesser collagen content (qualitatively and quantitatively) as compared to cattle caecum.

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