



Histoenzymic Distribution in Ileal Peyer's Patches of Buffalo during Prenatal Development

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ABSTRACT

The study was carried out on ileum of 15 buffalo fetuses ranging from 14.5 cm curved crown-rump length (CVRL) (93 days) to 100 cm CVRL (299 days) to elucidate the histoenzymic distribution of enzymes i.e. alkaline phosphatase (AKPase), Glucose-6-Phosphatase (G-6-Pase), Succinic dehydrogenase (SDH) and Diaphorases on ileal peyer's patches during their prenatal development. The fetuses were categorized into three groups based on their CVRL. In Group upto 20 cm CVRL, weak alkaline phosphatase, SDH activity was observed and activity of LDH and Non specific esterase (NSE) was absent. In 20 to 40 cm CVRL fetuses, strong AKPase and moderate granular G-6-PD activity was observed in the villi of small intestine and developing group of lymphocytes in submucosa. In > 40 cm CVRL fetuses, strong AKPase activity was observed in dome region of the lymphoid follicle that invaded the mucosa in ileum. However, moderate SDH activity was observed at the periphery. The activity of LDH in 20 to 40 cm CVRL fetuses and also in > 40 cm CVRL fetuses was very weak. Intense activity of NADH enzyme was observed in submucosal lymphoid aggregates in group III of ileum.

Keywords: Buffalo, histoenzymic, ileum, peyer's patches, prenatal

The main function of the gut is to absorb nutrients and provide protection against all the bacteria, viruses, toxins and other antigens present in the gut. This protection is mediated by the presence of lymphoid tissue in gut, known as Gut associated lymphoid tissue (GALT). Ileal peyer's patches comprise a part of GALT and are present in ileum occupying most of the ileal mucosa. So, the presence of many enzymes, including phosphatases, dehydrogenases and esterases in ileal lymphoid tissue play a major role in the defense as well as in the metabolism in the gut. Therefore, the localization of these enzymes in GALT is important and is achieved by enzyme histochemistry. Enzyme histochemistry serves as a link between biochemistry and morphology. Alkaline phosphatase (AKPase) is associated with the ionic exchange across the membrane and in the cells specialized for endocytosis and pinocytosis (Singh and Singh, 2015). Activity of diaphorases is indicative of mitochondrial activity as well as cytoplasmic electron transport. Succinic dehydrogenase (SDH) is an essential part of Kreb's cycle and also found in all aerobic cells. Glucose-6-phosphatase is a crucial

enzyme in the control of glucose homeostasis as it catalyses the reaction of gluconeogenesis and hydrolysis reaction i.e. glycogenolysis into glucose-6-phosphate into glucose and Pi. G-6-Pase is a hydrophobic protein which was observed to be embedded within endoplasmic reticulum of the luminal membrane (Mithieux *et al.*, 2004). Therefore these enzymes serve an important role in the physiological functions of the lymphoid tissue. The alterations in localization of these enzymes can be used as a diagnostic tool in tissue damage and carcinomas of the gut.

Several reports have been made on enzyme localization in intestine of mouse (Watanabe *et al.*, 1983), rat (Owen and Bhalla, 1983) and rabbit (Sabatakou *et al.*, 2000). Moreover, scanty literature is available in sheep (Raju *et al.*, 2012) regarding its localization at prenatal period. But there is no literature available on histoenzymology of ileal peyer's patches in buffalo during its prenatal period. Therefore, this study was undertaken to elucidate the location of various enzymes in ileal peyer's patches of

buffalo during its prenatal life.

MATERIALS AND METHODS

The present study was conducted on ileum of 15 buffalo fetuses. The approximate age of fetuses was calculated by the using following formula given by Soliman (1975):

$$Y = 28.66 + 4.496 X \text{ (CVRL} < 20 \text{ cm)}$$

$$Y = 73.544 + 2.256 X \text{ (CVRL} \geq 20 \text{ cm)}$$

Where Y is age in days and X is curved crown rump length (CVRL) in cm. Depending upon CVRL fetuses were divided into three groups i.e., Group I (Upto 20 cm CVRL), Group II (20 to 40 cm CVRL) and Group III (> 40 cm CVRL). Just after measuring CVRL, the intestines were collected from fetuses. A part of ileum was dissected and kept at -20°C. Cryostat sections of 10 µm thickness at -20°C were obtained on glass slides and incubated with different substrates to study distribution pattern of different enzymes (Table 1) viz; Phosphatases: Alkaline phosphatase (AKPase) and Glucose-6-Phosphatase (G-6-Pase), Non-specific esterase (NSE) and Oxidoreductases: Oxidases: Monoamine oxidase (MAO); Dehydrogenases: Succinic dehydrogenase (SDH), Lactic dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G-6-PD), Nicotinamide adenine dinucleotide diaphorase (NADH-d) and Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) (Chayen *et al.*, 1969).

RESULTS AND DISCUSSION

Group I: In Group I, weak alkaline phosphatase and succinic dehydrogenase activity was observed in epithelium of villi. However no enzyme activity was observed in submucosal region as there was no lymphoid tissue developed at this age. This observation was in accordance with the observations made on prenatal development of peyer's patches in buffalo by Kapoor and Singh (2015). However, the activity of lactic dehydrogenase was completely absent in ileum at this age group. Glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide-diaphorase activity observed in villous epithelium specifically was also very weak (Table 2). Moreover, activity of non-specific esterase was also absent in both villi and submucosal region.

Group II: At 152 days, strong alkaline phosphatase activity was observed in the villi of ileum. The developing aggregates of lymphocytes in submucosa also presented strong activity for alkaline phosphatase (Fig.1). The presence of alkaline phosphatase in lymphoid aggregates might be involved in inducing insitu transformation of lymphocytes and provide a suitable environment for lymphopoeisis as opined by Hosteller and Ackerman (1968). They further postulated that this enzyme might serve as a chemotactic agent, attracting blood borne lymphocytes.

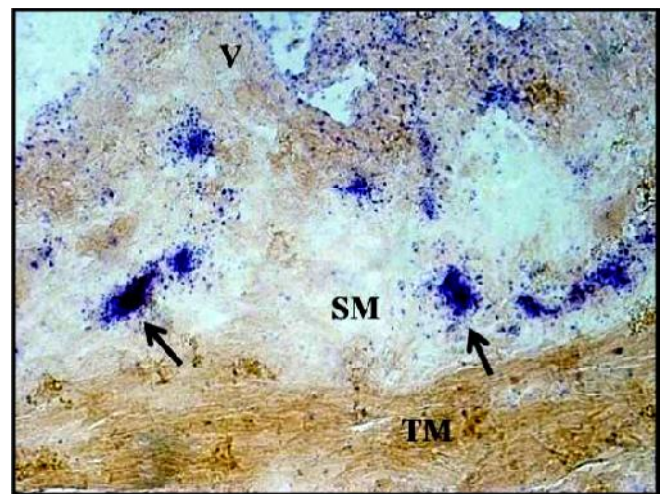


Fig. 1: Ileum of 152 days fetus showing strong AKPase activity in submucosal (SM) lymphoid aggregates (arrow), villi (V) and weak in tunica muscularis (TM). Azo dye method X400.

Glucose-6-Phosphatase activity was moderate in ileal villi at this age. However, the developing lymphocytic aggregations and the tunica muscularis showed intense activity of the enzyme (Fig. 2, Table 2). It is a key enzyme of gluconeogenesis and serves the function of gluconeogenesis in the tissue of small intestine along with the liver and kidney. Similarly, it was reported in mouse that Glucose-6-Phosphatase activity was present along the whole length of the intestinal tract of the mouse but less in ileum as compared to jejunum (Watanabe *et al.*, 1983). The activity of this enzyme was supported by the fact that the small intestine specifically utilizes glucose very actively, so the participation of this enzyme in glucose production necessitates the simultaneous release and uptake of glucose by this tissue.

Table 1: Histoenzymic methods used on cryostat sections of ileum of buffalo fetuses

Sr. No.	Enzyme	Substrate	Method	Reference	Incubation Time
A. Phosphatases					
(i)	Alkaline phosphatase (AKPase)	Naphthol AS-MX phosphate disodium salt in combination with Fast Blue RR	Simultaneous coupling azo dye method using substituted naphthols	Barka and Anderson (1963)	30 min
(ii)	Glucose-6-phosphatase (G-6-Pase)	Glucose-6-phosphate and lead nitrate	Lead nitrate method	Barka and Anderson (1963)	20 min
B. Oxidoreductases					
(i)	Succinic dehydrogenase (SDH)	Di-Na succinate	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
(ii)	Lactic dehydrogenase (LDH)	Na-DL lactate	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
(iii)	Glucose-6-phosphate dehydrogenase (G-6-PD)	Di-Na glucose-6-phosphate	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
(iv)	Monoamine oxidase (MAO)	Tryptamine hydrochloride	Standard method of bound enzyme by nitro BT method	Pearse (1972)	60 min
(v)	Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase)	Co-enzyme (NADPH)	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
(vi)	Nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase)	Co-enzyme (NADH)	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
C. Esterases					
	Non-specific esterase (NSE)	Alpha-naphthol acetate	Naphthol acetate method	Barka and Anderson (1963)	10 min

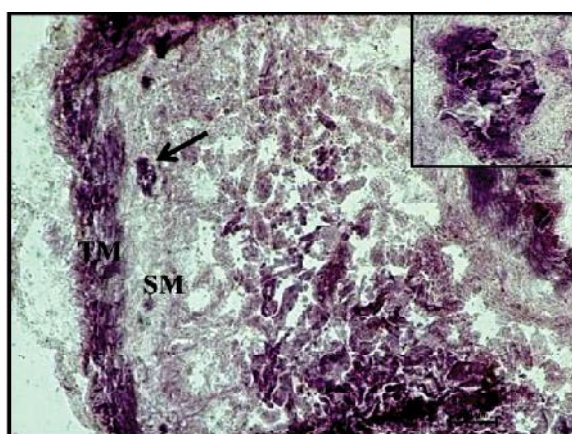


Fig. 2: Ileum of 152 days fetus showing strong G-6-Pase activity in villi (V), lymphoid aggregates (arrow, inset) in submucosa (SM) and tunica muscularis (TM). Lead Nitrate method X100.

At 154 days, weak to moderate activity of Glucose-6-phosphate dehydrogenase was observed in the villous epithelium. Moreover, the developing lymphocytic aggregates were also having moderate activity of glucose-6-phosphate dehydrogenase (Table 2). At 157 days, weak to moderate activity of succinic dehydrogenase was observed in the villous epithelium of ileum and in lymphocytic accumulations developing at this age (Fig. 3).

At this age, a moderate to strong activity of nicotinamide adenine dinucleotide diaphorase was observed in villi and in submucosal aggregates of lymphocytes. Very weak activity of non-specific esterase enzyme was observed in villous epithelium and moderate in the lymphocytic accumulations in this age group (Table 2).

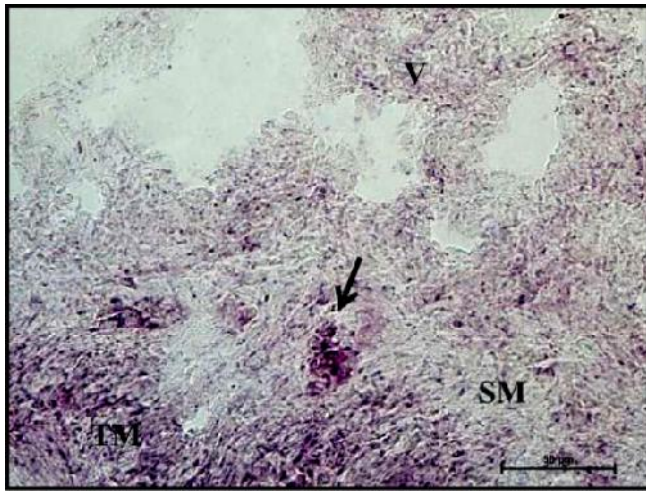


Fig. 3: Ileum of 157 days fetus showing weak SDH activity in villous epithelium (V) whereas moderate activity in lymphocyte aggregates (arrow) in submucosa (SM) and tunica muscularis (TM). Nitro BT method X400.

Group III: At 168 days, strong activity of succinic dehydrogenase was noticed in villous epithelium of small intestine whereas moderate activity was observed in lymphocyte accumulations at this age. Moreover, very weak activity of lactic dehydrogenase was observed at this age. At 172 days, strong activity of nicotinamide adenine dinucleotide- diaphorase was noticed in epithelium of villi (Table 2). Moreover, the tunica muscularis was having moderate activity of nicotinamide adenine dinucleotide-diaphorase while the clearly appreciable submucosal

lymphoid aggregates were having intense nicotinamide adenine dinucleotide diaphorase activity especially at the peripheral rims of the lymphocytes (Fig.4).

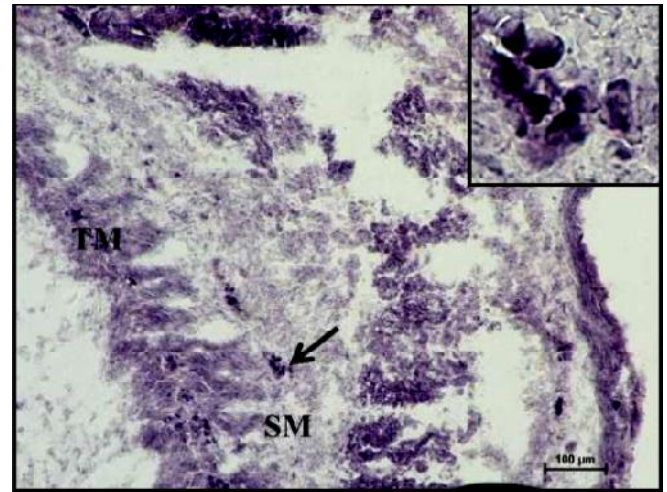


Fig. 4: Ileum of 172 days fetus showing strong NADH-diaphorase activity in villi, tunica muscularis (TM) and intense activity in submucosal lymphoid aggregates (arrow; inset). Nitro BT method X100.

At 299 days, strong activity of alkaline phosphatase was observed in dome region of the follicle that invaded the mucosa in ileum. This is probably because of the active migration of maturing lymphocytes towards the villi for their mode of action i.e., binding with the antigen to generate the immune response. Moreover, alkaline

Table 2: Histo enzymic distribution in foetal Ileal Peyer's patches of buffalo

Sr. No.	Enzymes	Villi			Lymphoid area			Capsule of Lymphoid follicle		
		Grp I	Grp II	Grp III	Grp I	Grp II	Grp III	Grp I	Grp II	Grp III
1	AKPase	+	+++	+++	0	++	+++	0	0	++
2	G-6-Pase	+	++	+++	0	++	+++	0	0	++
3	G-6-P-D	+	+ / ++	+ / ++	0	+	+ / + +	0	0	+ / + +
4	MAO	+	++	+++	+	+ / +	+++	0	0	++
5	SDH	+	++	+++	+	+ / +	++	0	0	++
6	LDH	0	+	+	0	0	+	0	0	+
7	NADH	+	++	+++	0	+	+++	0	0	+++
8	NADPH-d	+	++	+++	0	+	+++	0	0	+++
9	NSE	0	+	+	0	0	++	0	0	+

0 Not observed; + Weak; ++ Moderate; +++ Strong

phosphatase activity is correlated with ionic movements across the epithelium and cell differentiation. A moderate activity was observed in the crypt regions that were lying in between the domes of lymphoid follicles (Fig.5). This is in agreement with the observations made by Bjerknes and Cheng (1981) in mouse and Owen and Bhalla (1983) in rat. On the other hand, a very weak activity of this enzyme was noticed in tunica muscularis.

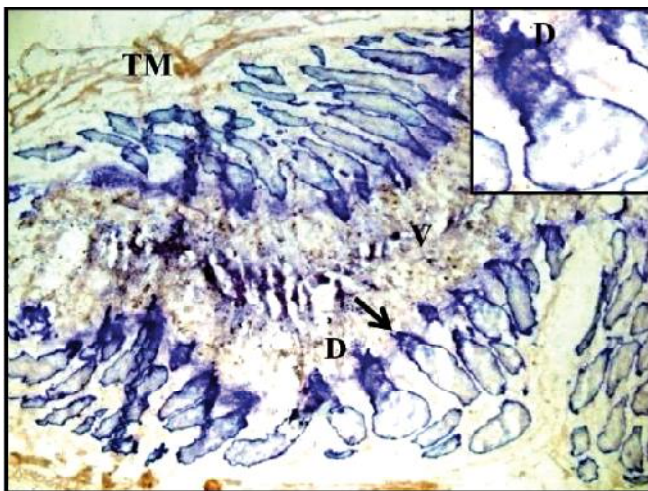


Fig. 5: Ileum of 299 days fetus showing strong AKPase activity in dome region (D, inset) of the follicle invading (arrow) the mucosa, moderate activity in crypts of villi (V) and weak in tunica muscularis (TM). Azo dye method X20.

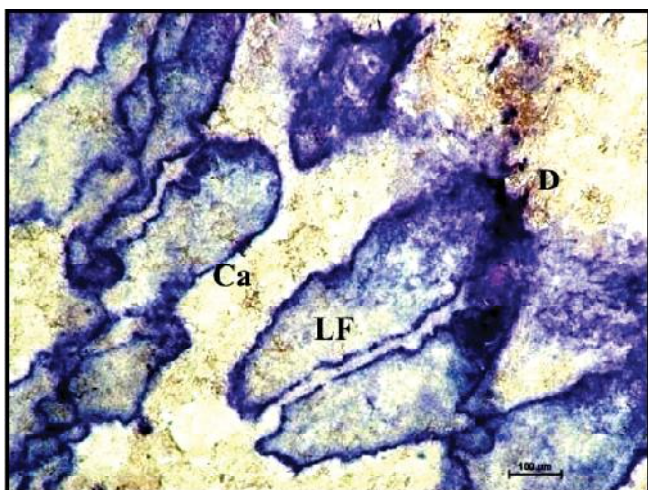


Fig. 6: Ileum of 299 days fetus showing strong AKPase activity in capsule (Ca) and dome region (D) of lymphoid follicles (LF). AKPase X100.

The capsule surrounding the lymphoid follicle was intensely positive for alkaline phosphatase activity (Table 2). Halleraker *et al.* (1990) also observed alkaline phosphatase enzyme activity in the follicle capsule in ruminants. The high endothelial venules found in the interfollicular region involved in the transport of lymphocytes toward's peyer's patches expressed negligible or very weak activity. Similar findings were made in mouse by Ropke *et al.*, (1972). (Fig. 6).

Monoamine oxidase showed weak positive granular perinuclear activity at this age (Table 2) and that played a major role in the regulation of cellular metabolism (Chayen *et al.*, 1969). At this age, a very strong activity of succinic dehydrogenase was observed in villous epithelium, especially in crypts. The cellular content of the lymphoid follicle at periphery showed moderate fine granular succinic dehydrogenase activity (Fig.7, inset).

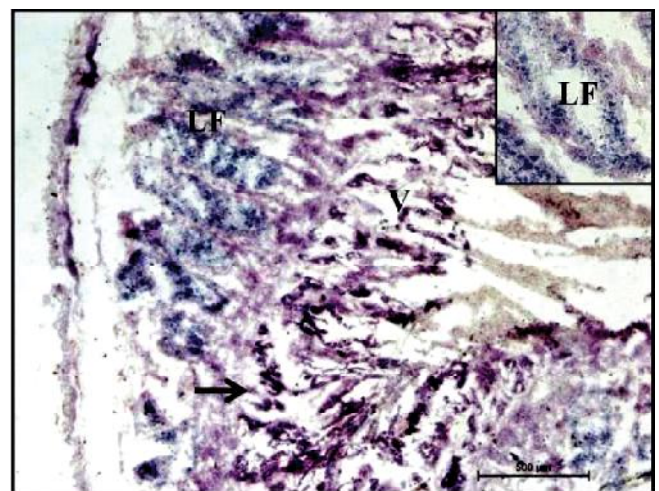


Fig. 7: Ileum of 299 days fetus showing strong SDH activity in villous epithelium (V), especially in crypts (arrow) and moderate activity in periphery of lymphoid follicle (LF, inset). Nitro BT method X40.

At 299 days, the activity of nicotinamide adenine dinucleotide diaphorase was intense in the capsule and within the follicle, especially on the periphery and at patchy locations in the center of the follicle (Fig. 8). Intense activity of enzyme may reflect differentiation and maturation of lymphocytes (Fennell and Pearse, 1961). A strong positive activity of nicotinamide adenine dinucleotide phosphate diaphorase was observed in lymphoid follicle and was indicative of mitochondrial

activity as well as including in cytoplasmic electron transport system (Chayen *et al.*, 1969).

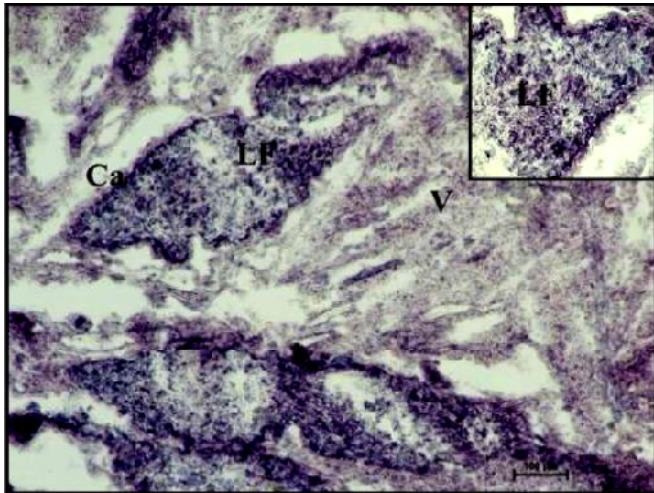


Fig. 8: Ileum of 299 days fetus showing moderate NADH-diaphorase activity in villi (V) and strong activity in capsule (Ca) and in lymphoid follicle (LF), especially on the periphery (inset). Nitro BT method X40.

In Group III, a strong activity of glucose-6-phosphate dehydrogenase was found in epithelium of villi and the capsule of the lymphoid follicles. Moreover, the cellular contents i.e., the developing lymphocytes at the periphery of the follicles were also strongly positive for glucose-6-phosphate dehydrogenase activity with fine granular reaction and the cells in the center were observed to have moderate activity at this age. The glucose-6-phosphate dehydrogenase enzyme is associated with the pentose phosphate shunt (Fennell and Pearse, 1961) and these pentose phosphates might be utilized for nucleic acid synthesis in lymphopoiesis (Fig. 9).

However, moderate activity of non-specific esterase was observed in lymphoid aggregates in submucosa. (Table 2). Only mild activity of non-specific esterase was reported in 26th or 28th day of foetal life in rabbit, although the activity was strong in 1 day old rabbits, in 19 day and older rabbits (Sabatakou *et al.*, 2000). Moderate non-specific esterase activity was observed in the submucosal nerve bundles around the lymphoid follicles. Nonspecific esterases are a group of cellular carboxylesterases which act as enzyme markers of monocytes or macrophages (Kolios *et al.*, 2002).

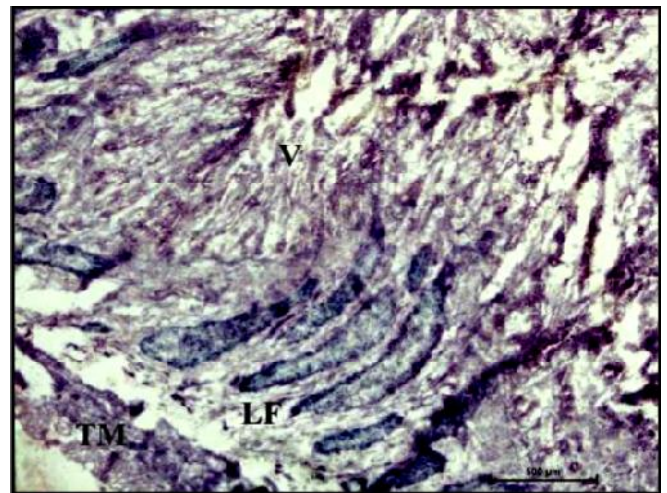


Fig. 9: Ileum of 299 days fetus showing moderate G-6-PD activity in villous epithelium (V), tunica muscularis (TM) and strong activity in lymphoid follicles (LF). Nitro BT method X40.

CONCLUSION

The present study revealed that the lymphoid tissue in ileum i.e., ileal peyer's patches of buffalo during prenatal period were observed with variable distribution of phosphatases, oxidoreductases and esterases at different gestational periods. Activity of these enzymes were absent at early gestational age group due to absence of lymphoid tissue at this age. However, moderate to strong activity was observed at mid gestational age, which progressively increased with advancement of age. Strong to intense activity of phosphatases was observed in the dome of the lymphoid follicles completely developed at late gestational period, which reflected increased migration of lymphocytes towards the villi from dome region. The high activity of diaphorases was because of the role played by the enzyme in differentiation and maturation of lymphocytes in lymphoid tissue.

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