



Histo-enzymatic Characterization of Small Intestine of Neonatal Piglets

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ABSTRACT

The present study was conducted on eight numbers of 0 and 7 day old neonatal piglets consisting of four animals in each group, irrespective of sex. Alkaline phosphatase and acid phosphatase have been investigated in the small intestine by means of light microscopic histochemistry. The reactivity of alkaline phosphatase was intense in absorptive epithelium, dome epithelium (DE) of the small intestine. The T-cell area and the neck portion of the lymphoid follicle showed a positive reaction. This activity was moderate in dome and follicular area of Peyer's patches whereas weak to negative activity was observed in interfollicular area and glandular epithelium throughout all segments of the small intestine in both the groups. The acid phosphatase activity was intense in glandular epithelium and absorptive epithelium; strong in dome area of Peyer's patches and FAE; moderate in follicular and interfollicular areas of Peyer's patches of small intestine.

Keywords: Histo-enzymatic, Small intestine, Peyer's patches, FAE, Piglets

Piglets during the suckling period, exposed to a variety of stresses. Stress factors can affect the growth and development of the new borne piglets. Intestine is the major organ responsible for the growth and development. The main function of the intestinal tract is the digestion and absorption of nutrients. The small intestine is the principal organ for this purpose. The epithelial surface increases the contact area between nutrients and absorptive cells and thereby multiplies digestive efficiency. A key element of this contact area is the brush border membrane. It consists of microvilli and a glycoprotein coating – the glycocalyx. The brush border membrane contains enzymes, crucial for the carbohydrate and protein digestion, and carrier proteins for the absorption of nutrients (Mosenthin, 1998). The Peyer's patches (PP) had significant bearing on the normal functioning of the digestive system and the gut-immune system during pre weaning period. Several studies had been carried out by various workers on different aspects of the gut-associated lymphoid tissues (GALT) of various domestic species like cattle and sheep (Yasuda *et al.*, 2006)

and pig (Talukdar, 1999 and Kalita *et al.*, 2017). However, literature pertaining to the histochemistry of the intestine in neonatal pig is meagre. Hence, the present investigation was intended to throw some light on this aspect.

MATERIALS AND METHODS

The present study was conducted on eight neonatal piglets divided into two age groups of 0 and 7 days of age, consisting of four animals in each group irrespective of sex. They were collected from the ICAR-AICRP/MSP on pig, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati. Tissue samples of intestine were collected soon after sacrifice of the animals and preserved immediately in liquid nitrogen at -196°C. The samples were then shifted directly to cryostat microtome (Shandon Finesse) which was maintained at minus 22°C. The frozen sections were cut at 8 µm thickness and were collected on clean slides. They were temporarily stored at minus 22°C and were then treated for histochemical demonstration of enzymes as shown hereunder:

- ❑ Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1978)
- ❑ Gomori's acid phosphatase method (Singh and Sulochana, 1978)

The histochemical characteristics of the small intestine were observed and interpreted with the help of Nikon E200 camera mounted microscope and Image Pro Express Ver-2.0 software. The photomicrographs of selected slides were taken for typical demonstration.

RESULTS AND DISCUSSION

In the present study, cryostat sections from all the segments of small intestine were subject to histochemical staining by respective protocols and the results are illustrated in Table 1.

Alkaline phosphatase

The different segments of the small intestine of 0 and 7 days old Hampshire piglets were processed for alkaline phosphatase activity. Alkaline phosphatase activity was intense in absorptive epithelium, dome epithelium (DE) of the small intestine (Fig. 1 and 2). However, this activity was weak to negative in Brunner's gland of duodenum and intestinal glands. The Peyer's patches were recorded in jejunum and ileum, however, more numbers were observed in the ileum of both the groups. The T-cell area and the neck portion of the lymphoid follicle showed a positive reaction. This activity was moderate in dome and follicular area of Peyer's patches whereas weak to negative activity was observed in interfollicular area and glandular epithelium throughout all segments of the small intestine in both the groups (Fig. 3). Similar observations were reported by Gautam (2015) in Peyer's patches of growing

Table 1: Histochemical characterization of small intestine of 0 and 7 day old hampshire piglets

Histoenzymes	Absorptive epithelium	Gland	Follicle	Dome area	FAE/DE	Interfollicular area
Alkaline phosphatase	++++	-/+	++	++	++++	+ / ++
Acid phosphatase	++++	++++	++	+++	+++	++

Gradation for intensity of histochemical reaction:

- = Negative + = Weak
 ++ = Moderate +++ = Strong
 ++++ = Intense

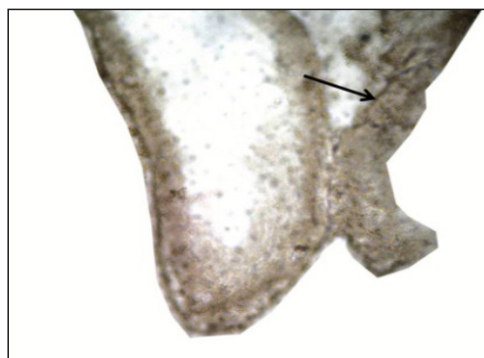


Fig. 1: Photomicrograph showing alkaline phosphatase activity in the villous epithelium (arrow) of duodenum in 7 day old Hampshire piglet. Gomori's, X400.

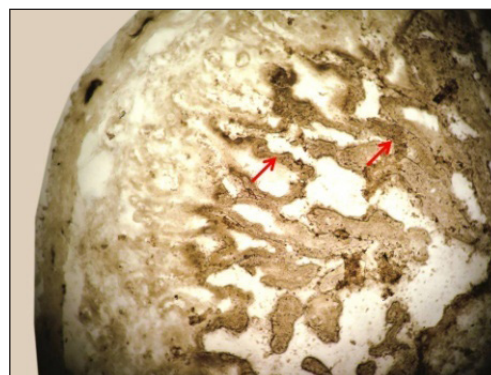


Fig. 2: Photomicrograph showing alkaline phosphatase activity in the villous and dome epithelium (arrow) of duodenum in 0 day old Hampshire piglet. Gomori's, X100.



Fig. 3: Photomicrograph showing alkaline phosphatase activity in the Peyer's patches area (arrow) of ileum in 0 day old Hampshire piglet. Gomori's, X100.

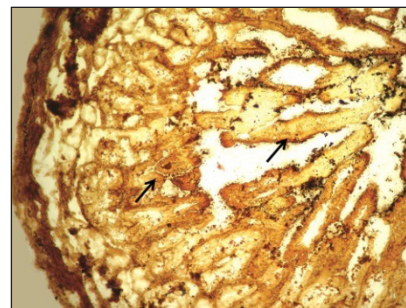


Fig. 4: Photomicrograph showing acid phosphatase activity in the glandular and absorptive epithelium (arrow) of ileum in 0 day old Hampshire piglet. Gomori's, X100.



Fig. 5: Photomicrograph showing acid phosphatase activity in the dome and FAE area (arrow) of ileum in 0 day old Hampshire piglet. Gomori's, X100.

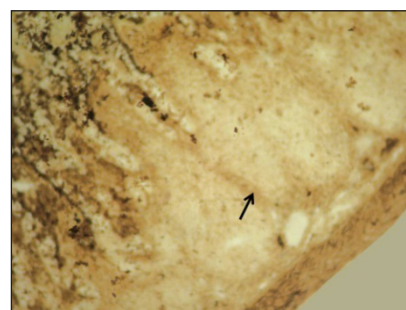


Fig. 6: Photomicrograph showing acid phosphatase activity in the interfollicular area (arrow) of duodenum in 7 day old Hampshire piglet. Gomori's, X400.

piglets. In the present study, alkaline phosphatase activity was moderate in the surface epithelium of duodenum and ileum; while the activity was strong in the jejunum indicating the preferred nutrient absorption site. The reactivity of alkaline phosphatase in the follicle associated epithelium (FAE) and dome area of Peyer's patches might be indicative of the presence of energy dependent active transport mechanism at these sites. The antigen transport through M cells present in the FAE was transcellular and active as opined by Schulz and Pabst (2013).

Acid phosphatase

In the present investigation, acid phosphatase, being a lysosomal enzyme activity was intense in glandular epithelium and absorptive epithelium (Fig. 4); strong in dome area of Peyer's patches and FAE (Fig. 5); moderate in follicular and interfollicular areas of Peyer's patches (Fig. 6) of small intestine. The intense reaction of this enzyme in the glandular and absorptive epithelium might indicate the presence of lysosomal activity in the

respective site. The dome area which is important for antigen capturing after the FAE, might be having lysozyme rich dendritic cells for presentation of particulate antigen and subsequent transport to other immune organs of the body. Bancroft (2008) opined acid phosphatase enzyme as a lysosomal enzyme. In the present study, some cells of the lamina propria and some in the epithelial lining were found to be positive for this enzyme activity and were in consonance to the findings of Gautam (2015) in growing piglets. This finding was also tuned to the findings of Dawson (1981) and Kudweis (1991); who reported that acid phosphatases are lysosomal enzymes present within the phagolysosomes of absorptive cells and macrophages within the lamina propria. Besides Kudweis (1991) detected acid phosphatase activity in the intestinal crypts in suckling piglets.

CONCLUSION

The present study was undertaken to determine the histochemical reaction of small intestine in 0 and 7 day old



neonatal piglets. The reactivity of alkaline phosphatase was intense in absorptive epithelium, dome epithelium (DE) of the small intestine. This activity was weak to negative in interfollicular area and glandular epithelium throughout all segments of the small intestine in all the piglets. The acid phosphatase activity was intense in glandular and absorptive epithelium in all the piglets throughout all segments of the small intestine.

REFERENCES

- Bancroft, J.D. 2008. Theory and Practice of Histological Techniques. 6th Edn., Churchill Livingstone, Elsevier Health Sciences.
- Dawson, I.M.P. 1981. The value of Histochemistry in the Diagnosis and Prognosis of Gastrointestinal Diseases. In: *Histochemistry: The Widening Horizons.*, Stoward P.J., Polak J.M., (Edns.) John Wiley and Sons, New York.
- Gautam, C. 2015. Antigen uptake and comparative histomorphology and ultrastructure of the histocompartments of Peyer's patches and solitary lymphoid nodules of intestine of growing piglet. Ph.D. Thesis submitted to the Assam Agricultural University, Khanapara, Guwahati, India.
- Kalita, A., Sarma, K., Talukdar, M., Deka, A. and Kalita, P.C. 2017. Gut integrity of neonatal piglets: a Histomorphological analysis. *J. Anim. Res.*, **7**(6): 1115-1121.
- Kudweis, M., Lojda, Z. and Julis, I. 1991. Histochemistry of acid phosphatase in small intestine mucosa in experimental coccidiosis in suckling piglets. *Vet. Med.*, **36**(2): 93-105.
- Mosenthin, R. 1998. Physiology of Small and Large Intestine of Swine- Review. *Asian-Australas. J. Anim. Sci.*, **11**: 608-619.
- Schulz, O. and Pabst, O. 2013. Antigen sampling in the small intestine. *Trends Immunol.*, **34**(4): 155-161.
- Singh, U.B. and Sulochana, S. 1978. A laboratory manual of histological and histochemical technique. 1st Edn., Kothari Medical Publishing House, Bombay, pp. 50-54.
- Talukdar, M. 1999. Gross anatomical, histomorphological and histochemical studies on the stomach and intestine of crossbred adult pig. Ph.D. Thesis submitted to the Assam Agricultural University, Khanapara, Guwahati, India.
- Yasuda, M., Jenne, C.N., Kennedy, L.J. and Reynolds, J.D. 2006. The sheep and cattle Peyer's patch as a site of B-cell development. *Vet. Res.*, **37**: 401.