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Enzymatic and Mucopolysaccharide Profile of the caecum in Aseel cross Birds

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Abstract

This present study characterized the histochemical profile of the caecum in Aseel cross birds with special emphasis on the distribution of mucopolysaccharides and phosphatase enzymes. The lamina propria, lymphoid nodules and goblet cells exhibited strong positivity for both neutral and acidic mucopolysaccharides. Periodic Acid-Schiff (PAS) staining had showed the intense reactivity in the villi, Crypts of Lieberkühn, collagen fibers and muscularis mucosa due to abundant neutral muco-substances. Strong PAS positivity was also observed in the submucosa and tunica muscularis whereas the tunica serosa had showed only mild Alcian blue reactivity due to comparatively lower concentration of acidic mucopolysaccharides. Alkaline phosphatase (ALP) activity was strongly localized on the apical surface of the glandular epithelium with moderate activity in the villus core and goblet cells whereas the lymphoid tissues and muscular layers exhibited mild reactions. Acid phosphatase (ACP) activity was moderately distributed throughout the villus core, apical glandular epithelium, lymphoid nodules, submucosa and tunica muscularis. The findings of the present study had demonstrated a distinct distribution pattern of glycoconjugates and phosphatase enzymes in the caecum indicate it has important absorptive, protective and immunological functions within the intestinal microenvironment.

Key words: *Enzymatic profile, Mucopolysaccharide Profile, Caecum, Aseel crossbred, native chicken*

Introduction

The native chickens are widely recognized for their significant adaptability to the diverse agro-climatic conditions, tolerance to environmental stress and greater resistance to endemic diseases as compared to the highly selected commercial poultry strains. Their ability to survive and perform efficiently under low-input production systems is due to their rich genetic diversity, natural scavenging behaviour and efficient utilization of locally available feed resources such as kitchen waste, insects, crop residues and green forages (Aini, 1990; FAO, 2010). The layer breeds depend highly on balanced feed, controlled housing and intensive healthcare unlike the commercial broiler and indigenous chickens sustain the production under harsh environmental conditions with minimal external inputs. Their hardiness, broodiness and adaptability make them highly suitable for the backyard and smallholder farming systems in the remote and resource-constrained regions (Horst, 1989; Padhi, 2016).

The native chickens also play significant socio-economic role in the rural livelihoods by providing readily available source of high-quality animal protein, supplementary income and employment opportunities for the economically weaker sections of society. The backyard poultry farming requires relatively low initial investment and provides regular returns through the sale of eggs, meat and live birds which in turn increase the benefit for small and marginal farmers, women and landless labourers (Kumaresan *et al*., 2008). In many rural households, the women are primarily involved in poultry rearing, which in turn contributes significantly to women's empowerment, household nutrition and livelihood security. Moreover, the indigenous poultry production serves as an important risk-buffering enterprise during periods of crop failure and financial stress which in turn enhance the food security and livelihood resilience among the vulnerable communities (FAO, 2014).

In India, the indigenous chickens constitute an important component of the poultry sector and account for nearly 33 percent of the total poultry population (Government of India, 2019). These native breeds have enormous genetic variability and serve as valuable

reservoirs of adaptive traits such as disease resistance, heat tolerance, broodiness, adaptability to harsh environments and efficient utilization of low-quality feed resources (Horst, 1989). Such characteristics are of immense significance for future breeding programmes aimed at developing climate-resilient and sustainable poultry strains. Furthermore, the indigenous chickens contribute to the rural biodiversity conservation and ecological sustainability through nutrient recycling, pest control and integration into low-input farming systems (FAO, 2010). Therefore, the conservation, characterization and genetic improvement of native chicken breeds are important not only for sustaining rural livelihoods and nutritional security but also for preserving the valuable poultry genetic resources for future generations.

Among the indigenous poultry breeds, Aseel birds are renowned for their hardiness, adaptability, disease resistance and superior meat quality. Despite their importance, the detailed information on regarding the histomorphology and histo-chemistry of the digestive organs especially the caecum, remains limited. The avian caecum is an important component of the gastrointestinal tract which is responsible for microbial fermentation, water absorption, digestion of fibrous feed materials and immune regulation. Gross anatomical observations revealed that the caecum in Aseel birds is paired and consists of three distinct regions such as proximal, middle and distal parts as similar to the findings reported by Thekra and Omar (2023). The paired caeca originate at the junction of the ileum and colo-rectum and extend caudally as blind tubular sacs. Morphologically, the proximal region appears wider and more vascular whereas the distal part gradually narrows towards the apex.

Histologically, the mucosal surface of the caecum is characterized by numerous villi of varying height and shape that increase the absorptive surface area. The villi are lined by simple columnar epithelium interspersed with goblet cells responsible for mucin secretion and protection of the mucosal lining. The abundance of goblet cells indicates active mucus production that facilitates lubrication and protects the intestinal epithelium from mechanical and microbial damage. Beneath the epithelial lining, the lamina propria consists of loose connective tissue richly supplied with blood vessels, lymphocytes and glandular components. The prominent aggregations of lymphatic nodules along with diffuse lymphoid tissues are present within the lamina propria which emphasizes the immunological significance of the caecum (Gal *et al*., 2024).

The presence of organized lymphoid nodules and diffuse lymphatic infiltration confirms that the avian caecum functions as an important gut-associated lymphoid tissue (GALT) which in turn contribute significantly to the local immune defense mechanisms. These lymphoid tissues participate in antigen recognition, immune surveillance and production of immune cells that protect the birds against enteric pathogens. Similar observations were reported by Majeed *et al*. (2009) who described the caecum as a major immunologically active organ due to the extensive distribution of lymphoid tissues within its mucosa and submucosa. Therefore, the structural organization of the caecum in Aseel birds has its dual role in digestion and mucosal immunity; therefore, the detailed histomorphological and histochemical investigations were required to understand the functional biology of indigenous poultry breeds.

Materials and Methods

The young and apparently healthy Aseel birds aged of 8 weeks were procured from a private poultry slaughterhouse for the present investigation. Immediately after slaughter, the abdominal cavity was carefully opened under aseptic conditions and the caecal segments were dissected out. The collected tissue samples were gently washed in normal physiological saline to remove the adhering intestinal contents and blood clots without damaging the mucosal surface. Representative tissue pieces from the different regions of the caecum were trimmed into small sections and fixed in suitable fixatives depending upon the nature of the histochemical and histoenzymatic procedures to be employed.

For the routine histological and histochemical studies, the tissue samples were fixed in neutral buffered formalin and other appropriate fixatives to ensure correct preservation of cellular architecture and chemical constituents. Following fixation, the tissues

were dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax following standard histological procedures. Paraffin sections of approximately 5–6 µm thickness were prepared with use of a rotary microtome and mounted on clean glass slides for histochemical staining. For histoenzymatic studies, fresh tissue samples were processed under cold conditions and cryosections of 15–25 µm thickness were obtained with use of a cryostat microtome to preserve the enzymatic activity (Shetty *et al*., 2020).

The prepared sections were subjected to various histochemical and histoenzymatic staining techniques to demonstrate the distribution of mucopolysaccharides and phosphatase enzymes in the caecal tissue. Combined Alcian blue–Periodic Acid Schiff (AB–PAS) staining technique was employed for the differentiation and localization of acidic and neutral mucopolysaccharides following the method described by Luna (1968). In this method, Alcian blue stains acidic mucopolysaccharides and glycosaminoglycans blue whereas PAS reaction demonstrated the neutral mucopolysaccharides and glycogen as magenta-colored substances. This combined staining technique is particularly useful to identify the nature and distribution of mucosubstances in the epithelial and glandular tissues.

For histoenzymatic localization of phosphatase enzymes, Gomori's lead nitrate method was used to demonstrate the alkaline phosphatase (ALP) and acid phosphatase (ACP) activities according to the procedures outlined by Singh and Sulochana (1996). Alkaline phosphatase activity serves as an important indicator of absorptive and transport functions of epithelial tissues whereas the acid phosphatase activity is associated with lysosomal activity and intracellular digestion. The stained sections were examined under a light microscope and the intensity as well as distribution of histochemical reactions were recorded and interpreted systematically.

Results and Discussion

The lamina propria of the caecum in Aseel birds was consisted predominantly of loose connective tissue and exhibited strong positivity for both neutral and acidic mucopolysaccharides as demonstrated by PAS and Alcian blue staining, respectively (Figure 1). The intense histochemical reaction due to the abundance of glycoconjugates and glycosaminoglycans within the connective tissue matrix which are essential to maintain the tissue hydration, lubrication, structural integrity and mucosal protection. Similar observations regarding the presence of mucosubstances in the intestinal connective tissue have been reported in avian species where these compounds contribute to epithelial protection and immune defense mechanisms.

The villi and Crypts of Lieberkühn also exhibited strong PAS positivity due to the presence of abundant neutral mucopolysaccharides within the epithelial lining and glandular structures. These findings were contrary to the observations of Pandit *et al*. (2018) in Uttara fowl, where comparatively lower PAS activity was reported in the crypt regions. Histologically, the crypts of Lieberkühn were lined by simple columnar epithelium interspersed with goblet cells. The positive PAS reaction was observed in the crypt epithelium which is associated with active secretion of neutral mucins which in turn play an important role to protect the intestinal mucosa from the mechanical injury and microbial invasion.

Prominent lymphatic nodules composed of densely aggregated lymphocytes along with diffuse lymphoid tissue were observed within the lamina propria among the intestinal glands. These lymphoid structures had exhibited positive reactions for both neutral and acidic mucopolysaccharides due to the presence of glycoproteins and mucosubstances associated with immune functions. The collagen fibers surrounding the lymphatic nodules and intestinal glands were strongly PAS positive due to high concentration of carbohydrate-rich extracellular matrix components. The glandular epithelium also showed PAS positivity, whereas the apical surface of the epithelial cells had demonstrated strong affinity for both PAS and Alcian blue staining (Figure 1) due to coexistence of neutral and acidic mucins (Ceccopieri and Madej, 2024)

The Goblet cells were distributed within the intestinal epithelium exhibited intense positivity for both neutral and acidic mucopolysaccharides (Figure 1). The dual staining pattern is due to mixed mucin secretion which in turn important for lubrication, mucosal protection,

maintenance of intestinal pH and prevention of microbial colonization. Similar findings were partially supported by Hawrra *et al*. (2024) who had reported the strong PAS positivity in goblet cells of the caecum in sheep and rabbits. However, Adriana *et al*. (2021) had observed the predominant Alcian blue positivity in goblet cells due to the species-specific variations in mucin composition. The presence of both acidic and neutral mucins in Aseel birds due to higher mucosal adaptability and protective mechanisms in the caecal environment.

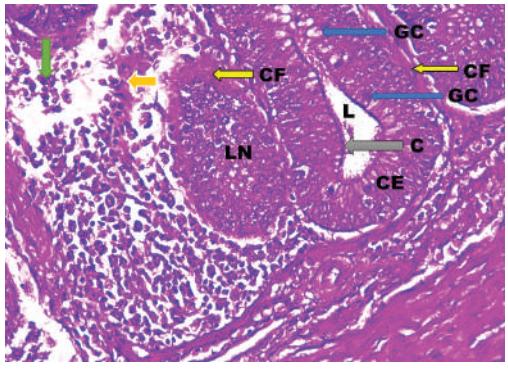

Fig. 1. Photomicrograph of the caecum showing the lamina propria layer. Green arrows indicating the Acid mucopolysaccharide reaction, orange arrow indicating the Neutral mucopolysaccharide reaction in the connective tissue of lamina propria. Presence of intestinal glands in the propria L: Lumen of the intestinal gland, CE: Columnar epiyjelium of the gland, C: Apical surface of the epithelium, GC: Goblet cell, LN: Lymph nodules, CF: Collagen fibres (Combined PAS –Alcian Blue ×400)

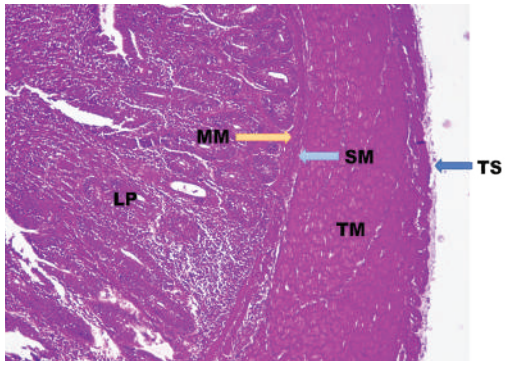

Fig. 2. Photomicrograph of the caecum showing the histological layers stained with PAS – Alcian blue, LP: lamina propria, MM: Muscularis mucosa, SM: Submucosa, TS: Tunica serosa (Combined PAS –Alcian Blue ×100)

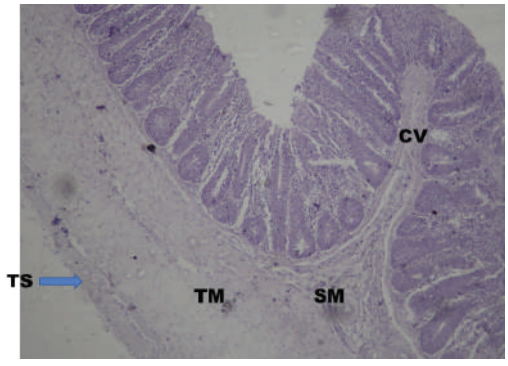

Fig. 3. Photomicrograph of the caecum showing the reaction towards Alkaline phosphatase; CV: Core of Villi, SM: Submucosa, TM: Tunica muscularis, TS: Tunica serosa (Alkaline phosphatase Gomori's method ×100)

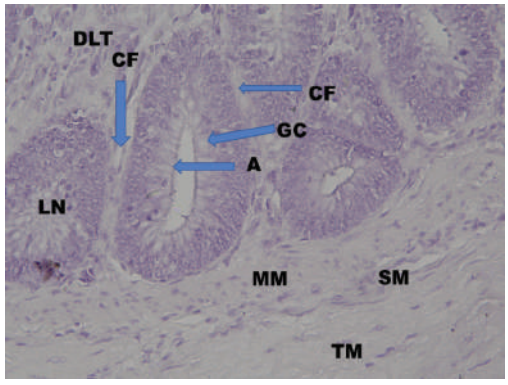

Fig. 4. Photomicrograph of the caecum showing the reaction towards Alkaline phosphatase. A: Apical portion of epithelium, GC: Goblet cells, CF: Collagen fibres surrounding the glands, LN: Lymphatic nodules, DLT: Diffuse Lymphatic tissue, MM: Muscularis mucosa, SM: Submucosa, TM: Tunica muscularis and TS: Tunica serosa (Alkaline phosphatase Gomori's method ×400)

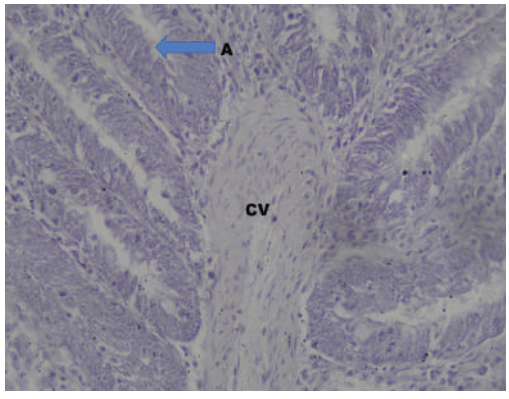

Fig. 5. Photomicrograph of the caecum showing Acid phosphatase activity in the central Villi core (CV) and in the apical portion of intestinal glands (A) (Acid phosphatase Gomori's method ×400)

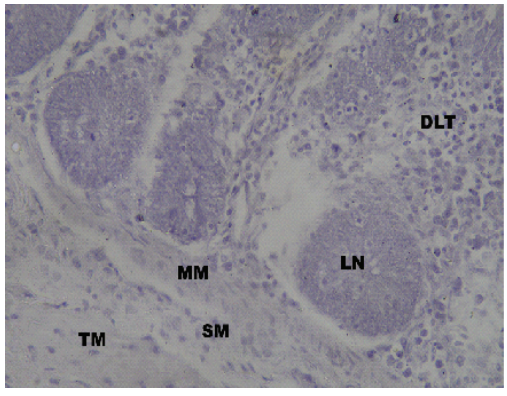

Fig. 6. Photomicrograph of the caecum showing Acid phosphatase activity in the Lymphoid tissues in the lamina propria. DLT: Diffuse lymphatic tissue, LN: Lymphatic nodules, MM: Muscularis mucosa, SM: Submucosa, TM: Tunica muscularis (Acid phosphatase Gomori's method ×400)

The muscularis mucosa was composed of smooth muscle fibers extending into the lamina propria and exhibited strong PAS positivity (Figure 2) due to the presence of carbohydrate-rich glycoproteins associated with muscle connective tissue components. The submucosa which is composed of loose connective tissue and also demonstrated the intense PAS activity. Similarly, the tunica muscularis consisted of inner circular and

outer longitudinal smooth muscle layers which had showed strong PAS positivity (Figure 2). These findings suggested the presence of glycogen and glycoprotein complexes associated with muscular metabolism and structural support. In contrast, the tunica serosa which is formed primarily of loose connective tissue and exhibited only mild Alcian blue positivity (Figure 2) due to lower concentration of acidic glycosaminoglycans. This mild Alcian blue reaction represent the presence of acidic mucosubstances that facilitate the lubrication and reduce the friction between visceral organs and the abdominal wall during intestinal movement (Parisa *et al*., 2019; Anwar *et al*., 2021).

The moderate alkaline phosphatase (ALP) activity was observed in the central core of the villi (Figure 3) due to active absorptive and transport functions within the caecal mucosa. The mild ALP activity was also detected in the lamina propria especially in areas containing diffuse lymphoid tissue (Figure 4). These findings were differed from the observations of Kritima and Opinder (2016) who had reported the moderate ALP activity in certain regions of the tunica mucosa of buffalo fetuses. The central regions of the lymphatic nodules had showed mild positivity for ALP activity (Figure 4) which was contrasts with the findings of Prashant and Nandeshwar (2022) who had reported the strong ALP activity in the caecal tonsils of Khaki Campbell ducks. The relatively lower ALP activity in lymphoid tissues of Aseel birds was die to species-related variations in enzymatic distribution and immune activity.

The apical surface of the glandular epithelium had exhibited strong alkaline phosphatase activity due to active membrane transport and absorptive functions. The Goblet cells had showed moderate ALP reactivity whereas the collagen fibers surrounding the glands had demonstrated only mild enzymatic activity. The muscularis mucosa and tunica muscularis had exhibited mild ALP reactions (Figure 4) due to lower metabolic and transport activity within the muscular layers (Rana *et al*., 2016).

The acid phosphatase (ACP) activity was moderately localized within the central villus core and the apical portions of the columnar epithelium lining the intestinal glands (Figure 5). The moderate ACP activity generally indicates the lysosomal activity and intracellular digestion associated with cellular turnover and phagocytic processes. The lymphatic nodules and diffuse lymphoid tissues within the lamina propria also had demonstrated the moderate ACP activity (Figure 6). This enzymatic activity is associated with macrophage-mediated phagocytosis and degradation of engulfed pathogens due to the immunological role of the caecal lymphoid tissue. However, Prashant and Nandeshwar (2022) had reported strong ACP activity in the lymphatic nodules of Khaki Campbell ducks due to species differences in immune enzyme localization.

The muscularis mucosa, submucosa and tunica muscularis also exhibited the moderate ACP activity (Figure 6) which are associated with lysosomal enzyme systems in tissue remodeling, cellular metabolism and maintenance of muscular integrity. Thus, the histochemical and histoenzymatic distribution patterns observed in the caecum of Aseel birds due to well-developed mucosal defense system and active absorptive and immunological functions essential to maintain the intestinal homeostasis (Rus *et al*., 2025).

Conclusion

The specialized distribution of glycoconjugates and phosphatase enzymes within the caecum due to highly organized functional adaptation that supported both the digestive and immunological activities. These histochemical and enzymatic components contributed to the mucosal protection, nutrient absorption, epithelial maintenance and local immune defense, thereby enabling the intestine to efficiently utilize nutrients where simultaneously protect the host against potentially harmful pathogens and environmental challenges.

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