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## Priyanka

Department of Veterinary  
Anatomy, College of Veterinary  
and Animal Science, Rajasthan  
University of Veterinary and  
Animal Sciences, Bikaner,  
Rajasthan, India

## Pankaj Kumar Thanvi

Department of Veterinary  
Anatomy, College of Veterinary  
and Animal Science, Rajasthan  
University of Veterinary and  
Animal Sciences, Bikaner,  
Rajasthan, India

## Sanwar Mal

Department of Veterinary  
Anatomy, Shekhawati  
Veterinary College, Sikar,  
Rajasthan, India

## Pura Ram

Department of Veterinary  
Anatomy, Apollo College of  
Veterinary Medicine, Agra Road,  
Jamdoli, Jaipur, Rajasthan,  
India

## Raj kumar Siyag

Department of Veterinary  
Anatomy, Apollo College of  
Veterinary Medicine, Agra Road,  
Jamdoli, Jaipur, Rajasthan,  
India

## Corresponding Author

### Priyanka

Department of Veterinary  
Anatomy, College of Veterinary  
and Animal Science, Rajasthan  
University of Veterinary and  
Animal Sciences, Bikaner,  
Rajasthan, India

## Histochemical studies on the proventriculus and gizzard of white leghorn

**Priyanka, Pankaj Kumar Thanvi, Sanwar Mal, Pura Ram and Raj Kumar Siyag**

### Abstract

The present study was conducted on 8-10 white leghorn chicken. The tissues were collected from the recently died birds from both genders. The tissues for histological and histochemical studies were collected from the proventriculus and gizzard. The lining epithelium, lamina propria, lamina muscularis of proventriculus and gizzard showed moderate positive reaction for carbohydrates in PAS stain. After treatment with the saliva, lining epithelium, lamina propria and tunica muscularis layer showed negative reaction for carbohydrates in PAS stain. Mucosubstances showed strong positive reactions in lamina propria, lamina muscularis and in submucosa.

**Keywords:** Proventriculus, gizzard and PAS stain

### Introduction

In birds the rod shaped proventriculus is located between the Oesophagus and the Ventriculus. Proventriculus is a glandular segment of the stomach that store food and initiates the digestion before moving it to the gizzard. Primarily proventriculus secretes hydrochloric acid and pepsinogen which helps the churning of food material that take place in ventriculus. Pepsinogen produces pepsin and it smashes the peptide bonds of amino acid of food material (Syihus Birger 2014) [13].

In birds' teeth and taste buds are absent, due to absence of teeth and taste buds birds eat hard items such as seeds and nuts. Birds with thick gizzards frequently pickup grit, little stones, sands and small shells that are collected in gizzard. When food and digestive juices enter the gizzard, the thick muscles and grit help grinding the food (McLelland 1975) [7].

The available literature revealed that the work on the gross and microscopic structure of the gizzard and proventriculus of white leghorn is still meagre. The present investigation has been planned to explain the gross and histological aspects of proventriculus and gizzard of white leghorn. The findings of this study will be useful to clinicians and para-clinician for the diagnosis and treatment of various ailments of this organ.

### Materials and Methods

The present research was carried out on 20 carcasses of adult white leghorn birds. Proventriculus and gizzard of recently dead birds were procured from the poultry farm of RAJUVAS, Bikaner. Histochemical studies on the research samples were conducted in the department of anatomy, CVAS, RAJUVAS, Bikaner. For light microscopic studies, the samples were fixed in 10% formalin for routine staining method and in Bouin's fluid for special staining for 48 to 72 hours and up to 18 hours respectively. The tissue was processed by Alcohol-xylene method using cedar wood oil (Luna.1968) [5], paraffin blocks were prepared and numbered. Sections of 5-6 m thickness were obtained then placed on albumenized slides and kept overnight in a hot air oven at 36 °C and finally stained for general Histomorphological and histochemical observations.

**Table 1:** The following stains were used in this study.

1.	McManus's method with saliva (PAS)	For glycogen	Singh and Sulochana, 1997.
2.	Mc Manus's method-PAS	For glycogen.	Singh and Sulochana, 1997.
3.	PAS- Alcian blue method pH 1.0	For Mucosubstances	Luna, 1968. [5]
4.	PAS- Alcian blue method pH 2.5	For Mucosubstances	Luna, 1968. [5]

**Results and Discussion**

**Mc Manus's method for Glycogen (PAS)**

The proventricular mucosa showed PAS positive reaction for glycogen that showed magenta colour. This was result was dissimilar with the findings of Zhu (2015b) [14]. The epithelium cells lining of proventriculus as a cavity was observed as the simple tubular glands which shown a weak positive reaction with PAS stain. The submucosal glands showed instance positive reactions (Fig. 12 and 16), according to Das *et al.* (2017) [2], the PAS reaction was moderate in the tunica mucosa of proventriculus of kadaknath fowl. The lining epithelium and lamina propria of gizzard showed strong positive reaction (Fig. 16). The result was similar with the Saffar and Samawy (2015) [10] in Mallard. The tunica submucosa, tunica muscularis also showed intanse positive reactions for carbohydrates (Fig. 15).

**McManus's method with Saliva for Glycogen (PAS)**

After treatment with saliva the proventricular mucosa, submucosa and tunica muscularis showed PAS negative reaction (Fig. 14). The result was dissimilar with Demirbag *et al.* (2015) [4], Any reactivity to Periodic Acid-Schiff (PAS) staining was not observed in both epithelium and glands of sparrow proventriculus. After treatment with saliva the mucosa of gizzard showed negative reaction for carbohydrates because glycogen was digested by human saliva. The tunica submucosa and tunica muscularis also showed negative reactions for carbohydrates after treatment with saliva (Fig. 10, 11 and 13). The result was similar with the reports of Pastor *et al.* (1988) [9] in chicken.

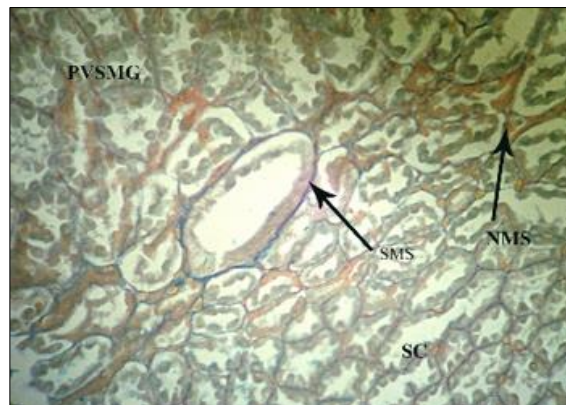
**PAS- Alcian blue reaction at pH- 1.0**

Submucosa of proventriculus showed moderate PAS reaction (Fig. 1). Red colour showed neutral mucosubstances and blue colour showed sulphated mucosubstances. The finding of present study was in conformity with the reports of Selvan *et al.* (2008) [11] in guinea fowl, Malwadkar (2013) [6] in Indian pond heron. The result was dissimilar with the Nahla *et al.* (2011) [8] the tunica submucosa was thick and consisted of the deep proventricular glands and these glands were shown negative or weak reaction with Alcian blue or PAS in cattle egret. The tunica mucosa, submucosa and muscularis of gizzard showed dark blue colour which indicates the moderately positive reaction for Sulphated mucosubstances (Fig. 2, 3, 4 and 5).

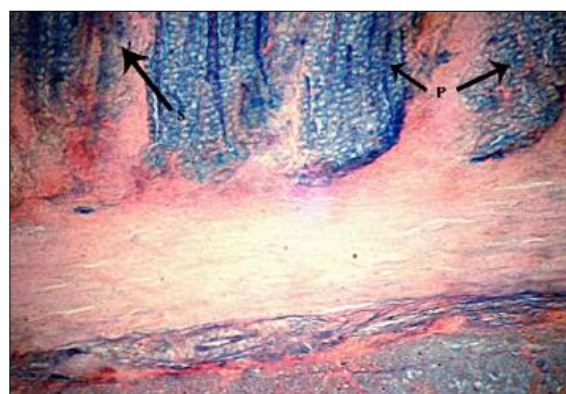
**PAS- Alcian blue reaction at pH- 2.5**

The epithelium of proventriculus showed an instance positive reaction. The finding of present study was in accordance with reports of Ahmed *et al.* (2011) [1] in Japanese quail. The submucosa showed moderate positive reaction (Fig. 8 and 9). Sulphated mucosubstances showed dark blue colour and neutral mucosubstances showed red colour. The finding of present study was in accordance with reports of Zhu (2015 b) [14] in black tailed crane. Tunica mucosa and tunica muscularis of gizzard showed strong positive reaction, whereas tunica submucosa showed moderate positive reaction for PAS Alcian blue pH- 2.5 stain (Fig. 6 and 7). Reddish

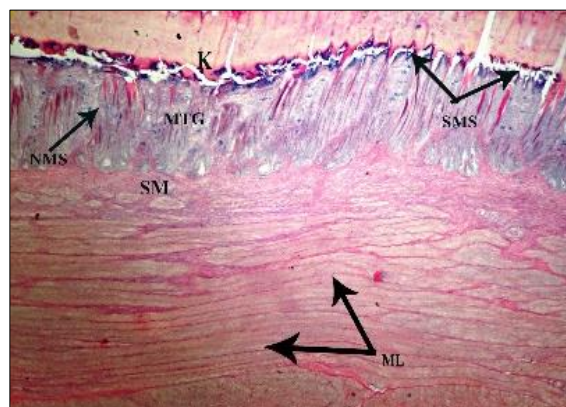
colour for neutral mucosubstances and dark bluish colour for acidic mucosubstances. The finding of present study was in accordance with reports of Nahla *et al.* (2011) [8] in cattle egret, Zhu (2015 b) [14] in black tailed crane.



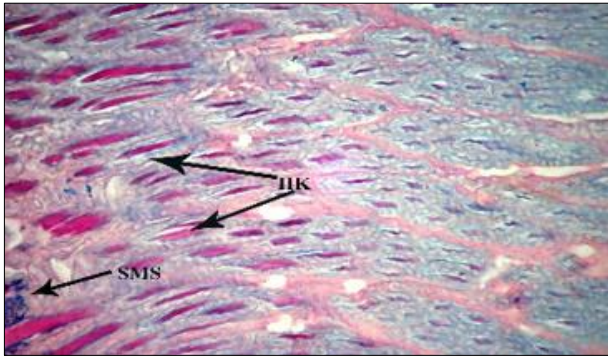
**Fig 1:** Photomicrograph of proventriculus showing PAS positive reaction in proventricular glands. S MS-sulphated mucosubstances. NMS-neutral mucosubstances SC-proventricular secretory cells. PAS alcian blue pH.0.400x



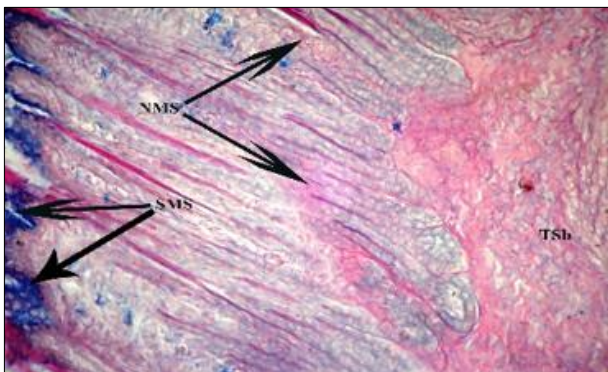
**Fig 2:** Photomicrograph of gizzard showing PAS positive reaction, P-Plicae Ventriculares, S- sulci b/w mucosal folds PAS alcian blue pH1.0, 400x



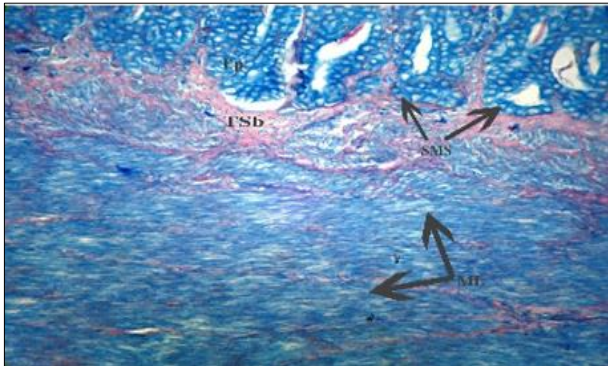
**Fig 3:** Photomicrograph of gizzard showing PAS Positive reaction in different layers K- Koloin, SMS-sulfated mucosubstances, SM-submucosa, NMS- neutral mucosubstances, MTG- mucosal tubular glands, ML- Muscle layer. PAS – Alcian blue stain for mucosubstances pH1.0, 100x



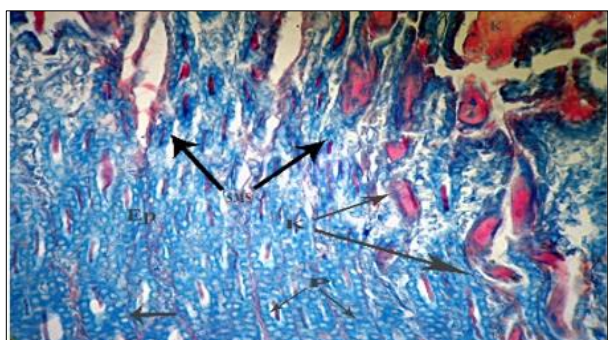
**Fig 4:** Photomicrograph of gizzard mucosal epithelial glands showing PAS positive reaction. HK- horizontal kaolin, SMS- sulphated mucosubstances. PAS Alcian blue P<sup>H</sup> 1.0, 400x



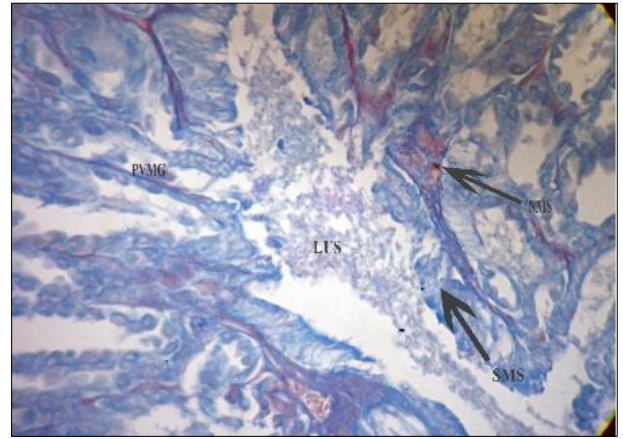
**Fig 5:** Photomicrograph of gizzard showing PAS positive reaction NMS- neural muscosubstances, SMS- sulphated mucosubstances, TSB- tunica submucosa PAS Alcian blue PH 1.0, 100x



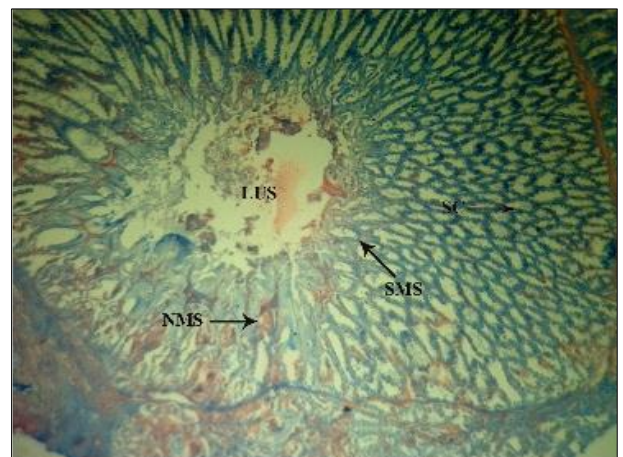
**Fig 6:** Photomicrograph of gizzard different layers showing PAS positive reaction Ep- mucosal epithelial gland, Tsb- Tunica submucosa, SMS- sulphated mucosubstances, ML- muscular layer. Alcian blue pH 2.5, 400x



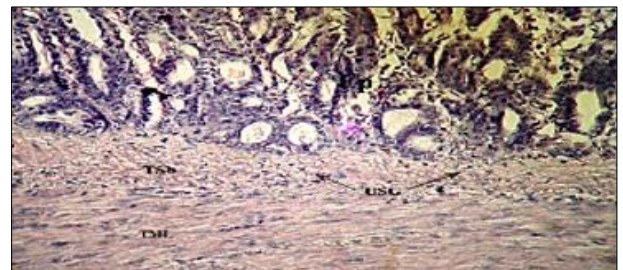
**Fig 7:** Photomicrograph of gizzard mucosal epithelial glands PAS – Positive reaction, Ep – mucosal epithelium, K- Kolin, P- Plaecae of gland, Black arrow show lining epithelium of mucosal gland, SMS – Sulphated mucosubstances Alcian blue pH 2.5, 400x



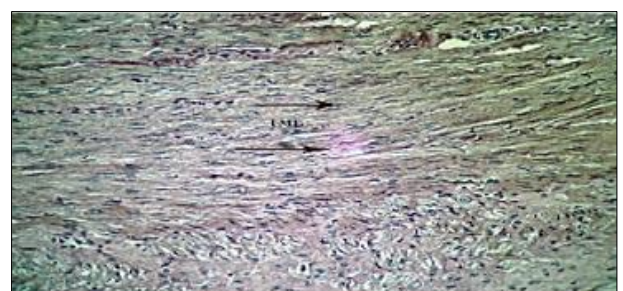
**Fig 8:** Photomicrograph of proventricular showing PAS positive reaction in mucosal glands, LUS- luminal surface, SMS- sulphated mucosal glands, NMS- neutral mucosubstances, PVMG- proventricular mucosal glands Alcian blue pH 2.5, 400x



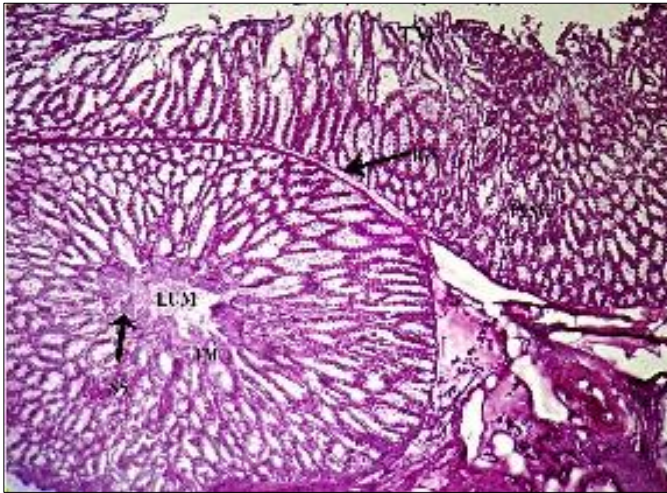
**Fig 9:** Photomicrograph of proventriculus showing PAS positive reaction LUS- luminal surface, SMS- sulphated mucosubstances, SC- secretory cells. PAS Alcian blue pH 2.5, 400x



**Fig 10:** Photomicrograph of gizzard showing PAS negative reaction in different layer. Ep – mucosal epithelium, Tsb – tunica submucosa, TML – Tunica muscularis layer, USG – Unstain glycogen PAS with Saliva stain, 400x

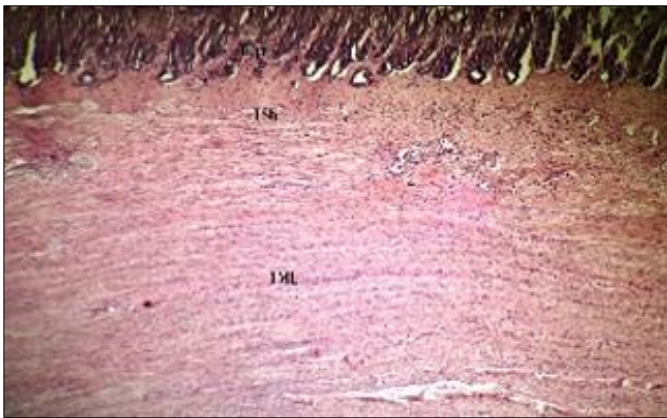


**Fig 11:** Photomicrograph of gizzard showing PAS negative reaction in tunica muscularis layer PAS with Saliva stain, 400x

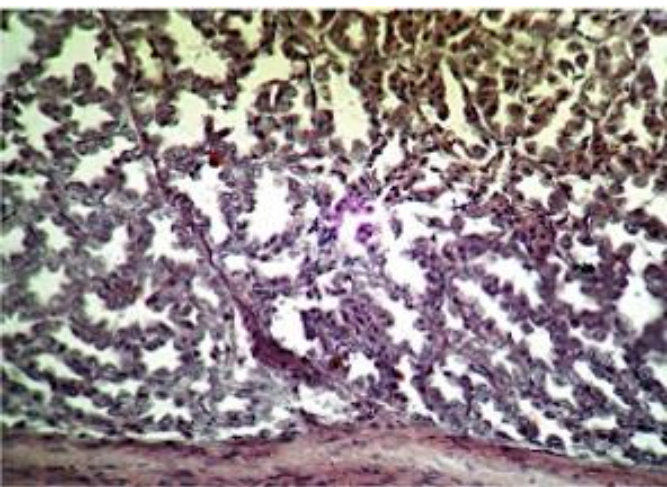


**Fig 12:** Photomicrograph of proventriculus showing PAS reaction Without saliva, LUM – luminal surface, IGS- interglandular septa PVSG- proventricular secretory glands, SS – secretory substances, TM- tunica mucosa PAS – without saliva, 100x

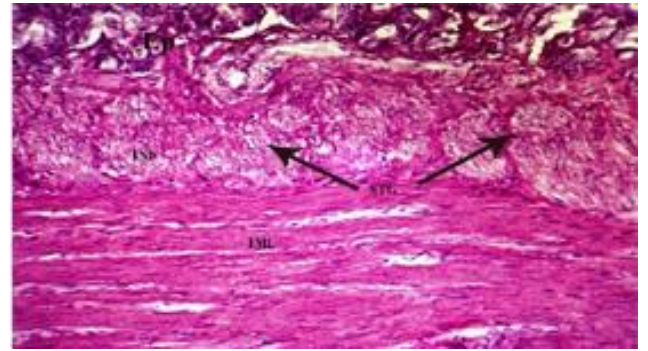
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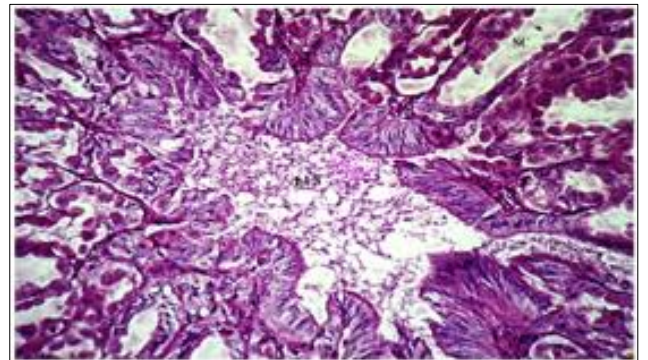
**Fig 13:** Photomicrograph of gizzard showing PAS saliva negative Reaction in different layers. EP – mucosal epithelium, TSb- Tunica submucosa, TML – tunica muscularis layer. PAS – with saliva stain, 100x



**Fig 14:** Photomicrograph of proventricular submucosal gland showing PAS negative reaction with saliva. PAS with Saliva stain, 400x



**Fig 15:** Photograph of gizzard showing PAS positive reaction without saliva in tunica mucosa, runica submucosa and muscular layer, Ep-mucoal epithelium, TSb-Tunica submucosa, TML- tunica Muscularis layer, STG – stain glycogen. PAS without Saliva strain 400x



**Fig 16:** Photomicrograph of proventricular gland showing PAS positive reaction without saliva. LUS- luminal surface of glands, SC- secretory cells. PAS without Saliva stain 400x

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