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Comparative Glycoconjugates Histochemistry of the Proventriculus of the Chicken, Ducks and Geese

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ABSTRACT

A lectin histochemical study was performed on formalin-fixed paraffin-embedded tissue sections of proventriculus from three mature chickens, three mature ducks and three mature geese. The purpose of this study was to focus on the comparative glycoconjugates of the chicken, ducks and geese proventriculus. The distribution of glycoconjugates in the proventriculus of chicken, ducks and geese was studied using eight (LCA, ConA, PNA, RCA 120, WGA, DBA, UEA and PHA-E4) horse raddish peroxidase labeled lectins and the peroxidase activity was visualized by Dako cytomation (DAB). The neutral mucopolysaccharides were restricted on the supranuclear part of the epithelium of the mucosal folds (plicae) and negative in the basal part (sulci) epithelium in all species under investigation. The examined parts of the proventriculus (plicae epithelium, sulcui epithelium, ducts of the glands and glandular tissue) were labeled to all lectins used in case of geese. The ducks proventriculus showed expression to all lectins except glandular duct epithelium with D glucose, N acetylegalactosamine and L fucose. The proventriculus of chicken was labeled to all lectins except DBA which bind to N acetyle galactosamine. Moreover, the glandular tissue and sulci epithelium were negative to D glucose and D galactose respectively. In conclusion, all investigated species showed neutral mucopolysaccharides with different sugar residues to protect the mucosa and glandular tissue from the harmful effect of the acid content.

1. INTRODUCTION

The stomach structure of birds presents variations that depend on the dietary habits of each species (Turk, 1982; Ogunkoya and Cook, 2009). The proventriculus and gizzard constitute the first important site of enzyme activity. The main function of proventriculus is production of gastric juice and propulsion of juice and food in to the gizzard, which is the main site of gastric proteolysis (Colin, 2015).

Mucin forms a gel layer over the epithelial surface as a lubricant and protective barrier to physical damage by the luminal contents (Neutra and Forstner, 1987). Carbohydrates on epithelial cell surfaces play an important role as attachment sites for different microorganisms like bacteria and viruses (Pohlmeyer et al., 2005). In recent years, lectin histochemistry has developed into a useful tool to study various aspects of cell differentiation and cell-to-cell interaction (To¨pfer-Petersen, 1999; Gabius, 2001). It is significant that most plant and animal lectins have been classified into a rather limited number of carbohydrate-binding groups (Goldstein and Poretz, 1986). These include the mannose/glucose- binding lectins, the galactose-binding lectins, the N-acetylgalactosamine-binding lectins, the N-acetylglucosamine-binding lectins, the L-fucose binding lectins, sialic acid-binding lectins, and lectins with complex carbohydrate-binding sites. Glycoconjugates, which are large glycoprotein secreted by mucous cells, play important roles in gastric defense mechanisms against aggressive factors such as gastric acids, pepsin and pathological microorganisms (Kunisaki and Sugiyama, 1992). So, the aim of our study is to focus on the comparative

glycoconjugates of chicken, duck and geese proventriculus.

2. MATERIALS AND METHODS

Cross section samples of the proventriculus of the chicken, ducks and geese were fixed in bouin's solution for 18-24 hours at room temperature. The samples were extensively washed in 70% ethanol. Thereafter, the samples were dehydrated in graded series of ethanol (80%, 90%, 95% and absolute), cleared in lemosol and embedded in paraffin wax. Sections 3-5 microns thickness were mounted on uncoated and coated slides with 3-aminopropyltrieth-oxy-silane.

2.1. Conventional histochemistry:

For general description of glycolconjugates, the slides were stained with Periodic Acid Schiff (PAS) for neutral mucopolysaccharides (Bancroft and Stevens, 1996).

2.2.Lectin histochemistry:

Lectin binding sites were demonstrated by means of horse radish peroxidase (HRP). The slides were deparaffinized, then rehydrated using descending grades of ethanol until distilled water. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide (H2O2) in methanol for 30 minutes at room temperature, thereafter the sections were incubated in 1% goat serum albumin (DAKO, USA) in phosphate buffer saline (PBS) for 20 minutes to minimize nonspecific staining. Subsequently, the slides were incubated with HRP conjugated lectins (J Oil Mills, Tokyo, Japan) for one hour at room temperature. Lectins were generally used at concentrations 5- 20 µg/ml PBS pH 7.6 (Table 1). All sections were washed in PBS 3 times (3x5min.). The peroxidase activity was visualized by Dako cytomation (Liquid DAB; 3, 3' diaminobenzidine; and substrate chromogen system) (DAKO, USA) for 30 minutes at room temperature. Finally, the sections were washed in distilled water (3x5min.), counterstained with Meyer's haematoxylin, dehydrated, and mounted with Mount-Quick (Daido Sangyo co., Japan). Lectin specificities were adopted to Debray et al. (1981), Spicer and Schulte (1992) and Danguy (1995).

To examine the specificity of lectin staining, control sections were prepared by one of the following: addition of inhibitory sugars to the respective lectin solution, substitution of unconjugated lectins for the horse radish and finally the exposure of sections to Dakocytomation without lectins. The staining intensity was classified by two independent observers into 4 categories: no labeling (-ve), weak labeling (+), moderate labeling (++), and strong labeling (+++).

Table 1: the lectins used and their sugars binding specificities:

Lectin group*		Name	Sugar binding specificity	Concentration	Binding	Inhibitor sugar
Glucose binding lectins	LCA	Lens culinaris	α-Man	5µ/ml	HRP	Man
	Con A	Concanavalin A	α-D- Man, α-D-Glc	10µ/ml	HRP	Man
Galactose binding lectins	PNA	Peanut agglutinin	Gal β1-3GalNAC	20µ/ml	HRP	Gal
	RCA 120	Ricinus communis 120	Gal β1-4GlcNAC	5µ/ml	HRP	Gal
Glucosamine binding	WGA	Wheat germ agglutinin	β-D-GlcNAC	5µ/ml	HRP	GlcNAC
Galactosamine binding	DBA	Dolicos biflorus	GalNAC α 1-3 GalNAC	20µ/ml	HRP	GalNAC
Fucose binding lectin	UEA-1	Ulex europaeus -1	α-L-Fuc	20µ/ml	HRP	Fuc
Non specific	PHA-E4	Phaseolus vulgaris	D-GalNAC	5µ/ml	HRP	GalNAC

Gal: Galactose, GalNAC: N-acetylgalactosamine, Glc: glucose, GlcNAC: Nacetylglucosamine, Fuc: fucose, Man: mannose.

3. RESULTS

The neutral mucopolysaccharides were restricted on the supranuclear part of the epithelium of mucosal folds (plicae) and negative in the basal part (sulci) epithelium in the species under investigation. The reaction neither demonstrated in the glandular tissue in all species nor the duct of the gland in chicken, while the duct epithelium of duck was strongly positive and only brush border of the duct cells in geese was labelled (panel 1; Fig. A, B,C).

3.1.Glycohistochemical finding

The distribution of glycoconjugates in the proventriculus of chicken, duck and geese was studied using eight (LCA, ConA, PNA, RCA 120, WGA, DBA, UEA and PHA-E4) horse raddish peroxidase labeled lectins as shown in (Table 1). The binding sites of lectins on the epithelium of plicae, duct of submucosal gland and glandular tissue were varied according to the type of lectin as shown in (Table 2). The connective tissue was stained with all lectins under investigation and the variation is as follow:

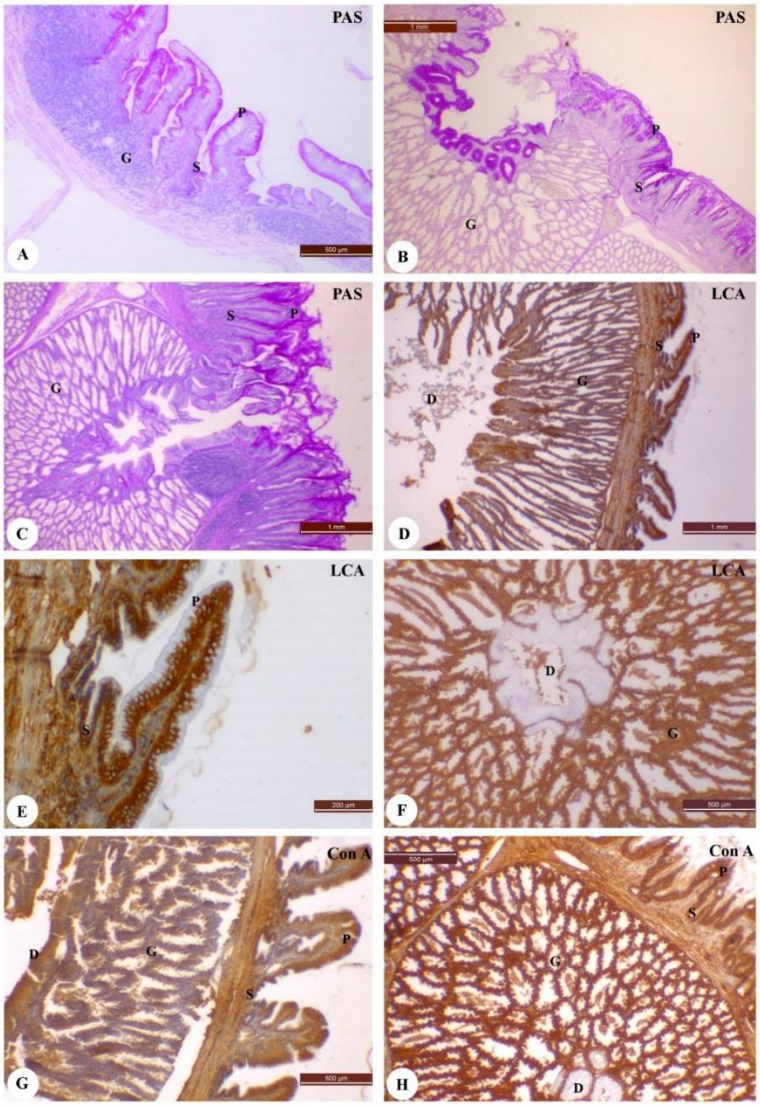
3.2.D-glucose binding lectins

As demonstrated by LCA and ConA, the binding sites in the epithelium of the plicae were confined to the perinuclear and basal region and weak to absent in the supranuclear region in all species under investigation. The epithelium of the sulci was more intense in duck. The glandular tissue showed no binding sites in chicken while in duck and geese were labeled and be more intense in geese. The duct of submucosal gland was labeled in chicken and geese but unlabeled in duck. The reaction in the epithelium of duct in chicken was confined to the perinuclear and basal part of the cells while be diffuse in the geese (panel 1; Figs: C, D, E, F, G).

3.3. Galactose binding lectins

As shown by PNA and RCA 120, the galactose binding site was exclusively observed in the perinuclear region of epithelium of plicae and sulci of chicken and duck and in the supranuclear

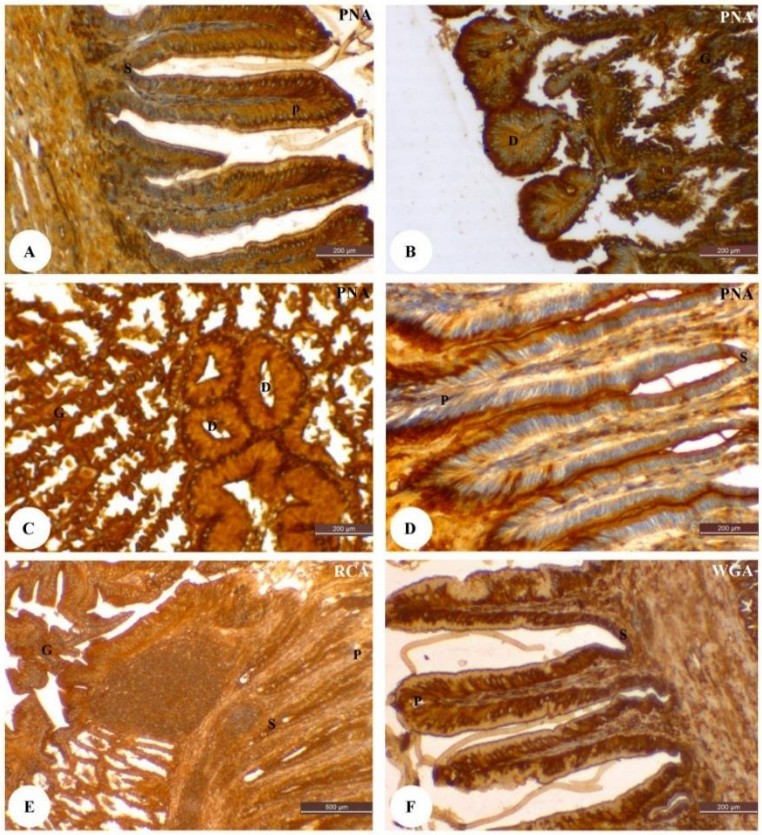
region in geese. The brush border in the epithelium of plicae showed an intense reaction in all species. The glandular tissue was labeled with PNA and RCA 120 in all species, although the geese glands showed labeling in the cells boundaries. The duct epithelium showed moderate to intense reaction to these lectins (panel 2; Fig. A, B, C, D, E).



Panel 1. Histomicrograph of the proventriculus of chicken (A), ducks (B) and geese (C) showing PAS positive reaction in the surface epithelium of plicae (P) and sulci epithelium (S) with negative reaction to sub mucosal glands (G). D-glucose binding lectin (LCA) in the chicken proventriculus (D) showing positive reaction to the plicae (P) and sulci epithelium (S) as well as the duct of sub mucosal glands (G). D-glucose binding lectin (LCA) in the duck proventriculus (E) showing positive reaction in the peinuclear and basal reigions of plicae (P) and sulci epithelium (S) as well as the duct of submucosal glands (G). Moreover the submucosal glands of duck (F) showed positive reaction (G) while the duct epithelium is unlabelled (D). D-glucose binding lectin (ConA) in the duck proventriculus (G) showing positive reaction in the plicae (P) and sulci epithelium (S) as well as the glandular duct epithelium (D) while the glandular tissue (G) is unlabelled. D-glucose binding lectin (ConA) in the geese proventriculus (H) showing positive reaction in the plicae (P) and sulci epithelium (S) as well as the glandular tissue (G) while the glandular duct (D) is unlabelled.

3.4. Glucosamine binding lectin

As demonstrated by WGA, the glucosamine binding sites was observed in the brush border and perinuclear and basal part of cells of the plicae in chicken and duck, while it was diffuse in these cells in the geese. The glandular tissue was diffusely labeled with WGA in duck and geese, while labeled in the cells boundaries in chicken. The epithelium of submucosal gland duct was diffusely labeled in duck and geese, while showed intense reaction in the apical part of the cells in chicken (panel 2; Fig, F) and (panel 3; Fig. A).



Panel 2. Histomicrograph of galactose binding lectin (PNA) of duck proventriculus (A) showing positive reaction in the peinuclear part of the plicae (P) of the mucosal folds and negative reaction in the sulci epithelium (S). Galactose binding lectin (PNA) of geese proventriculus (B) showing positive reaction in the boundaries of glandular tissue (G) and the supranuclear part of glandular duct (D). Galactose binding lectin (PNA) of chicken proventriculus (C) showing positive reaction in both glandular (G) and duct epithelium (D). Galactose binding lectin (PNA) of geese proventriculus (D) showing positive reaction in supranuclear and secretory material of plicae (P) as well as weak reaction in the sulci epithelium (S). Galactose binding lectin (RCA 120) of duck proventriculus (E) showing positive reaction in perinuclear and supranuclear parts of plicae (P) and sulci (S) epithelium as well as glandular (G) and duct (D) epithelium. Glucosamine binding lectin (WGA) of chicken proventriculus (F) showing positive reaction in perinuclear and basal parts of plicae (P) and sulci (S) epithelium.

3.5. Galactosamine binding lectin

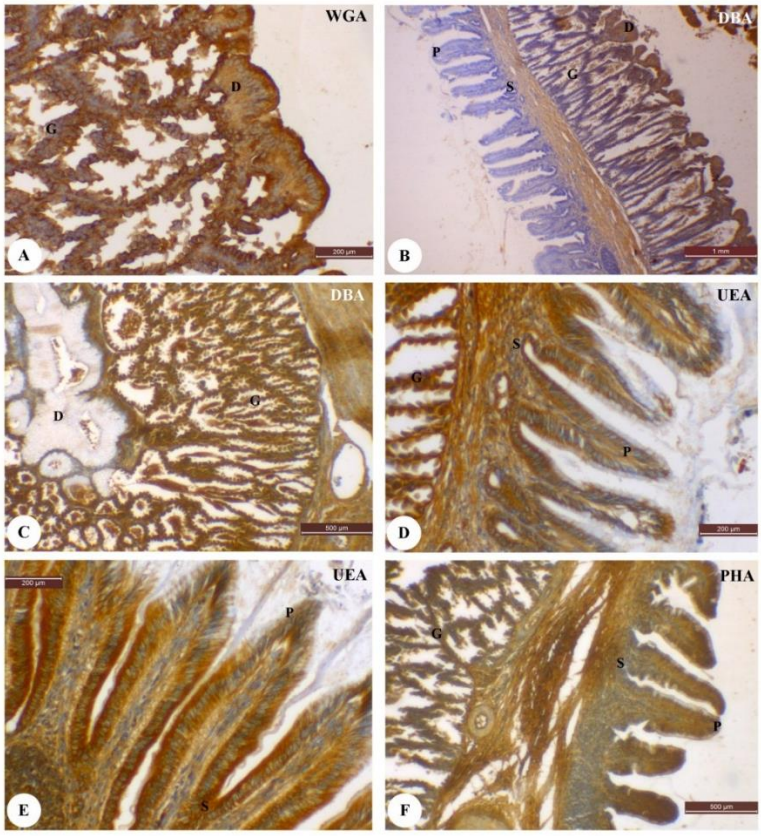
As shown by DBA, the galactosamine binding sites were diffuse in the epithelium of the sulci of duck and geese as well as in the epithelium of the plicae in geese, while that of duck the conjugation was in the perinuclear and apical part of epithelium. The whole plicae and sulci epithelium was unlabeled in chicken. The glandular tissue was intensely labeled in duck and geese while in chicken, it was unlabeled. The duct epithelium was labeled in chicken and geese, while in duck, it was unlabeled (panel 3, Fig. B, C).

3.6. Fucose binding lectin

The lectins of this group represented by UEA showed intense reaction to the perinuclear and basal part of epithelium in the plicae and sulci in chicken and duck, while it was diffuse in geese. The glandular tissue was conjugated with this lectin in all species. The submucosal gland duct exhibited intense reaction in chicken and geese, while in duck, it was unlabeled (panel 3; Fig. D, E).

3.7. Lectin with complex carbohydrates binding sites

From this group PHA-E4 was intensely conjugated with perinuclear and basal part of epithelium of the plicae and sulci in all species except geese where the sulci epithelium observed diffuse in reaction and chicken sulci epithelium was negative. The glandular tissue was moderate labeled in duck and geese, while in chicken, it was unlabeled. The duct reacted intensly with this lectin in chicken and geese, while in duck, it was unlabeled (panel 3; Fig. F).



Panel 3. Histomicrograph of Glucosamine binding lectin (WGA) of chicken proventriculus (A) showing positive reaction in the apical part of glandular duct (D) and the cell boundaries of glandular tissue (G). Galactosamine binding lectin (DBA) of chicken proventriculus (B)showing positive reaction in the ducts of glands (D) and negative reaction in the glandular tissue (G), plicae (P) and sulci (S) of mucosal fold. Galactosamine binding lectin (DBA) of duck proventriculus (C) showing positive reaction in the glandular tissue (G) and negative reaction in the duct epithelium (D). Fucose binding lectin (UEA) in the geese proventriculus (D) showing diffuse positive reaction in the plicae (P) and sulci (S) epithelium as well as glandular tissue (G). Fucose binding lectin (UEA) in the duck proventriculus (E) showing positive reaction in the apical part of plicae (P) and sulci (S) epithelium. The nonspecific carbohydrates binding lectin (PHA-E4) in the duck proventriculus (F) showing positive reaction in the perinuclear and basal part of plicae (P) and sulci (S) epithelium as well as glandular tissue (G).

Table 2: lectin-binding sites of proventriculus of chickens, ducks and geese

Lectin group	Acronym	Chickens				Ducks				Geese			
		PE	SE	GDE	GE	PE	SE	GDE	GE	PE	SE	GDE	GE
I. D-Glucose binding lectins	LCA	++	+++	++	-	++	+++	-	+	++	++++	+++	++
	Con A	++	+++	+++	-	++	+++	-	+	++	++++	++++	++
II. D-Galactose binding lectins	PNA	+++	-	+++	++	++	+++	+++	++	++++	+++	+++	+
	RCA 12o	+++	-	+++	++	++	+++	+++	++	+++	+++	+++	++
III. N-acetyl D-glucosamine (GlcNAc) binding lectin	WGA	++	+++	++	+	++	+++	++	++	++	+++	++++	++
IV. N-acetyl D-galactosamine (GalNAc) binding lectin	DBA	-	-	+	-	+++	+++	-	+++	+++	+++	++++	+++
V. L-Fucose binding lectin	UEA-1	+	+	+++	++	+	++	-	+	+	+++	+++	++
VI. lectins with complex carbohydrates-binding sites	PHA-E4	++	-	+	-	+++	++++	-	+++	+++	+++	+++	++

PE: plicae epithelium, SE: sulci epithelium, GDE: glandular duct epithelium, GE: glandular epithelium.

4. DISCUSSION

In mammals and birds, the mucosa of the stomach is covered by a layer of thick mucus, which is vital to protect the cell linings of that organ from the highly acidic environment within it. The major macromolecular constituents of normal mucus are the mucins. Mucins are complex carbohydrate secreted by different types of epithelial cells and glandular tissues of alimentary tract (Cheah and Ramachandran, 1994).

The proventriculus has two types of glands: the superficial simple tubular glands and the profound proventricular glands, which are lined by oxynticopeptic cells. The oxynticopeptic cells secrete both hydrochloric acid and the enzyme precursor pepsinogen, hence combining the functions of the mammalian peptic and parietal cells (Hodges, 1974). Hydrochloric acid, mucus and a digestive enzyme called pepsin are secreted in the proventriculus and start the process of breaking down the structure of the food material the bird has eaten (Liman et al., 2010).

Our results showed the presence of neutral mucopolysaccharides demonstrated by PAS on the surface epithlium, plicae epithlium and their absence in the sulci epithlium and glandular tissue in all species under investigation. These results refer to a major role of neutral mucopolysaccharides in the protection of the lining epithlium of the proventriculus. These finding coincide with that of Ogunkoya and Cook (2009) who stated that the epithelium is covered with a layer of mucins which protects it from chemical, enzymatic and mechanical damage and pathogenic microorganisms. Gastric mucins of birds present variations that depend on the dietary habits of each species (Zhu, 2015).

PAS positive mucin granules were found to occupy the supra nuclear area of the cells as reported by our results and Colin , 2015in domestic fowl and Shyla et al. (1992) in ducks. These granules were found to be abundant in the cells lining the apical part of the plica than in the cells lining the sulci (Senthamil Selvan et al., 2008). Epithelial mucins can be subdivided into secretory and membrane-associated forms. The secretory forms are synthesized, stored, and then released by exocytosis from the apical surface of secretory cells to form the overlying mucus gel. Moreover, the secreted mucins are divided into two subgroups, gel-forming and soluble mucins. In contrast, the membrane associated forms have a hydrophobic membrane-spanning domain that serves to anchor them to the plasma membrane (Hattrup & Gendler, 2008; Thornton et al. 2008).

Our results demonstrated that, the sugars residues in the surface epithelium, plicae epithlium and sulci epithelium showed positive expression of D-glucose, D-galactose, N-acetyleglucosamine, N-acetylegalactosamine and L-fucose in all species under investigation, except N-acetylegalactosamine in chicken. The lectins binding sites showed that D-glucose, D-galactose, N-acetyleglucosamine, N-acetylegalactosamine and L-fucose in the apical cytoplasm of the cells of the plicae and sulci epithelium are correlated to the Golgi area described by the electron microscope. This is considered the area of the organelle responsible for adding carbohydrates to the secreted protein (Aire, 2002).

Our results showed that the glandular epithelium is labeled to all lectins in geese. In case of chicken, all sugar residues under investigation could be detected except N-acetylegalactosamine. While in ducks, L-fucose and N-acetylegalactosamine were not expressed in the glandular duct epithelium. These results may attribute to glucose and mannose residues are abundant in the compound with ion transport function (Spicer and Schulte, 1992; Blackmore and Eisoldt, 1999). In addition, the presence of a-D-mannose in the cell surface of the lining epithelium might indicate that this sugar plays a role in regulating cell-to-cell recognition and cell adhesion (Burk et al., 1979; Roberson and Armstrong, 1979). Moreover, N acetyleglucosamine residues regulate membrane interaction and membrane permeability (Blackmore and Eisoldt, 1999; Topfer-Petersen, 1999). Galactose residues are important for cell-cell adhesions (Spicer and Schulte, 1992; Topfer-Petersen, 1999) and are also considered markers of cell differentiation (Spicer and Schulte, 1992). Galactose-β (1→3)-D-N-acetylgalactosamine complexes participate in the transport of fluids and ions (Spicer and Schulte, 1992).

These neutral sugars may be contributed to protective function, acting in neutralizing gastric acidity and may transport macromolecules across cell membrane (Domenoghini et al., 1998 and Parillo et al., 2001 and 2002). This may augment our results in that most of the lectins used in our study produced similar staining intensities in oxyntic cells (labeling of the intracellular membrane system and of the plasma membrane) and chief cells (labeling of the apical plasma membrane).

In conclusion, all investigated species showed neutral mucopolysaccharides with different sugar residues to protect the surface mucosa and glandular tissue of the proventriculus from the harmful effect of the acid content.

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