



**Lectin-Based Histochemical Analysis of Sugar Residues in the Gastrointestinal Tract of New Zealand White Rabbit\***

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**Abstract:** The gastrointestinal system of mammals exhibits interspecific variability both physically and functionally. The diversity in the gastrointestinal system reflects unique and functional traits among vertebrate animals. Lectin histochemistry is a method that uses lectins to identify and attach to glycosidic linkages in polysaccharides, glycoproteins, and glycolipids. This particular binding enables the identification of complex structures by determining terminal sugars, hence elucidating physiological or pathological alterations in cells, intercellular relationships, and intracellular transport mechanisms. The present study aimed to identify and illustrate the distribution and density of glycans throughout the gastrointestinal system of the New Zealand White rabbit, spanning from the stomach to the rectum, utilizing lectin histochemistry. Paraffin sections of Bouin-fixed rat tissues were taken 5-µm thick and were examined for the binding of lectins specific to GalNAc (HPA), Gal (PNA), GlcNAc (WGA), and mannose and/or glucose (Con A) using the lectin histochemical method. The lectin binding patterns in the stomach both the small and large intestines exhibited alterations, signifying a diverse composition of carbohydrates. The data indicate that mucin glycosylation differs across various anatomical locations and likely represents a responsive mechanism tailored to local physiological requirements.

**Keywords:** Gastrointestinal system, intestine, lectin histochemistry, rabbit, stomach.

**Yeni Zelanda Beyaz Tavşanın Gastrointestinal Sistemindeki Şeker Kalıntılarının Lektin Bazlı Histokimyasal Analizi**

**Öz:** Memelilerin gastrointestinal sistemi hem fiziksel hem de işlevsel olarak türler arası değişkenlik gösterir. Gastrointestinal sistemdeki çeşitlilik, omurgalı hayvanlar arasında benzersiz mimari ve işlevsel özellikleri yansıtır. Lektin histokimyası, lektinleri kullanarak polisakkaritler, glikoproteinler ve glikolipidlerdeki glikozidik bağlantıları tanımlayan ve bunlara bağlanan bir yöntemdir. Bu özel bağlanma, terminal şekerleri belirleyerek karmaşık yapıların tanımlanmasını sağlar ve böylece hücrelerdeki fizyolojik veya patolojik değişiklikleri, hücreler arası ilişkileri ve hücre içi taşıma mekanizmalarını açıklar. Mevcut çalışma, lektin histokimyasını kullanarak Yeni Zelanda Beyaz tavşanın mideden rektuma kadar uzanan gastrointestinal sistemi boyunca glikanların dağılımını ve yoğunluğunu tanımlamayı ve göstermeyi amaçlamaktadır. Bouin fiksasyonlu sıçan dokularının parafin kesitleri 5 µm kalınlığında alındı ve lektin histokimyasal yöntemi kullanılarak GalNAc (HPA), Gal (PNA), GlcNAc (WGA) ve mannoz ve/veya glikoza (Con A) özgül lektinlerin bağlanması açısından incelendi. Midedeki ve hem ince hem de kalın bağırsaklardaki lektin bağlanma desenleri, karbonhidratların çeşitli bir bileşimini gösteren değişiklikler gösterdi. Veriler, musin glikozilasyonunun çeşitli anatomik konumlarda farklılık gösterdiğini ve muhtemelen yerel fizyolojik gereksinimlere göre uyarlanmış bir yanıt mekanizmasını temsil ettiğini göstermektedir.

**Anahtar kelimeler:** Bağırsak, gastrointestinal sistem, lektin histokimyası, mide, tavşan.

**Introduction**

The gastrointestinal (GI) system of mammals reveals interspecific diversity physically and functionally, albeit numerous striking similarities. This diversity in the GI system represents distinct structural and functional characteristics among vertebrate species (Treuting et al., 2018). Despite these observable dif-

ferences, the GI tract throughout all mammalian species is, by its function, continuously exposed to mechanical and chemical insults caused by ingested foods. In addition, the release of various digestive enzymes, bile acids, and hydrochloric acid into the gastrointestinal environment creates an environment in need of protection for the epithelium, which is responsible for many vital functions (Bansil and Turner, 2006). The epithelial surface of the GI tract is protected against abrasion, bacteria, parasitic infections, and noxious chemicals by a mucus coating composed primarily of water and mucins (Cornick et al., 2015; Caspe et al., 2024).

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GI mucins are chiefly produced by goblet cells. Mucins in membrane-bound and soluble-secreted forms are glycoproteins consisting predominantly of O-linked oligosaccharides (Bansil and Turner, 2006). Their functional tasks include lubricating and protecting the epithelial cells from mechanical, physical, and chemical abrasions. They also provide suitable anatomical sites for attachment and entry of pathogenic organisms into the underlying epithelium (Robbe et al., 2004; Kim and Ho, 2010). Therefore, detachment of this layer renders the GI tract more vulnerable to pathogen invasion. In this context, information obtained from commonly used laboratory animals, including mice, rats, and rabbits, is of the utmost importance for further understanding the structure and function of the human GI tract and its associated pathologies (Oglesbee and Lord, 2020). Therefore, a deeper understanding of glycohistochemical differences between the GI tract of various animal species may help us choose the proper animal model for providing valuable insights into glycohistochemical characterization of the human GI tract in health and disease.

Many GI diseases have been reported that may result from aberrant protein glycosylation (Reily et al., 2019; Verhelst et al., 2020; Morosi et al., 2021). The GI tract produces many glycoconjugates that may vary by species, sex, gender, anatomical region, cell type, functional status, and pathological scenario (Schumacher et al., 2004; Kudelka et al., 2020). Therefore, studying changes in glycoconjugate composition would help further understand their functional and pathological importance throughout the GI tract. Lectin histochemistry is a valuable means to identify differences between glycosylation patterns of glycoproteins (Corfield, 2017). While previous studies have indicated that lectin binding specificity mostly indicates the respective monosaccharide binding preference, the lectins used are likely to recognize different, more complex structures present in the sequence (Brooks, 2024). Recent bioinformatics studies have revealed that the majority of plant lectin sequences encode chimerolectins composed of more than one protein domain. In fact, these analyses showed that many lectins are multidomain proteins in which one lectin domain binds to one or more protein domains that serve another function (De Coninck and Van Damme, 2021). Therefore, lectin histochemistry studies are valuable for disease diagnostics and comparative purposes. These studies can detect variations between normal and pathologic conditions of given tissues, between different regions in the same organ, or between homologous areas of specimens of different ages, sexes, or species.

Several studies have reported the distribution of sugar residues in vertebrate GI tracts (Galotta et al., 2009; Boonzaier et al., 2013; Scillitani and Mentino, 2015; Tano De La Hoz et al., 2016; Gomez-Santos et

al., 2017). However, the literature review reveals only patchy evidence of glycan expression in the New Zealand White rabbit GI tract (Galotta et al., 2009; Zanuzzi et al., 2010). Therefore, we aimed to show the distribution of glycans in the GI tract of the New Zealand White rabbit from the stomach to the rectum and whether there are density differences using lectin histochemistry and image analysis.

## Material and Methods

### Animals

In this study, the gastrointestinal tract from the stomach to the rectum of ten adult male New Zealand rabbits with a weight range of 4-5 kg purchased from a local rabbit breeder in Isparta, Türkiye, were used as material. The Ethical Board of Experimental Animal Production and Experimental Research Laboratory at Süleyman Demirel University (SDU-HÜDAL B.30.2.SDÜ.0.05.06.00-186) provided approval for this study.

### Tissue Processing

Samples of the gastrointestinal tract were collected from anesthetized animals using ketamine/xylazine (80/12 mg/kg; Alfasan/Bayer). After anaesthetizing, the abdominal cavities of the animals were opened. Samples harvested from the stomach, small, and large intestines were fixed in Bouin's solution for 15 h, dehydrated in a sequence of increasing ethanol concentrations, cleaned in xylene, and embedded in paraffin. Sections of 5-7 µm in thickness were cut and mounted on albumin-coated slides.

### Lectin Histochemistry

Lectin histochemistry was employed to demonstrate the distribution and density of glycans in the gastrointestinal tract regions of the New Zealand White rabbit. Following this protocol, the sections were rinsed in distilled water subsequent to dewaxing in xylene and rehydrating in decreasing concentrations of ethanol. The sections were immersed in 0.3% H<sub>2</sub>O<sub>2</sub> (v/v) 100% methanol at room temperature for ten minutes to inhibit endogenous peroxidase activity. Following rinsing in distilled water and washing in 0.01 M PBS (Phosphate Buffered Saline) (pH 7.2) with 1% BSA (Bovine Serum Albumin), the sections were treated for 30 minutes at room temperature with a panel of Horseradish Peroxidase (HRP)-conjugated lectins and subsequently washed in PBS (Çınar et al., 2016). Table 1 lists the HRP-conjugated lectins utilized in this study, along with their binding specificities and optimal concentrations. The sections were exposed to DAB (3,3-diaminobenzidine tetrahydrochloride) to visualize the lectin binding sites at room temperature for 10 minutes. Sections were counterstained in Mayer's Haematoxylin. Then, the sections were rinsed in distilled water, dehydrated in increas-

ing concentrations of alcohol, cleaned in xylene, permanently mounted with Entellan, examined under a light microscope (Olympus, CX 41), and photographed using DP72 digital camera (Olympus, Tokyo, Japan) mounted on the microscope. Control sections were immersed in PBS without the lectins for 30 minutes at room temperature, after which the same procedure was followed.

opposite pattern of staining was seen in the pyloric gland region. The surface mucous cells and some gastric pits were negative, while cells in the glands were intensely stained (Figure 1B). The cardiac glands showed a strong positive reaction to PNA in the different regions of the gastric mucosa. PNA reaction was confined to the surface mucous cells and the lower portions of the cardiac gland regions

**Table 1.** Lectins used in the study and their binding specificity

Source of lectin	abbreviation	Oligosaccharide specificity	Dilution	Company	Catalog no.
<i>Helix pomatia</i>	HPA	GalNAc $\alpha$ (1,3)GalNAc>Gal $\beta$ (1,3)GalNAc	10 $\mu$ g/ml	Sigma	L6387
<i>Arachis hypogaea</i>	PNA	Gal $\beta$ (1,3)GalNAc>Gal $\beta$ (1,4)GlcNAc>Gal	25 $\mu$ g/ml	Sigma	L7759
<i>Canavalia ensiformis</i>	Con A	$\alpha$ -D-Man	50 $\mu$ g/ml	Sigma	L6397
<i>Triticum vulgaris</i>	WGA	GlcNAc( $\beta$ 1,4GlcNAc)1-2> >Neu5Ac	$\beta$ GlcNAc 20 $\mu$ g/ml	Sigma	L3892

**Evaluation of the Staining**

Staining in each experiment was evaluated by at least two independent observers from three histological sections from each sample and scored according to their intensity as (+++) strong reaction, (++) moderate reaction, (+) weak reaction, and (-) negative (Manning et al., 2004).

(Figure 1C). A comparable distribution of PNA lectin binding sites was seen in the pyloric gland region (Figure 1D).

Conversely, Con-A displayed a moderate reaction in the cardiac, fundic, and pyloric gland regions (Figure 2A-C). WGA exhibited greater intensity of binding pattern in the fundic gland region, with a more pronounced positive reaction in the higher sections of

**Results**

**Table 2.** Reaction of lectins with sugar residues in gastrointestinal tract regions of New Zealand White rabbits at different intensities

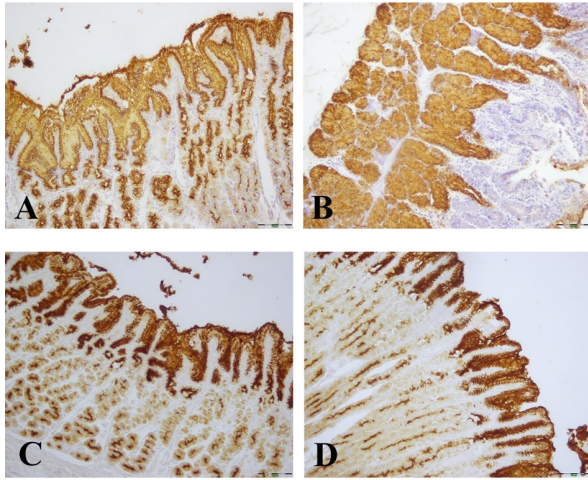
	HPA	PNA	Con A	WGA
<b>Cardia</b>	+++	+++	++	+++
<b>Fundus</b>	+++	+++	++	+++
<b>Pylorus</b>	+++	+++	++	+++
<b>Duodenum</b>	+++	-	++	+++
<b>Jejunum</b>	+++	-	++	+++
<b>Ileum</b>	+++	++	+	++/+++
<b>Proximal Colon</b>	+++	-/+	++	+++
<b>Distal Colon</b>	++	-/+	++	+++
<b>Caecum</b>	-	-/+	++	+++
<b>Rectum</b>	+	-/+	+	+++

The results of lectin histochemistry indicated that lectins reacted with sugar residues in the gastrointestinal tract areas of the New Zealand White rabbit at varying intensities. Table 2 summarizes our main findings.

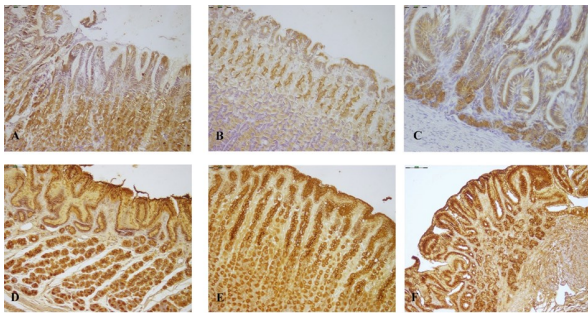
**Stomach**

The upper region of the cardiac and fundic glands showed a stronger positive reaction to HPA. This positive reaction was observed in the surface mucous cells, gastric pits, and gastric glands (Figure 1A). The

the glands compared to their bases. The mucus released over the gastric mucosa had a substantial positive reaction to WGA in the cardiac, fundic, and pyloric gland regions. This strong reaction was seen in the surface mucous cells, stomach pits, and gastric glands (Figure 2D-F).



**Figure 1.** Lectin-binding sites of cardia, fundic, and pylorus regions. A. Cardia, Lectin HPA, Scale bar: 100 µm; B. Pylorus, Lectin HPA, Scale bar: 100 µm; C. Cardia and D. Fundus Lectin PNA, Scale bar: 100 µm.

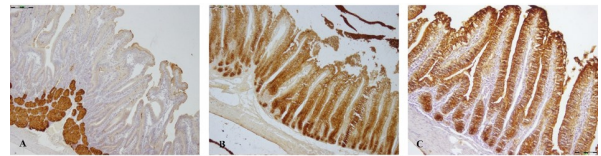


**Figure 2.** Lectin-binding sites of cardia, fundic, and pylorus regions. A. Cardia, Lectin Con A, Scale bar: 100 µm; B. Fundus, Lectin Con A, Scale bar: 100 µm; C. Pylorus, Lectin Con A, Scale bar: 50 µm; D. Cardia, Lectin WGA, Scale bar: 100 µm; E. Fundus, Lectin WGA, Scale bar: 100 µm; F. Pylorus, Lectin WGA, Scale bar: 100 µm.

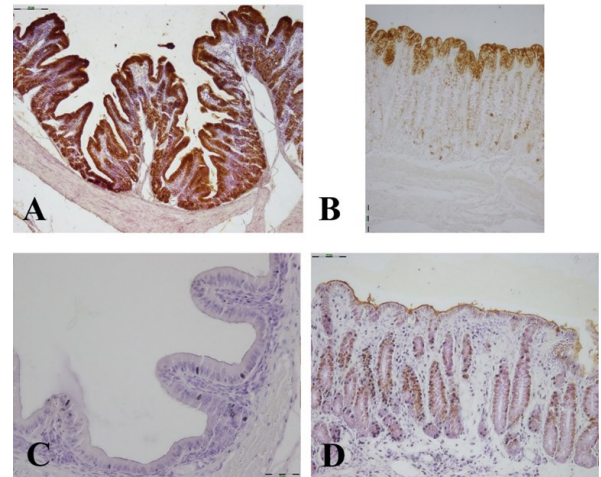
**Small and Large Intestine**

A strong reaction to HPA was observed in the brush border, villi, and Brunner's glands within the duodenum (Figure 3a.A). HPA exhibited enhanced binding to the jejunal epithelium, but cells within the Lieberkühn crypts displayed intense staining (Figure 3a.B). HPA labeling exhibited increased reactivity in the brush border of the crypts, villi, and Lieberkühn glands in the ileum (Figure 3a.C). In the proximal colon, HPA binding was observed throughout the whole crypts (Figure 3b.A). Conversely, in the distal colon, only the cells of the few Lieberkühn glands at

the lower portion of the crypts exhibited a moderate reactivity to HPA (Figure 3b.B). HPA did not react with all regions of the caecum (Figure 3b.C). HPA exhibited a pronounced reaction with the brush border and the Lieberkühn glands in the rectum (Figure 3b.D).

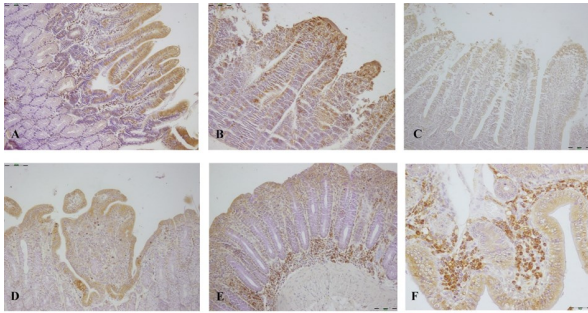


**Figure 3a.** HPA-binding sites of the small intestine regions. A. Duodenum, Scale bar: 200 µm. B. Jejunum, Scale bar: 200 µm. C. Ileum. Scale bar: 100 µm.



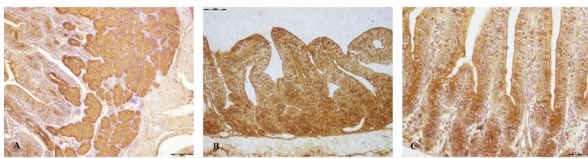
**Figure 3b.** HPA-binding sites of the small and large intestine regions. D. Proximal colon, Scale bar: 200 µm. E. Distal Colon, Scale bar: 100 µm. F. Caecum, Scale bar: 50 µm. G. Rectum. Scale bar: 100 µm.

The binding of Con A to the brush border of the duodenum and jejunum crypts and villi was stronger than that observed in the ileum while exhibiting weak reactivity in the upper regions of Brunner's gland. Goblet cells predominantly exhibited no reaction in the small intestines (Figure 4A-C). The proximal (Figure 4D), distal (Figure 4E), and caecal (Figure 4F) epithelia exhibited moderate Con A lectin binding, although certain cells within the Lieberkühn glands displayed weak staining.

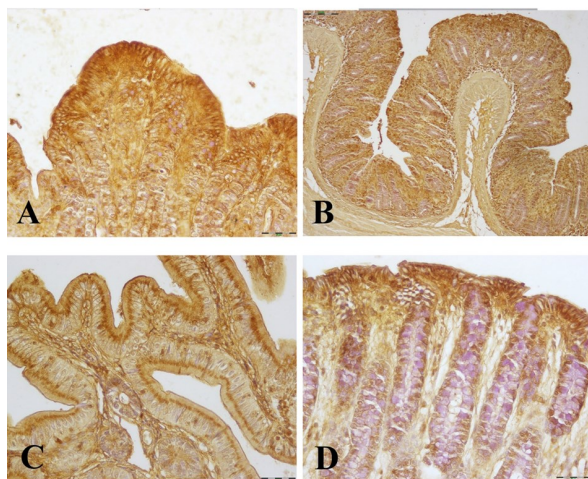


**Figure 4.** Con A-binding profile of the small and large intestine regions. A. Duodenum, Scale bar: 100 µm., B. Jejunum, Scale bar: 100 µm., C. Ileum, Scale bar: 100 µm., D. Proximal colon, Scale bar: 100 µm., E. Distal Colon, Scale bar: 100 µm., F. Caecum, Scale bar: 50 µm.

WGA exhibited considerable intensity of binding in the duodenum and jejunum, but cells in the ileum had lower staining (Figure 5a). WGA labeling was pronounced in the brush borders of the large intestine (Figure 5b). WGA exhibited a pronounced reaction in both the proximal and distal colon and a mild reaction with the Lieberkühn glands in the rectum (Figure 5b.D).

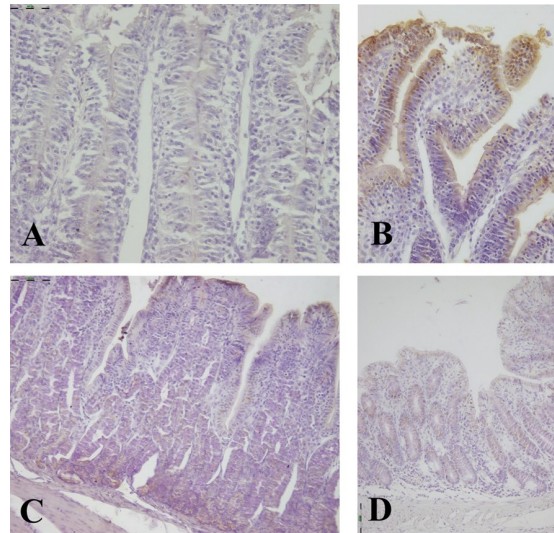


**Figure 5a.** WGA-binding profile of the small intestine regions. A. Duodenum, Scale bar: 50 µm., B. Jejunum, Scale bar: 100 µm., C. Ileum, Scale bar: 100 µm.



**Figure 5b.** WGA-binding profile of the large intestine regions. D. Proximal colon, Scale bar: 50 µm., E. Distal Colon, Scale bar: 200 µm., F. Caecum, Scale bar: 50 µm., G. Rectum, Scale bar: 50 µm.

PNA exhibited no reaction in the duodenum and jejunum, whereas a moderate reaction was noted in the ileal epithelial cells and brush border (Figure 6 A-B). The signals of both PNA-lectins were markedly weak in the crypts and the brush edge (Figure 6 C-D). PNA staining was very weak at both the crypts and brush border in the large intestine (Figure 6 C-D).



**Figure 6.** PNA-binding profile of the small and large intestine regions. A. Jejunum, Scala Bar: 50 µm, B. Ileum, Scala Bar: 50 µm, C. Proximal colon, Scale bar: 100 µm, D. Distal Colon, Scale bar: 100 µm.

### Discussion

Certain proteins facilitate biological recognition, binding, and attachment to particular targets. This binding is highly specific to sugar residues and depends mostly on the ability to bind to surface sugars found in the animal, mainly human, digestive tract. Upon binding to specific targets, lectins can induce agglutination of fetal or neoplastic cells, promote lymphocyte proliferation, and impede tumor growth both in vivo and in vitro (Ghasempour and Freeman, 2023). Lectins are a widely used method to identify specific glycan motifs and determine their tissue and cellular localization in histochemical studies, providing important information about glycan diversity and their specific roles (Akimoto and Kawakami, 2014; Gomez-Santos et al., 2017; Sugahara et al., 2017; Tano de la Hoz et al., 2017; Ghasempour and Freeman, 2023). Many studies indicate that lectins attach to the glycoconjugates of the gastrointestinal structures in mammalian species, facilitating the comparison of intra- and inter-species variations in glycoconjugate distribution (Scillitani and Mentino, 2015; Strobel et al., 2015).

The lectin histochemistry approach, employed to map

out tissue glycosylation, has been extremely helpful in uncovering modifications in cellular glycosylation linked to cancer progression and metastasis. It has enabled the development of glycosylated biomarkers that may predict prognosis, and improve early diagnosis and screening efforts. This method is particularly effective for analyzing gastrointestinal carbohydrate changes in animal models designed to understand the pathogenesis of human colorectal diseases such as carcinoma and inflammatory bowel disease (Sharma and Schumacher, 2001; Enomoto et al., 2024). Prior research has indicated that the characteristics and prevalence of terminal sugars on cell surface glycoconjugates are altered in cancer cells within human colon adenocarcinoma (Arab et al., 2013; Strobel et al., 2015).

The constant exposure of intestinal epithelium to dietary constituents, local microbiota, and diverse microorganisms complicates the assessment of each component's involvement in both healthy and pathological conditions. Moreover, as many of these factors work synergistically, alterations in intestinal carbohydrate composition rely on multiple variables (Zanuzzi et al., 2010). The digestive system of rodents has a crucial ecological and physiological role, particularly due to its adaptability to various conditions and the histological structure of the colon depending on water and carbohydrate absorption (Moghaddam et al., 2009). Tano de la Hoz et al. (2017) asserted that observable variations in lectin (Con A, WGA, DBA, SBA, PNA, and UEA-I) histochemistry in the rodent colon may result from the interaction between mucins and bacteria traversing the colonic groove.

Strobel et al. (2015) evidenced the existence of GlcNAc on the mucosal surface, chief cells, neck cells, and parietal cells via WGA lectin binding models in the digestive system of bats. Their study on bats revealed that GalNAc was present in the glycocalyx of enterocytes in the small intestine, whereas similar cells were absent in the large intestine. WGA exhibited considerable reactivity in the duodenum, jejunum, and large intestine, while WGA labeling was less pronounced in the ileum. HPA exhibited pronounced reactivity in the small and large intestine regions, except the caecum. In cancer cells, cell surface terminal sugars and extracellular matrix glycoconjugates appear to be extensively modified. Studies showed that the positivity of glycoconjugates containing GalNAc and GlcNAc increased for different grades of neoplasia in colon adenocarcinoma (Arab et al., 2013).

A moderate PNA binding pattern was observed in the epithelial and mucous neck cells of the pyloric stomach region in *Rhinella icterica* (Machado-Santos et al., 2014). We observed a strong reaction to PNA in the same region. Another study indicated that the surface mucosal cells of the cardia, gastric pits, and

gastric gland areas in monkeys exhibited a pronounced positive reactivity to WGA. The positive reaction to WGA was observed in the fundic gland region, confined to the upper part of the glands and absent from their bases. The surface mucosal cells in the pyloric region and gastric pits exhibited negativity, although certain cells in the body and the base of the glands demonstrated mild staining. The same researchers indicated that the surface mucosal epithelium and foveolar epithelium in the cardiac, fundic, and pyloric gland regions had a negative reaction to Con-A, whereas the body and base of the glands demonstrated a substantial positive reactivity to Con-A (Fayed et al., 2010). We found moderate Con-A staining in the cardiac, fundic, and pyloric gland areas.

A study indicates that alterations in particular lectins and specific regions of the small intestine (PNA in the duodenum and jejunum and WGA in the jejunum) were primarily observed during *Solanum glaucophyllum* intoxication (Zanuzzi et al., 2010). In parallel with these findings, our study revealed that PNA did not exhibit reactivity in the duodenum, whereas WGA lectin labeling demonstrated a strong reaction in the jejunum. Another study indicated that Con-A and PNA labeling was moderate to strong in the jejunum and ileum of *Lagostomus maximus*, but WGA labeling was more pronounced in the jejunum compared to the ileum (Tano De La Hoz et al., 2016). Galotta et al. (2009) investigated the binding patterns of some lectins in the intestines of horses, pigs, and rabbits. Con A yielded negative results in the jejunum and ileum across all three species. WGA lectin binding was seen in the jejunum and ileum of all three species. PNA lectin binding was not present in the jejunum and ileum of pigs and rabbits, whereas it was detected at considerable levels in the same regions of horses. Furthermore, Con A binding was absent in the colons of horses, pigs, and rabbits, and exhibited minimal reactivity in the rectum. However, PNA exhibited no reactivity in the colon and rectum of all examined species (Galotta et al., 2009). Conversely, in our study, Con A showed reactivity in both the small and large intestines. PNA exhibited no reaction in the duodenum and jejunum, while a moderate reaction was noted in the epithelial cells and brush border of the ileum. PNA labeling was markedly weak in the crypts and brush border of the large intestine.

Normal gastrointestinal lectin histochemistry has shown that lectins can be used as reliable markers not only for cell identification but also for the search for similar glycans with subtle differences in sugar sequences. In conclusion, this study demonstrates that lectin binding patterns across the rabbit gastrointestinal tract reveal different compositions of carbohydrates. The observations indicate that mucin glycosylation differs among various anatomical locations, possibly forming a responsive system tailored to local

physiological needs. This data may also aid in establishing a comprehensive histochemical framework to serve as a comparative baseline for future research focused on the dietary physiology of this species. Additional investigations into the alterations in glycan expression in diseased situations may clarify their biological roles and functional importance.

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