Indonesian Journal of Veterinary Sciences
Vol. 3. No. 2 September 2022, Page. 63-68
DOI:10.22146/ijvs.v3i2.89085, ISSN 2722-421X (Online)
Available online at https://jurnal.ugm.ac.id/ijvs

Sugar Residue Distribution Profile of the Male Sugar Glider *(Petaurus breviceps) Intestinal Mucosae*

Bram Aditia[1], Gregorius Tunggal Sadewa Dirgantara Putra[1], Zephania Kusumaningasih Yulianto[1], Faizal Achmad Hidayat[1], Fransiscus Giovanni[1], Ariana[2*]

[1]Undergraduate Program Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia.
[2]Department of Anatomy, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author: ariana@ugm.ac.id

[1], A

Received: May, 11, 2022, Accepted: August 9, 2022, Published: September 1, 2022

Abstract

The sugar glider is a popular exotic pet animal endemic to Australia, Tasmania, Papua New Guinea, and Indonesia. Sugar gliders are often kept as pets and in zoos, yet information on histological characteristics of sugar gliders is limited. This study aims to determine the distribution profile of various sugar residues in the intestines of *Petaurus breviceps* using lectin histochemical staining. Samples were taken from healthy adult *Petaurus breviceps* and stained with the lectins: Concanavalin-A (Con-A), Dolichos Biflorus Agglutinin (DBA), Peanut Agglutinin (PNA), Ricinus Communis Agglutinin (RCA), and Ulex Europaeus Agglutinin-1 (UEA-1). The data obtained were analysed qualitatively with four categories of reactivity, namely negative, weak, moderate, and strong. The results of research on *Petaurus breviceps* intestines showed that the PNA lectin was weak positive in the duodenum, ileum and cecum indicated that there was a small amount of Galβ1-3GalNAc. The DBA lectin was weak to strong positive in the cecum and colon indicated that there were small to a lot amount of GalNACα1-3GalNAc. The UEA-1 lectin was weak to strong positive in the cecum, colon dan rectum, indicated there were small to a lot amount of α-L-Fucose. The RCA-1 lectin was moderate positive in the cecum, indicated there were moderate amount of β-D-Galactose. The Con-A lectin weak to moderate positive in the colon and rectum, indicated there were small to moderate of α-Mannose. This study concluded that there were numerous sugar residues indicated by various lectin reactivities in the intestine of *Petaurus breviceps*.

Keywords: intestine; lectin; *Petaurus breviceps*; residues; sugar

Introduction

Sugar gliders (*Petaurus breviceps*) are small omnivorous marsupial animal endemic to Australia, Tasmania, Papua New Guinea, and Indonesia (Booth, 2003; Kubiak, 2021). Their small size and appearance have made them popular animals around the globe and often kept as pets or in zoos (Booth, 2003). There is a well-established pet trade for sugar gliders across Europe, Asia, and North America (Kubiak, 2021). Their increasing popularity shows a need to increase our knowledge about sugar gliders as they may be presented at any veterinary practice (Raftery, 2015).

Sugar gliders have a simple gastrointestinal tract similar to other carnivorous mammals (Johnson-Delaney and Orosz, 2009; Quesenberry *et. al.,* 2011). The gastrointestinal tract is protected by a layer of mucus which consists mainly of mucin. Mucins are glycoprotein that are composed by a high content of oligosaccharides. These intestinal mucins are synthesized by goblet cells found throughout the intestinal wall. Studies on mammals have shown varying distribution of goblet cells and mucin subtypes based on their glycosylation pattern (Tano De La Hoz *et. al.,* 2016). These differences are caused by factors such as genetic (Buisine *et. al.,* 1998; Jass and Walsh, 2001), enzyme expression (Buisine *et. al.,* 1998), age and diet (Montagne *et. al.,* 2004; Galotta *et. al.,* 2009), physiology (Brinck *et. al.,* 1995; Sakamoto, 2000), and pathological processes (Brinck *et. al.,* 1995; Kandori, 1996).

Currently, there is limited information regarding glycosylation patterns in the sugar glider.

This study aims to determine the distribution of various sugar residues in the intestines of male *Petaurus breviceps* in normal condition by using lectin histochemical staining. Lectins are proteins that can bind to polysaccharides or glycoconjugates (Bender and Murray, 2015). They have high affinity and specificity for specific sugar residue that can be found in intestinal mucins (Narita and Numao, 1992).

Materials and methods

Procedures in this study has received approval from the Ethics Commission of the Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia with ethical clearance number 011/EC-FKH/Int./2022. This research used two adult male *Petaurus breviceps* to obtain the intestinal samples. While in captivity, the animals were provided with commercial feed and sliced fruits, and occasionally with crickets and mealworms. They were fasted a day prior to euthanasia. They were anaesthetized with an intramuscular injection of ketamine (20 mg/kg BW) and xylazine (2 mg/kg BW), after that, the animals were perfused with NaCl 0.9% and continued with Phosphate Buffer Saline (PBS) formalin 10%. The digestive system was taken out quickly after the body showed signs of stiffness. The intestines were then processed to be immunohistochemically stained using FineTest® SABC KIT (Wuhan Fine Biotech Co. Ltd), DAB Substrate Kit (Wuhan Fine Biotech Co. Ltd) and Biotinylated Lectin Kit I (Vector Lab.): Concanavalin-A (Con-A), Dolichos Biflorus Agglutinin (DBA), Peanut Agglutinin (PNA), Ricinus Communis Agglutinin (RCA), and Ulex Europaeus Agglutinin-1 (UEA-1). The specificity of lectins used in this study was shown in Table 1

Table 1. Lectins used in this study

Lectins	Specificity
Peanut Agglutinin (PNA)	Galβ1-3GalNAc
Dolichos Biflorus Agglutinin (DBA)	GalNACα1-3GalNAc
Ulex Europaeus Agglutinin-1 (UEA-1)	α-L-Fucose
Ricinus Communis Agglutinin (RCA)	β-D-Galactose
Concanavalin-A (Con-A)	α-Mannose

The collected samples were then fixed in 10% formalin for 24 hours. Samples were processed using the paraffin method and paraffin blocks were cut to a thickness of 8 µm. The slides were at first deparaffinized and rehydrated, then washed with running water They were submerged in a boiling citrate buffer solution and put in an incubator at 60°C for 1 hour, the slides were left at room temperature for 20 minutes to cool down. Endogenous peroxidase activity in the sample was blocked by submerging the slides under H_2O_2 : methanol solution for 30 minutes. After submersion, the slides were washed using PBS 3 times for 5 minutes each. Non-specific reactions in the sample are limited by administrating 50 µl of FineTest® blocking serum working solution to each slide and left in a chamber for 1 hour. Tissue samples were then stained with lectins: Con-A (20 µg/ml), DBA (20µg/ml), PNA (10 µg/ml), RCA (10 µg/ml), and UEA-1 (5 µg/ml). Slides were placed back in the chamber and incubated in a refrigerator at 4°C for 24 hours.

Slides were taken out from the refrigerator and left at room temperature from 15 minutes to thaw. They were washed with PBS. Then, each slide was given 50 µl of the SABC reagent working solution and incubated in the chamber for 60 minutes. They were washed with PBS and given 50 µl FineTest® DAB substrate kit reagent each in a dark room until stain discoloration appeared. Immediately after the colour developed, the slides were rinsed with distilled water and counterstained with Harry's haematoxylin for 30 seconds. After being counterstained, the slides were washed under running water for 10 minutes, then the slides dehydrated and clearing before being mounted and observed using light microscopy.

Data analysis was carried out descriptively qualitatively on the intensity of lectin reactivity using diaminobenzidine. The intensity of lectin reactivity was indicated by the presence of a brown color on the tissue. The observation results are divided into four categories: negative (-), weak (+), moderate (++), and strong (+++) based on the observer's subjectivity. Some samples were not observable as they had expired prior to staining procedure and the results were not available (N/A)

Results and discussion

Lectin reactivity indicates the presence of the specific sugar residue corresponding to the lectin

specificity as shown in Table 1. In this study, the aspect of lectin reactivity to sugar residues was observed in Goblet cells of the organ observed. Overall, the slides of sugar gliders intestinal tract which was incubated with DBA, UEA-1, RCA-1, Con A, and PNA lectins showed varying results. Reactions are localized in the Goblet Cells (GC) of *Petaurus breviceps*. The result of lectin staining in this study is summarized in Table 2.

Table 2. Lectin binding pattern in the Petaurus breviceps intestinal mucosae

	PNA	DBA	UEA-1	RCA-1	Con A
Duodenum	+[a]	N/A	N/A	N/A	N/A
Jejunum	-	-	-	-	-
Ileum	+[a]	-	-	-	-
Cecum	+	+~+++	+	++	-
Colon	-	+++	++	N/A	+[b]
Rectum	-	-	+++	-	++

-negative;+weakly positive; ++moderately positive;+++strongly positive;[a] GC located at the luminal surface of the villi; [b]Heterogenous staining (some GC were positive while some others were negative); N/A data not available

Lectin staining in the intestine showed binding pattern. Lectin binding were with PNA showing a weak reaction in the duodenum (Figure 1A), ileum and cecum (Figure 1B). Lectin binding with PNA, in the duodenum stained weakly at the goblet cells near the luminal surface of the villi, in the ileum stained weakly in the goblet cells at the luminal surface and in the cecum stained weakly at the goblet cells of the intestinal glands. The colon and rectum showed no reaction of lectin binding with PNA. The *Petaurus breviceps*'s jejenum, ileum and rectum staining using the DBA lectin showed no reaction. The cecum showed varying results ranging from weak positive to strong positive. Goblet cells show a light brown to dark brown color that varies in a certain area. The colon (Figure 1C) shows a strong positive result which is indicated by a brown color that surrounds the goblet cells of the intestinal glands. The reactivity of sugar residues with the UEA-1 lectin in the jejenum and ileum showed a negative result, the cecum were weakly positive, the colon was moderately positive, and the rectum (Figure 1D) had a strong positive reactivity. The distribution of sugar residues was not detected using the RCA lectin in the jejenum, ileum and rectum, whereas the cecum (Figure 1E) showed a moderate positive result with a brown coloring. The results of jejenum, ileum dan cecum staining using the Con-A lectin showed negative. The colon (Figure 1F) showed weak positive results with heterogeneous results because some Goblet cells showed weak positive results, while other Goblet cells showed negative result. The rectum showed moderately positive results.

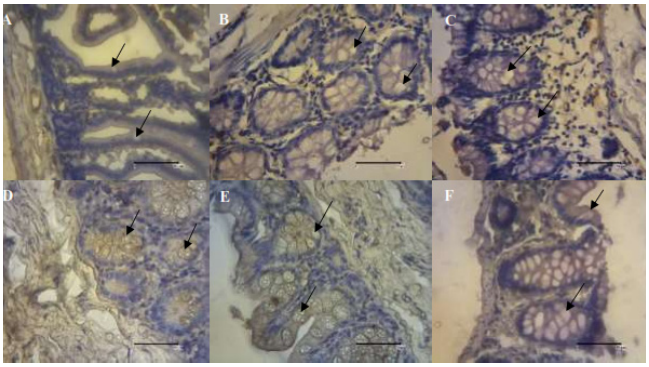

Figure 1. Lectin binding profile of the Petaurus breviceps intestine. (A) Duodenum incubated with PNA, (B) Cecum incubated with PNA, (C) Colon incubated with DBA, (D) Rectum incubated with UEA-1, (E) Colon incubated with RCA-1, (F) Colon incubated with Con-A. Arrow: Goblet cell. Scale bar: A-F 50µm

Discussion

Glycoconjugate sugar residues can be found in the mucin produced by Goblet cells of the intestinal tract both on the surface and in the intestinal glands (Martin *et. al.,* 2019). Lectin binding pattern observed in this study showed PNA, DBA, RCA-1, UEA-1, Con-A staining in the intestine of the *Petaurus breviceps*. The intestine of *Petaurus breviceps* stained using the PNA lectin showed a weak positive result in the duodenum and cecum, and negative in the jejenum, colon and rectum. This indicates that the intestine produces sugar with Galβ1-3GalNAc terminal with a small to non-existent amounts. The results of the reactivity between the sugar Galβ1-3GalNAc with PNA lectins in the intestine are similar to the results of the study of Galotta et al. (2009), where the intestines of rabbits and pigs, especially in the jejenum, colon and rectum showed negative results.

The DBA lectin showed varying results ranging from weak positive to strong positive in the Goblet cells of the *Petaurus breviceps* intestinal glands, which indicates that the tissue

has sugar residues of GalNAcα1-3GalNAc. In the cecum and colon, sugar residues with terminal GalNAc*α1*-3GalNAc are found to be varied from small to abundant amount. Hirabayashi (2014) states that the sugar GalNAcα1-3GalNAc is one of the sugars that is abundantly distributed in intestinal epithelial cells, both in complex and less complex animals, and is one part of the structure of O-glycans in the mucin component. Similar studies on omnivores as mice by Kandori et al. (1996) observed small to large amount of GalNACα1-3GalNAc. Negative results of DBA lectin indicate the absence of the sugar residue jejenum, ileum and rectum *Petaurus breviceps* of GalNACα1-3GalNAc.

The reactivity of sugar residues with terminal *α-L*-Fucose using the UEA-1 lectin in the intestine of *Petaurus breviceps* showed varying reactivity. The pattern of lectin reactivity in the intestine of *Petaurus breviceps* as compared with omnivorous animals such as pigs (Galotta *et. al.,* 2009) and mice (Kandori *et. al.,* 1996) showed a nearly close distribution. Pig jejunum and ileum have moderate to large amount terminal α-L-Fucose residues, colon and rectum have a small to large amount terminal residues. The Duodenum and ileum of mice have unreactive to moderate amount of α-L-Fucose whereas cecum and colon have large amount of terminal residues. Negative results of UEA-1 lectin indicate that the absence of the sugar residue jejenum dan ileum *Petaurus breviceps* seems have no terminal of *α-L-Fucose*, while the cecum have a small amount, the colon have a moderate amount and the rectum have a large amount of α-L-Fucose.

Negative results of RCA-1 indicate the absence of the jejunum, ileum and rectum sugar residue in the *Petaurus breviceps* intestines, so it can be said that there was no sugar residue with *β-D-Ga*lactose terminal in this organ. The distribution of *β*-D-Galactose sugar residues were detected using the RCA lectin in the cecum. Compared with omnivorous animals such as pigs (Galotta *et. al.,* 2009), the jejunum and ileum showed unreactive to a lot amount of α-L-Fucose, while there were a large amount of terminal residues to moderate reavtivity amount of β-D-Galactose in the colon and the rectum.

The Con-A lectin showed varying results ranging from negative to moderate positive in the Goblet cells of the *Petaurus breviceps* intestinal glands, which indicates that the tissue have a small amount of sugar residues of *α- Ma*nnose. Compared with animals that have similarities as omnivores, Kandori et al. (1996) examined the distribution of *α- Ma*nnose sugar residues in the Goblet cells of the duodenum, ileum, cecum, and colon of mice showing negative results. Galotta et al. (2009) also examined glycoconjugates with the ConA lectin in Goblet cells in pigs. Negative results were obtained in the jejunum, moderate positive results in the ileum Goblet cells and weak positive results were found in the colon of the pigs.

The results of our study have similarities with the results of a study on Malayan pangolins which have an insect diet, one of many food items that sugar gliders naturally eat (Booth, 2003; Kubiak, 2021). It was reported that the residues of glycoconjugates in large intestinal are higher than in small intestinal epithelium of the Malayan pangolin (Suprasert *et. al.,* 2007). The sugar residue N-Acetyl galactosamine has a role in the transport of fluids and ions, as well as regulation of membrane interactions and permeability (Spicer and Schulte, 1992; Blackmore and Isolde, 1999). Fucose sugar residues have a role in intercellular adhesion and in regulating the diffusion of substrates between cells (Spicer and Schulte, 1992; Blackmore and Isolde, 1999). Glucose and mannose sugar residues have functions in ion transport in cells, while galactose sugar residues are involved in intercellular adhesion and as markers of cell differentiation (Spicer and Schulte, 1992).

These differences could be attributed to the variety glycosylation pattern appearing in the intestinal mucin. Mucins are highly heterogeneous and differ between species and individual within the same species itself (Linden *et. al.,* 2008). The pattern of mucin glycosylation is determined by the gene that regulates the enzyme glycosyltransferase or glycosidase (Buisine *et. al.,* 1998). They are affected by pathological or physiological changes occurring in the body (Brinck *et. al.,* 1995) such as age (Montagne *et. al.,* 2004), diet (Galotta *et. al.,*

2009), and diseases (Miettinen, 1983; Jacobs and Huber, 1985; Ota *et. al.,* 1988; Narita and Numao, 1992; Kandori *et. al.,* 1996; Blonski *et. al.,* 2007). Host and environmental factors such as diet can also affect the composition of the mucin in an organism. Montagne et al. (2004) said that the composition of mucin and the properties of mucus are influenced by the protein, fibre, and anti-nutritional content of the food. In addition to diet, age can also affect the mucin composition of an organ. In the case of intestinal mucin, the activity of the gut microflora plays an important role in the glycosylation pattern as they normally degrade mucin (Spicer and Schulte,1992; Buisine *et. al.,* 1998).

Conclusion

This study showed the normal sugar distribution pattern found in the intestine of the male *Petaurus breviceps*. Small amount of Galβ1-3GalNAc sugar residue is present in the duodenal, ileal and caecal l part of the intestine. The residual sugar of GalNAcα1-3GalNAc is not produced in jejunum, ileum dan rectum while a small to abundant amounts in the cecum and colon. The sugar residue of α-L-Fucose is no produced in jejunum and ileum, a small to large amounts in all parts of the large intestine. The sugar residue of β-D-Galactose is not produced in jejunum, ileum and rectum, while sufficient quantities in the cecum. The sugar residue of α-Mannose is not present in the jejunum, ileum and cecum, but still present in small to moderate amounts in the colon and rectum This information could be used to compare and analyze changes in sugar distribution pattern in the intestine of the *Petaurus breviceps* under various conditions for further research on the sugar glider.

References

Blackmore, P. F., and Isolde, S. (1999) The neoglycoprotein mannose–bovine serum albumin, but not progesterone, activates T-type calcium channels in human spermatozoa. *Mol. Hum. Reprod.* 5: 498-506.

Blonsky, K., Mile-Langosch, K., Bamberger, A., Osterholz, T., Butler, C., Berger, J., Loening and Schumacher T U. (2007) Ulex europeus Agglutinin-I Binding as a Potential Prognostic Marker in Ovarian Cancer. *Anticancer Res.* 27: 2785-2790.

Brinck, U., Bosbach, R., Korabiowska, M., Schauer, A., and Gabius, H-J. (1995) Lectin-binding sites in the epithelium of normal human appendix vermiformis and in acute appendicitis. *Histol Histopathol.* 10: 61-70.

Buisine, M., Devisme, L., Savidge, T., Gespach, C., Gosselin, B., Porchet, N., and Auberta, J. (1998) Mucin gene expression in human embryonic and fetal intestine. *Gut.* 43: 519-24.

Galotta, J. M., Marquez, S. G., Zanuzzi, C. N., Gimeno, E. J., Portiansky, E. L., and Barbeito, C. G. (2009) Lectin binding pattern of intestinal goblet cells in horse, pig and rabbit. *Ani. Bio. J.* 1: 49-58.

Hirabayashi, J. (2014) *Lectins Methods and Protocols.* Humana New York. NY. 302

Jacobs, L., and Huber, P. W. (1985) Regional distribution and alterations of lectin binding to colorectal mucin in mucosal biopsies from controls and subjects with inflammatory bowel diseases. *J. Clin. Invest* 75: 112-118.

Jass, J., and Walsh, M. (2001) Altered mucin expression in the gastrointestinal tract: a review. *J. Cell Mol. Med.* 5: 327-51.

Johnson-Delaney, C. A., and Orosz, S. E. (2009) *Applied Clinical Topics in Extoic Companion Mammal Medicine.* Association of Exotic Mammal Veterinarians. Wisconsin. 38.

Kandori, H., Hitayama, K., Takeda, M., and Doi, K. (1996) Histochemical, Lectin-Histochemical and Morphometrical Characteristics of Intestinal Goblet Cells of Germfree and Conventional Mice. *Exp. Anim* 45: 155-60.

Kubiak, M. (2021) *Handbook of Exotic Pet Medicine.* John Wiley & Sons. UK. 125-6.

Linden, S., Sutton, P., Karlsson, N., Korolik, V., and McGuckin, M. (2008) Mucins in the Mucosal Barrier to Infection. *Mucosal Immunology* 1: 183-197.

Martin, S. P., Seeberger, P. H., and Silva, D. V. (2019) Mucins and Pathogenic Mucin-Like Molecules Are Immunomodulators During

Infection and Targets for Diagnostics and Vaccines. *Frontiers in Chemistry* .7:1-13.

Miettinen, M., Holthofer, H., Lehto, V., Miettinen, A., and Virtanen, I. (1983) Ulex europaeus I lectin as a marker or tumors derived from endothelial cells. *Amer. J. of Clin. Path.* 79: 32-36.

Montagne, L., Piel, C., and Lalles, J. (2004) Effect of Diet on Mucin Kinetics and Composition: Nutrition and Health Implications. *Nut. Rev.* 62: 105-14.

Narita, T., and Numao, H. (1992) Lectin binding patterns in normal, metaplastic, and neoplastic gastric mucosa. *J. Histchem. Cytchem* 40 (5): 681-687.

Ota, H., Nakayama, J., Katsuyama, T., and Kanai, M. (1988) Histochemical comparison of specificity of three bowel carcinoma-reactive lectins, griffonia simplicifolia agglutinin-II, peanut agglutinin and ulex europaeus agglutinin-I. *Acta Pathol Jpn.* 38 (12): 1547-1559.

Quesenberry, K., Orcutt, C., Mans, C., and Carpenter, J. (2011) *Ferrets, Rabbits and Rodents Clinical Medicine and Surgery.* Elsevier Health Sciences. Missouri. 385-386.

Raftery, A. (2015) Sugar Gliders (*Petaurus breviceps*). *Companion Animal* 20 (7): 422-426.

Sakamoto, K., Hirose, H., Nizuka, A., Hayashi, M., Futamura, N., Kawamura, Y., and Ezaki, T. (2000) Quantitative Study of Changes in Intestinal Morphology and Mucus Gel on Total Parenteral Nutrition in Rats. *J. Surgic. Res.* 94 (2): 99–106.

Spicer, S. S., and Schulte, B. A. (1992) Diversity of cell glycoconjugates shown histochemically: a perspective. *J. Histochem. Cytochem.* 40 (1):1-38.

Suprasert, A., Liumsiricharoen, M., Pongket, P., Prapong, T., Doungern, A., and Prompa, N. (2007) Histology and Glycoconjugates Histochemistry in the Small and Large Intestinal Epithelium of the Malayan Pangolin, Manis javanica. *Kasetsart J. (Nat. Sci.)* 41: 186 – 191.

Tano De La Hoz, M. F., Flamini, M. A., and Díaz, A. O. (2016) Comparative analysis of the morphology, ultrastructure, and glycosylation pattern of the jejunum and ileum of the wild rodent Lagostomus maximus. *Anat. Rec.* 299 (5): 630-642.