

Morphological investigation of intestine structure of the Sunda Porcupine (*Hystrix javanica*)

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Manuscript received: 8 August 2022. Revision accepted: 12 September 2022.

Abstract. Yuliasuti, Prawira AY, Wahid ME, Nisa' C, Agungpriyono S. 2022. Morphological investigation of intestine structure of the Sunda Porcupine (*Hystrix javanica*). *Biodiversitas* 23: 4793-4799. The intestine is an important part of the digestive system, which plays a role in the enzymatic process of food and the absorption of nutrients for survival. Sunda porcupine is one of the wild and protected animals in Indonesia and belongs to the group of rodents that have hindgut fermenter characteristics. Therefore, this study aims to investigate the intestine structure of the Sunda porcupine as an important part of the digestive system. Three adults of Sunda porcupine were euthanized in accordance with ethical approval of the Animal Care and Use Committee (ACUC) of IPB University (No. 92-2018 IPB). Gross anatomy of the macrostructure and length of the intestine, as well as histology features, such as general structure and carbohydrate content using PAS and AB pH 2.5 staining method, were observed. The organ consisted of the small and large intestine, with the duodenal ampulla and large caecum as the prominent structure. In this study, body weight and length correlated positively to intestinal length. The histological observation showed the presence of Brunner's gland in the duodenum and it has the highest villi compared to others, while villi was absent in the large intestine. Furthermore, the small intestine exhibited various intensities with PAS and AB stain, while the large intestine had strong intensity in the goblet cell. Brunner gland in duodenum demonstrated the moderate intensity of PAS stain compared to the goblet cell in the mucosa. This study provides information about the intestine structure of Sunda porcupine as the herbivore and hindgut fermenter.

Keywords: Caecum, digestive system, duodenal ampulla, hindgut fermenter, intestine

INTRODUCTION

The intestine is an important part of the digestive system, which plays a role in the enzymatic process of food and the absorption of nutrients for the survival of an animal. Variations in the size and structure of the small to the large intestine are found due to the different types of feed for each animal. In mammals, there are generally 4 types of digestive systems, namely ruminant and non-ruminant foregut fermenter, hindgut fermenter, and autoenzyme-dependent digester (Furness et al. 2015). Sunda porcupine (*Hystrix javanica*) is one of the wild, endemic, and protected animals in Indonesia (van Weers 1979; Gomez 2021). Based on the natural diets of porcupines (Hystricidae and Erethizontidae), they are considered herbivores that consume a variety of browse, grass, bulbs, roots, or fruits (Mori et al. 2017). In captivity, *Hystrix brachyura*, close relatives to *Hystrix javanica*, feed on sweet corn, sweet potato, guava, jicama, and water spinach (Farida et al. 2019). The Indonesian *Hystrix* sp are known to live in the forest and hillside plantations, and they also make their holes in the ground or rocks (Farida 2015).

Sunda porcupine belongs to the group of rodents that typically have autoenzyme-digester and hindgut fermenter characteristics. This type of animal has a large energy requirement based on body weight compared to large animals such as horses. The small intestine plays a major role in the production of enzymes along with the pancreas to digest various feeds consumed by animals. A well-developed caecum serves as a site for food fermentation and produces the energy needed for the body. Microbes located in the hindgut produce enzymes similar to those found in the rumen of fermented foregut animals (Furness et al. 2015). A group of rodents, arboreal squirrels (Sciuridae), show an adaptation of digestive tract length to its feeding habit. Smaller arboreal squirrels are known to have longer small intestines, feeding mainly on fruits and seeds (Mitsuzuka and Oshida 2018). According to several reports, herbivorous rodents can achieve digestive efficiencies of a magnitude similar to horses and substantial CH₄ production is part of the digestive process (Hagen et al. 2015, 2019a, 2019b).

Captive breeding is one of strategy to conserve wildlife outside its natural habitat, also called ex-situ conservation, and requires special knowledge, expertise, and courage. The basic knowledge that must be possessed in capturing

wild animals, such as porcupines, includes biology and ecology, reproduction, habitat, behavior, feed, disease management, and other aspects/maintenance techniques (Farida et al. 2019). Several reports showed that gastrointestinal diseases occur in porcupines, such as bacterial infections, including *Proteus mirabilis* and *Shigella flexneri*. It can also be affected by parasite infection, such as *Giardiasis*, *Cryptosporidiosis*, *Echinococcus ortleppi*, *Linguatula serrata*, *Trichuris* sp, and *Gireterakis girardi* (Nugroho and Purwaningsih 2015; Rajabloo et al. 2015; Hodžić et al. 2018; Coppola et al. 2020; Cavallero et al. 2021; Nisa et al. 2021). In order to support the captive breeding activity of the Sunda porcupine, the study of the digestive system is required. Previous studies on the digestive system of the Sunda porcupine were limited to several aspects, including the anatomy and distribution of endocrine cells in the stomach and pancreas (Wulansari 2012; Budipitojo et al. 2016a, 2016b). There is also no published study about the intestinal structure, but that of its distant relatives, such as the African porcupine (*Hystrix africaeaustralis*), has been studied by van Jaarsveld (1983), and van Jaarsveld and Knight-Eloff (1984). Apart from its special skin characteristics due to the quill (Prawira et al. 2018, 2019, 2022), the digestive system of Sunda porcupine needs to be studied to identify the morphological adaptation to the feeding diet. Therefore, this study aims to investigate the intestinal structure of the Sunda porcupine as an important part of its digestive system.

MATERIAL AND METHODS

Animals

Three adults of Sunda porcupine weighing about 5-9 kg were used. The animals were captured under permission from the Directorate General of Forest Protection and Nature Conservation, Ministry of Forestry, Indonesia (SK.386/KSDAE/SET/KSA.2/10/2017). All the procedures were performed in accordance with the ethical approval of the Animal Care and Use Committee (ACUC) of IPB University (No. 92-2018 IPB). The animals were anesthetized by the combination of 10% HCl ketamine (Ilium Ketamil, Troy Laboratories, Glendinning, NSW, Australia) and 2% xylazine HCl (Ilium Xylazil, Troy Laboratories) at doses of 2.5 mg/kg body weight (BW) and 1 mg/kg BW intramuscularly at the dorsal part of the tail before euthanasia with exsanguination method and infiltration using the paraformaldehyde 4% to the tissue through the heart. Afterward, the digestive organ from tongue to anus was removed from the body and immersed in paraformaldehyde 4% for fixation at room temperature for 3 days before removal into 70% alcohol for the stopping point.

Macroscopic observation

Gross anatomy was carried out using topographical and macroscopic observations, as well as the intestine length measurement. The measurement was performed on the small intestine comprising the duodenum, jejunum, and

ileum; caecum, large intestine, and rectum. The intestinal length was analyzed with correlation analysis to body weight; each part was then analyzed descriptively.

Histomorphology observation

Each sample from the small intestine, caecum, large intestine, and rectum was histologically observed using the paraffin-embed method. The tissue was sectioned at 5 μ m of thickness, while hematoxylin and eosin (HE) staining was performed as standard histological procedure. Meanwhile, other sections were stained with Periodic Acid Schiff (PAS) and Alcian Blue pH 2.5 (AB) to observe the mucin characteristic in the intestine. Positive PAS staining produced a magenta color, which indicated the presence of the neutral carbohydrate type of secretion, while AB pH 2.5 gave bright blue color as an indicator for the acid type of secretion. Semi-quantitative measurement of the carbohydrate content was conducted by scoring based on the color intensity. The score was indicated by (+++) for highly positive, (++) moderately positive, (+) weakly positive, and (–) negative reactions, each part was then analyzed descriptively.

RESULTS AND DISCUSSION

Macroscopic structure

The intestine of the Sunda porcupine had a total length of 437.86 ± 22.20 cm, as shown in Table 1, while the body weight showed a positive correlation to the length, as demonstrated in Table 2. The small intestine is divided into duodenum, jejunum, and ileum, with jejunum being the largest portion in 59.80%. The duodenum had a duodenal ampulla, which was an enlargement area in the proximal part close to the stomach (Figure 1). The jejunum was found to be the longest part and hanged by mesenterium with the mesenteric vasculature. The last part, the ileum, was short and formed an ileocecal junction to the caecum. Meanwhile, the large intestine consists of the caecum, colon, and rectum. The caecum was found to be a prominent part in the large intestine, measuring 44.23 ± 5.06 cm in length, which is around 10.1% of the total intestinal length (Table 2). Its wall had haustra, semilunar folds, and taenia continuing into the colon, which is divided into the ascending, transversal, and descending. Haustra and taenia were also observed in the proximal part of the ascending colon, but semilunar fold was absent. The transversal colon was shorter than those of ascending and descending colon. The descending colon where the fecal formation was observed ended into the rectum.

Histological structure

Histologically, the intestinal wall of the Sunda porcupine consisted of mucosa, submucosa, muscular, and serosa layers, the intestinal villi were observed in the small intestine and absent in the large intestine. The number of intestinal villi was most numerous in the duodenum and decreased towards the ileum, the surface was lined by a simple columnar epithelium (Figure 2). Furthermore, the intestinal glands or crypts of Lieberkühn were found in the

mucosal layer, while Goblet cells were observed in the epithelium and intestinal glands. The number of goblet cells was most frequent in the colon as demonstrated in Figure 2. Lymphatic nodules were found in the lamina propria of the duodenum, jejunum, ileum, caecum, colon, and rectum, while the submucosa consisted of connective tissue, fat cells, nerve fibers, and blood vessels. The connective tissue was dense in the submucosal part of the large intestine, then in the submucosa of the duodenum, numerous Brunner's glands were observed with a large lumen. The gland cells were cuboid with basally located nuclei, while the muscularis externa consisted of inner circular and outer longitudinal layer, the inner circular was thicker than the outer longitudinal layer. In addition, the muscularis externa was thicker in the large intestine except for the caecum compared to the small intestine.

PAS and AB staining results exhibited various intensities in intestine tissue as shown in Table 3, the mucin characteristic showed a mixed type of neutral and acid carbohydrate, especially in the duodenum and large intestine. The jejunum and ileum with moderate intensity

of PAS staining indicate a lower neutral carbohydrate content compared to the duodenum. The staining results on Brunner's glands showed a strong positive reaction on AB staining and a moderate positive reaction on PAS, indicating acid carbohydrates. Goblet cells in the duodenum, both in the villi and in the intestinal glands, showed a positive reaction with strong intensity on AB and PAS staining. The jejunum exhibited a strong positive reaction on AB staining and a moderate positive on PAS. Meanwhile, in the ileum, goblet cells demonstrated weak positive results on PAS staining and strong positive results on AB. The type of neutral mucin produced by goblet cells in the intestine generally changed from the small to the large intestine. The neutral intensity of carbohydrates in the duodenum showed strong color and decreased along the small intestine to the ileum but increased in the caecum and colon, while goblet cells in the colon exhibited strong positive color on PAS and AB staining. This result differs from acid mucin, where the color showed consistent intensities along the alimentary tract, as presented in Figure 3.

Table 1. Intestine length of Sunda porcupine

Parameters	Animals			Average	Intestine parts percentage (%)
	1	2	3		
Body Weight (kg)	6	9	5	6.67 ± 2.08	
Head-Body (HB) length (cm)	63	70	58	63.67 ± 6.02	
Total Intestine length (TIL) (cm)	438.2	459.9	415.5	437.87 ± 22.2	
Small Intestine (cm)	302	313.7	291	302.23 ± 11.35	69.02
Duodenum	26	40.2	22.5	29.57 ± 9.37	6.75
Jejunum	266	260.5	259	261.83 ± 3.69	59.80
Ileum	10	13	9.5	10.83 ± 1.89	2.47
Large intestine (cm)	136.2	146.2	124.5	135.63 ± 10.86	30.98
Caecum	42.2	50	40.5	44.23 ± 5.07	10.10
Colon	72	78	70	73.33 ± 4.16	16.75
Rectum	22	18.2	14	18.07 ± 4	4.13

Table 2. Correlation analysis of body weight and intestine length

Parameters	Body Weight	Head-Body length	Total Intestine length
Body Weight	1		
Head-Body length	0.982	1	
Total Intestine length	0.957	0.994	1
Small Intestine	0.965	0.997	0.999
Duodenum	0.999	0.971	0.939
Jejunum	-0.076	0.109	0.216
Ileum	0.994	0.956	0.919
Large intestine	0.947	0.990	0.999
Caecum	0.997	0.967	0.933
Colon	1	0.983	0.957
Rectum	0.268	0.441	0.535

Note: The value close to 1 indicates strong positive correlation between parameters. The value close to -1 indicates strong negative correlation between parameters. The value close to 0 indicate weak positive / negative correlation between parameters

Table 3. Score of color intensity in PAS and AB stain

Intestine parts	Parameters	PAS	AB
Duodenum	Enterocyte	+	-
	Goblet cell	+++	+++
	Brunner gland	++	+++
Jejunum	Enterocyte	+	-
	Goblet cell	++	+++
Ileum	Enterocyte	+	-
	Goblet cell	+	+++
Caecum	Enterocyte	+	-
	Surface Goblet cell	+++	+++
	Basal Goblet cell	+++	+++
	Enterocyte	+	-
Colon	Surface Goblet cell	+++	+++
	Basal Goblet cell	+++	+++
	Enterocyte	+	-

(+++), for highly positive, (++) for moderately positive, (+) for weakly positive, and (-) for negative reaction

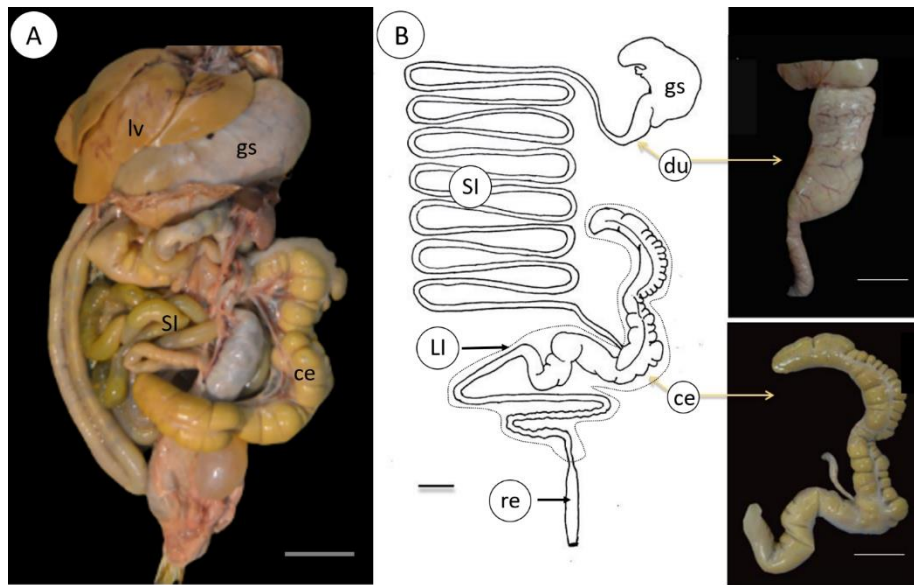


Figure 1. Gross anatomy of the intestine of Sunda porcupine. (A) Gross observation of the digestive system; (B) Schematic drawing of the intestine tract, showed enlargement of the duodenum (du), ampulla, at the proximal part while the caecum (ce) was the prominent structure of the large intestine (LI). lv: liver, gs: gastric, SI: small intestine, re: rectum, du: duodenum, ce: caecum. Bar: 5 cm

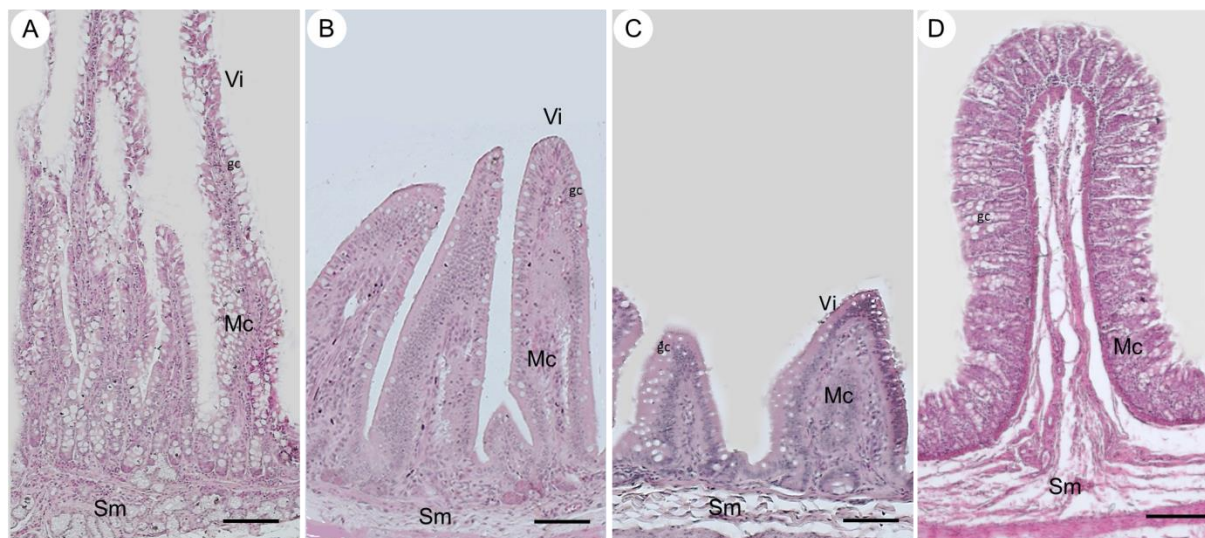


Figure 2. Villi (Vi) structure of the intestine of Sunda Porcupine. The villi of duodenum (A) was the longest with numerous goblet cells compared to the jejunum (B) and ileum parts (C). Structure of villi retracted towards the proximal caecum. The villi were absent in the colon (D) and it had dense connective tissue in the submucosa layer (Sm). Bar 100 μ m

Discussion

The intestine structure of Sunda porcupine is included in the herbivore hindgut fermenter type, which is characterized by a large caecum. Several rodents that possess this characteristic are Guinea Pig, chinchilla, and rat (Grant 2014). In addition, most rodents are also included in the auto-enzyme digester type that produces their own enzymes to digest food rather than using bacterial enzymes, this group has a simple stomach that plays a role in protein hydrolysis by gastric acid. Moreover,

auto-enzyme digestion occurs in the small intestine with enzymes produced from the pancreas and enterocytes. Fibers and plant cells that enter the digestive tract are digested in the large intestine, especially in the caecum by fermentation. The populations of hindgut bacterial colonies express digestive enzymes similar to those found in the rumen, thereby enabling enzymatic digestion of substances from plants that are difficult to digest to produce products having great energy potential (Furness et al. 2015).

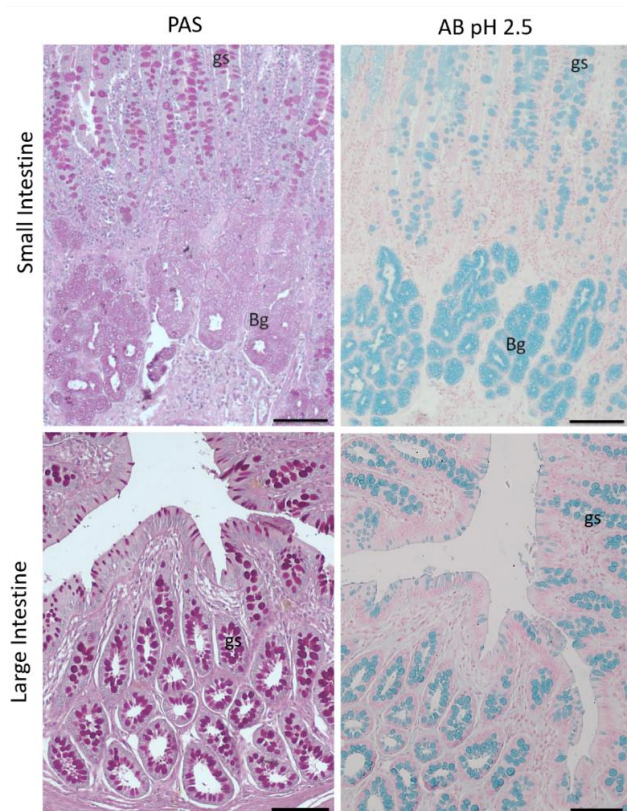


Figure 3. The PAS and AB pH 2.5 stain on the small and large intestine. (A) PAS staining on duodenum of small intestine; (B) AB pH 2.5 staining on duodenum of small intestine; (C) PAS staining on colon of large intestine; and (D) AB pH 2.5 staining on colon of large intestine. Bg: Brunner gland, gc: goblet cell. Bar: 100 μ m

According to Mori et al. (2017) and Farida et al. (2019), the types of diet for *Hystrix* sp and *Erethizon dorsatum* are browse, grass, bulbs, roots or fruits, sweet corn, sweet potato, guava, jicama, and water spinach, hence, they are categorized as herbivores. However, Coltrane (2012) classifies the American porcupine (*Erethizon dorsatum*) as a facultative specialist herbivore, in other words, it can consume feeds that are difficult or rarely consumed by other herbivores due to the presence of certain chemicals or mechanical traits in the feed. The adaptation of the digestive system in the *Erethizon dorsatum* that supports its ability to consume difficult feed is the larger caecum size than the hindgut fermenter herbivore of similar size (<20 kg). In the rodent *Akodon cursor*, the caecum length is only about 4.15% (Finotti et al. 2012), while in the arboreal squirrel *Callosciurus* sp, it is 5.17% of the total intestinal length (Mitsuzuka and Oshida 2018). However, body weight affects the length of the cecum of arboreal squirrels, in the species *Petaurista* sp, which weighs twice as the genus *Callosciurus* sp, the caecum portion is about 13.85% of the total intestinal length. This is also influenced by the different types of feed consumed by the two squirrel genera, where *Petaurista* sp has a diet of leaves, fruit and seeds, while *Callosciurus* sp feeds on fruit and seeds (Mitsuzuka and Oshida 2018). The leaves have more fiber

that is difficult to digest, hence, the adaptation of the caecum to be longer in the digestive system is needed to support the digestion process. Based on the results, the caecum length is 10% of the total intestinal length and about one-third of the large intestine. This size is quite large, indicating that the caecum plays an important role in the Sunda porcupine's diet pattern, which consists of fiber and fruits from the feed.

Furthermore, the length of the small intestine had a strong positive correlation (~ 1.0) to body weight and length. This result differs from several other herbivore rodents, such as *Petaurista* sp, *Callosciurus* sp, and *Lagostomus maximus* (Hagen et al. 2015; Mitsuzuka and Oshida 2018). In *Petaurista* sp, the length of the small intestine is inversely proportional to body weight which implies that the smaller the animal, the longer the small intestine. Meanwhile, in *Callosciurus* sp, the length of the small intestine correlates negatively with body weight but is still positively correlated with body length (Mitsuzuka and Oshida 2018). Compared to *Lagostomus maximus*, the length of the small intestine is positively correlated with the body weight and length, but the value is not as strong as that of the Sunda porcupine, which only ranges from 0.35-0.45 (Hagen et al. 2015). This variation needs to be investigated further because the small intestine plays a role as the main enzymatic digestion site for various nutrients, hence, the length might affect the enzymatic ability and absorption of nutrients for energy needs. Based on the results, the small intestine length of the Sunda porcupine is 69% of the total intestinal length, while *Petaurista* sp and *Lagostomus maximus* have lower percentages, namely 52.98% and 56.28%, respectively. A high percentage of small intestine length was found in *Callosciurus* sp, which has the smallest body weight of 74% (Hagen et al. 2015; Mitsuzuka and Oshida 2018). Hindgut fermenters, such as rodents, have large energy demands based on body mass that are greater than larger species such as a horse. A small rodent requires considerably more food to meet its energy demands, and the passage rate through the GI tract is much faster than a horse on the same diet. Besides, fermentation combines with auto-enzymatic digestion in the foregut to allow small herbivores to recycle nutrients for additional absorption (Grant 2014).

Hagen et al. 2019a showed that the Indian crested porcupine (*H. indica*) has an efficient digestive system with feed retention times in the intestines similar to that of other large rodents and horses. In addition, this hedgehog also does not require high-energy feed to survive, such as feed that is generally given in captivity, including sweet corn, sweet potato, guava, and jicama, because the porcupine can maintain its body weight from fibrous feed only (Farida et al. 2019; Hagen et al. 2019a, 2019b). The close relation between *H. indica* and *H. javanica* might cause similar physiological characteristics in their digestive system.

The surface of the intestinal mucosa is covered by a layer of mucus in the form of a viscoelastic gel. The mucus plays a role in lubricating the intestinal mucosa and protecting the epithelial layer from mechanical damage and pathogens invasion. In addition, the mucus layer also provides a microenvironment for certain microflora and

produces a biofilm on the mucosal surface, which is very important in intestinal health (Pelaseyed et al. 2014). Mucus is a constantly changing mixture containing secretions and exfoliated epithelial cells. The main factor that determines the function and physical components of mucus is mucin, which is a glycosylated protein. Mucins can be categorized into two types based on their charge, namely neutral and acid. Recent studies on mammals showed that the distribution of goblet cells and the type of mucin varies along the gastrointestinal tract and other specific factors, such as species, age, diet, and bacterial components (Liquori et al. 2012; Mastrodonato et al. 2013; Tano et al. 2014).

The characteristics of the mucin produced by the goblet cells of the small intestine were generally of the mixed type, a mixture of neutral and acid, but the composition changed along the intestine from the duodenum to the ileum. The mucin produced by the goblet cells in the duodenum is a balanced mixture of neutral and acidic mucus, while those produced by Brunner's glands are more acidic. In guinea pigs, this composition is slightly different with characteristic weak reactions of PAS and AB in Brunner's glands, indicating that the mucin composition contains a few neutral or acidic carbohydrates. In contrast, chinchillas showed a strong reaction to PAS and AB staining indicating a large amount of neutral and acidic carbohydrates in their secretions (Gal et al. 2019). The mucin characteristics in the Sunda porcupine's duodenum resemble those of the Persian squirrel, which exhibited a strong PAS staining intensity in goblet cells but was weak in Brunner's glands (Tootian et al. 2013). The characteristics of mucin in the ileum of the Sunda porcupine are dominated by acid carbohydrates. This is different from some rodents because the mucin secreted in the ileum tends to be mixed, such as in Persian squirrel (*Sciurus anomalus*) and plains viscacha (*Lagostomus maximus*), which show moderate reactions in their ileum goblet cells (Tootian et al. 2013; Tano et al. 2016). In the rodent *Meriones libycus*, the ileum contains more neutral carbohydrates, but overall, the goblet cells in the gastrointestinal tract produce neutral and acid mucin, similar to *Acomys dimidiatus* and *Meriones rex* (Johnson et al. 2016).

The caecum and colon of the Sunda porcupine have mucin characteristics similar to some other rodents, consisting of neutral and acidic mucin type, especially in the basal part of the epithelium. This characteristic was also found in guinea pig (*Cavia porcellus*) (Chende et al. 2021), plains viscacha (*Lagostomus maximus*) (Tano et al. 2017, 2019), *Meriones libycus*, *Meriones rex*, and *Acomys dimidiatus* (Johnson et al. 2016). Mucus plays an important role in the large intestine of herbivore hindgut fermenters to support the fermentation process. The mucus trapping mechanism found in rodents allows the large intestine to retain bacteria from the small intestine as well as the colon and then direct back to the caecum through antiperistaltic movements. The purpose of this mechanism is to collect bacteria used to ferment food particles that are difficult to digest (Grant 2014).

The gut structure of the Sunda porcupine represents herbivore of the hindgut fermenter type characterized by a large caecum size. The relatively long small intestine indicates that the enzymatic process can occur more optimally, culminating in a maximum digestion process and absorption of nutrients. Furthermore, histological characteristics of mucins are species-specific, as indicated by predominant mixed types and varying carbohydrate acids in the intestinal tract.

ACKNOWLEDGEMENTS

The authors are grateful to the Indonesia Endowment Fund for Education (LPDP), Ministry of Finance for the scholarship; Directorate General of Nature Protection, Ministry of Environment and Forestry for permitting the use of the animals for this study; Directorate of Human Resource Qualification, the Directorate General of Resources for Science and Technology and Higher Education (DG-RSTHE), Ministry of Research, Technology and Higher Education of the Republic of Indonesia; and *Program Magister Doktor untuk Sarjana Unggul* (PMDSU) No. 29/SP2H/PTNBH/DRPM/2018 and 1468/IT3.11/PN/2018. The authors also to thank the Reviewers for taking the time and effort necessary to review the manuscript. We sincerely appreciate all valuable comments and suggestions which helped us to improve the quality of the manuscript. The authors also thank Desrayni Hanadhita, Anisa Rahma, and Andi Hiroyuki for their assistance in this study. The authors declare that they have no conflict of interest.

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