Pertanika J. Trop. Agric. Sci. 40 (1): 19 - 34 (2017)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Comparative Histological Evaluations of the Sublingual Salivary Glands of EBN Swiftlets (*Aerodramus fuciphagus*) in Man-Made Houses and Natural Caves

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ABSTRACT

One of the most precious edible bird's nests (EBN) is constructed by the white-nest swiftlet (*Aerodramus*). However, different swiftlet populations might have different food intakes as a result of their different habitat sources. This situation will likely influence the secretion of the salivary gland. EBN is built from the saliva of the swiftlets. The major function of the salivary gland is to secrete saliva. This study was conducted with the aim of defining and comparing the histological structures of the sublingual salivary gland and its mucin content found in two separate populations of house-farm and cave white-nest swiftlets. Samples were collected from Seri Iskandar, Perak, Malaysia (04º20.824´N, 100º52.826´E) and Gomantong caves, Sabah, Malaysia (5º31.46.5´N, 118º4.29.6´E). It was found that the largest visible salivary gland present in both populations was the sublingual gland. The glands were stained with hematoxylin and eosin (H & E) stain and a combination of Alcian blue (AB) with periodic acid-Schiff (PAS) stain. The H&E stain displayed a broad range of cytoplasmic, nuclear and extracellular matrix features. The parenchyma of the cave swiftlet population appeared foamy due to high mucous secretion whereas the cells of the house-farm population could clearly be seen to be separated because of less mucous secretion. There was a clear difference in density and abundance of mucous acini cells in which the samples from the cave population were compacted with these cells. AB-PAS stains revealed full complement of tissue proteoglycans and acidic-mucin, neutral-mucin and mixtures of acidic and neutral mucins. The cave population exhibited higher concentrations of acidic, neutral, and mucins mixture compared with those from the house-farm. This is probably

ARTICLE INFO

Article history:
Received: 17 October 2014
Accepted: 10 November 2016

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ISSN: 1511-3701

caused by several combinations of factors such as difference in dietary habit, habitat preference and age of the swiftlet.

Keywords: white-nest swiftlets, edible bird's nest (EBN), sublingual salivary gland, saliva, house-farm swiftlets, cave swiftlets

INTRODUCTION

A few species of swiftlets (genus *Aerodramus*) build edible nests that are consumed by humans, known as the 'caviar of the East' or as a medicinal food (Marcone, 2005). Edible bird's nest (EBN) refers to the nest produced by several different swiftlet species. Human consumption of these nests has been regarded as a symbol of wealth, power and prestige, while its use for its medicinal value by traditional Chinese medicine practitioners dates as far back as the Tang (618-907 AD) and Sung (960-1279 AD) dynasties (Lim & Cranbrook, 2002). The majority of EBNs traded worldwide comes from two heavily exploited species, the white-nest swiftlet (WNS) and the black-nest swiftlet (*A. maximus*). This species distribution ranged from the Nicobar Islands in the Indian Ocean to the sea caves in the coastal regions of Thailand, Vietnam, Indonesia, Borneo and the Palawan Islands of the Philippines (Lim, 2000; Lim & Cranbrook, 2014). Based on the recent systematic review by Cranbrook et al. (2013), WNS are divided into two large allopatric species, namely the grey-rumped Swiftlet *Aerodramus inexpectatus*, with subspecies *A. i. germani* and *A. i. perplexus*, and Thunberg's or the brown-rumped Swiftlet *Aerodramus fuciphagus*, with subspecies *A. f. fuciphagus* and *A. f. vestitus*. Species identification in the field has proven to be challenging because of the limited variation in size and plumage colouration of the swiftlets. Stresemann (1931) has characterised these birds based on their tarsal feathering, rump and shaft colouration as well as the length of the wing, tail and their furcation. The type of the nest was also considered to be one of the reliable taxonomic indicators among swiftlets as shown by Medway (1966a). In this study, the taxonomic classification for the species studied follows that of Cranbrook et al. (2013), in which the cave population is identified as *A. f. vestitus*, while the current domestic house-farm population is a potential hybrid species drawn from genetic mixing of two species of WNS (i.e. *A. inexpectatus* and Thunberg's swiftlet *A. f. fuciphagus*).

EBN is the nest of the swift that is made from its saliva, which contains sialylglycoconjugates (Matsukawa et al., 2011). The composition of the swiftlet's saliva resembles that of salivary mucin. Many studies have been carried out on the tonic effects of EBN, and it has been shown that EBN stimulates mitosis hormones and the growth factor for epidermal growth, resulting in repair of cells and stimulation of the immune system (Ng et al., 1986; Kong et al., 1987). The average crude protein content in the EBN has been reported by Marcone (2005) to be at 62%-63% and by Kathan and Weeks (1969) to be at 32.3%. Researchers have also found several carbohydrate

molecules in EBN including new sialic acid-containing compounds and glycoconjugates (Martin et al., 1977; Pozsgay et al., 1987; Reuter et al., 1989; Wieruszeski et al., 1987; Kakehi et al., 1994; Yu-Qin et al., 2000). However, the importance of sialic acid residues in EBN is still not clear.

The number and arrangement of salivary glands vary among the species. In mammals, there are three main pairs of salivary glands: (i) the submandibular (ii) the sublingual, which lie under the tongue, and (iii) the parotid, which lies at the back of the mouth between the upper and the lower jaw (Tomasi & Plaut, 1985). In birds, adaptation of the salivary glands is based on the type of food consumed. In general, a species that relies on a relatively soft diet has less developed salivary glands while insectivores and seed eaters have more developed and functional salivary glands (King & McLelland, 1984; Blanks, 1993; Taib & Jarrar, 2001). In contrast, swiftlets have numerous minor salivary glands in their lingual apparatus. This modification allows the swiftlets to produce massive amounts of salivary secretion, which may manifest significantly during the nest-building process (Shah & Aziz, 2014). Although the salivary glands of most birds are not conspicuous as those in mammals, the comparative morphology has been studied since the 1880s (e.g. Batelli & Giacomini, 1889) and most avian glands are histologically described as the mucous type (Jerret & Goodge, 1973). In addition, the presence of serous cells has also been recorded in the salivary glands of quail, *C. coturnix* (Taib & Jarrar, 1998).

The primary function of salivary glands is to secrete saliva, a fluid composed of water, electrolytes and various multifunctional proteins (Koller et al., 2000). The basic protective mechanism mediated by saliva is bacterial clearance. Saliva is also an essential fluid for the health of human teeth and oral mucosal surfaces and for maintaining microbial balance and supporting other oral functions. Other than that, saliva also contains antifungal and antiviral substances that make it part of the mucosal immune system (Tomasi & Plaut, 1985). On the other hand, swiftlets use saliva to construct their nest (Goh et al., 2001), and this is considered one of the swiftlet's prized assets because there are no other organisms with such ability. Swiftlet nests are constructed at the vertical concave of a cave wall in a half bowl-like shape into which the swiftlets' eggs are hatched (Marcone, 2005). The objectives of this study were to define the histological structures of the sublingual glands and to determine the mucin type of the glands from two different swiftlet populations. It was hypothesised that there are differences in the morphology and the amount of protein concentrations in the sublingual glands of swiftlets from house-farm and cave populations.

MATERIALS AND METHODS

Sample and Data Collection

During mist-netting, a number of captured swiftlets were released as there was no visible bulking salivary gland underneath the throat and lower mandible. Only 14 birds were selected based on this criterion.

It was believed that the swiftlets used were adult birds based on their overall body size as well as the presence of bulking salivary glands found under the lower mandible. Seven WNS were captured in Seri Iskandar (04º20.824´N, 100º52.826´E), where the distance of the sampling area from the bird-house is approximately 500 m. The other seven swiftlets were captured inside the Gomantong caves (5º31.46.5´N, 118º4.29.6´E). The swiftlets from Seri Iskandar, Perak were captured on 3 January, 2013, whereas the swiftlets from Gua Gomantong, Sabah were captured on 18 December, 2013. Only 12 birds with well-developed sublingual glands (three for each group) out of the 14 birds were selected to standardise the comparison. Only a small number of samples was collected for each group due to lack of cooperation from the house-farm/cave owners. The 20-m mist net (2.5 m height and 2 cm x 2 cm mesh size) with two shelves was deployed in the free land of Seri Iskandar, Perak, and the swiftlets were enticed by playback calls using a portable speaker (G-Shark S938). When the birds hit the net, they were quickly caught and transferred into wooden cages, which were then covered with cloth to reduce the stress of the birds. The birds were quickly transported to a laboratory in the Institute of Biosciences, Universiti Putra Malaysia (UPM). Sampling at Gua Gomantong, Sabah was conducted by deploying the nets directly to heights closest to the bird nests. Subsequently, the birds were euthanised and dissected at the Regional Veterinary Laboratory, Department of Veterinary Services and Livestock Industries, Kota Kinabalu, Sabah. The study protocol was approved by the UPM Animal Ethics Committee (AUP: 12R144/Apr12-March13).

Gross Examination

Before performing the dissection, the swiftlets were euthanised using approximately 1 mL pentobarbitone sodium (Nembutal®) at a dose of 80 mg/kg intravenously, which was injected through the brachial ulna vein (Close et al., 1996). The feather around the lower mandible was gently removed using alcohol; this was carried out carefully to prevent any distortion to the salivary glands. Subsequently, the dissection was carried out to expose the sublingual glands. The glands were subjected to gross examination under stereomicroscope (Nikon SMZ1500, Tokyo, Japan), and the weight of the glands was measured using a three-decimal place weighing balance (B303-S analytical balance, Mettler Toledo, Switzerland). Following that, the gender of the swiftlets was determined by observing the sexual reproductive organs.

Microscopic Examination

The tissues of the gland were then removed and fixed in Bouin's solution for 16 to 24 h and washed every 2 h using 50% alcohol for a total of three times and preserved with 70% alcohol (Adnyane et al., 2011). The samples were then transferred into a cassette, processed for 16 h and then embedded in paraffin wax (Bancroft & Gamble, 2008). The blocks were serially sectioned into 4-µm thickness using a microtome

(Leica RM-2155 rotary microtome; Leica Microsystem Inc., Bensheim, Germany). The sections were then deparaffinised, hydrated through graded alcohol with water and stained with hematoxylin and eosin (H&E) to demonstrate the general histological architecture of the tissue (Spector & Goldman, 2006). The tissue was also stained using a combination of Alcian blue-periodic acid-Schiff (AB-PAS) with a pH of 2.5 for the differentiation of neutral and acid mucins (Spicer & Meyer, 1960; Bancroft & Gamble, 2008). The slides were mounted with cover slips using the mounting medium (Entellan®, Merck, Germany) and left for 24 h in the open air. Finally, the stained slides were examined under a light microscope equipped with an image analyser (Olympus BX51; Olympus Optical Co. Tokyo, Japan).

Statistical Analysis

The data providing sublingual gland weight were calculated based on actual and relative weight, which was expressed as mean ±SD. Statistical comparisons were conducted between the two WNS populations from different habitats. Data were analysed using an independent-sample t-test for parametrics (IBM SPSS Statistic Ver. 21). The significant level was set at $p<0.05$.

H_0 = Mean relative weight of sublingual glands was the same for both the house-farm and cave population

H_1 = Mean relative weight of sublingual glands was significantly different for the house-farm and cave population.

RESULTS

Gross Examination

Gross examination of the salivary glands showed that there was a pair of major salivary glands (i.e. sublingual glands) present ventral to the lower mandible of the swiftlet (Figure 1). However, this structure

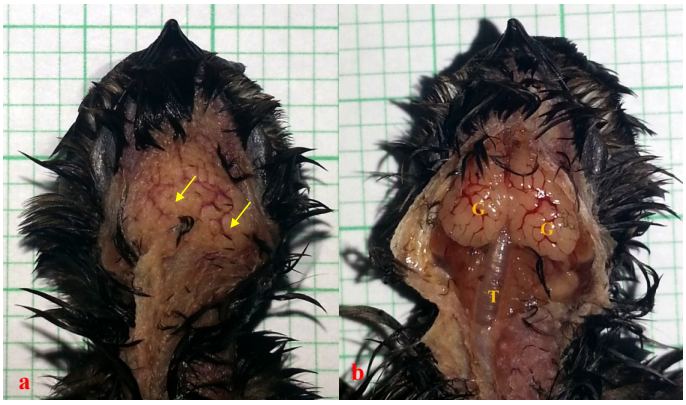

Figure 1. Photographs of the bird (a) before dissection showing the submandibular glands lying underneath the skin ventral to the lower mandible (arrows) (note that the feather around the lower mandible was removed) and (b) after dissection showing the exposed submandibular glands. The glands are enlarged and lobulated. G = submandibular glands; T = trachea; 1 grid = 1 mm

could only be seen clearly once the feather under the lower mandible of the bird was removed. The glands were present in a pair, and they were greatly enlarged compared with the other major salivary glands. An observation of the 3D images under the stereomicroscope showed a well-developed gland that appeared as a 'brain-like' coiled tubular structure with a soft white to pinkish appearance of the sublingual glands (Figure 2). The weight and the relative weight of the sublingual glands between the house-farm and cave WNS population are shown in Table 1.

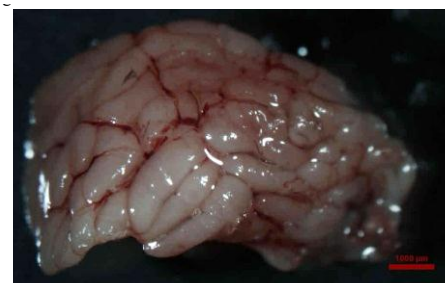

Figure 2. Photograph of the sublingual gland under high magnification (microvisualisation) showing the coiled tubular structure of the gland that appeared as a brain-like coiled tubular structure with soft white to pinkish colour. Magnification: x25; Scale: 1 mm

Table 1
Mean Values of Whole Body Weight and Sublingual Glands (Grams) of WNS from House-Farm and Cave Population

Location	House-Farm			Cave		
Sex	Male	Female	Mean (Total)	Male	Female	Mean (Total)
No. of animal	3	3	6	3	3	6
Body weight (g)	8.520 ± 0.494	9.033 ± 0.354	8.777 ± 0.476	10.283 ± 0.580	10.500 ± 1.212	10.39 ± 0.858
Sublingual gland weight (g)	0.050 ± 0.030[a]	0.079 ± 0.031[b]	0.065 ± 0.032[c]	0.072 ± 0.015[a]	0.050 ± 0.039[b]	0.061 ± 0.029[c]
Relative sublingual gland weight (%)*	0.604 ± 0.366[c]	0.879 ± 0.363[d]	0.721 ± 0.324**	0.702 ± 0.176[c]	0.500 ± 0.418[d]	0.574 ± 0.253**

*The relative sublingual gland weights was calculated based on the sublingual gland weight (g)/body weight (g) and presented in percent (%).
[a, b, c, d, e] The mean value of the sublingual glands with similar letter was not significantly different ($p>0.05$).
**Comparison between these two populations showed a significant p-value ($p<0.05$).

As shown in Table 1, the mean body weight of the cave population was 10.28±0.58 g for males and 10.50±1.21 g for females, whereas the house-farm population had a lower mean body weight (8.52±0.49 g for males and 9.03±0.35 g for females). The weight of the sublingual glands of the cave WNS was greater than that of the house-farm population in males, but the weight of the gland in females from the house-farm population was greater than that of the cave population although the body weight was slightly lower. However, based on the statistical test, the relative weight of the

sublingual glands between both populations was not significantly different ($p>0.05$). Comparison between these two populations was significantly different at $p<0.05$.

Microscopic Examination

Based on the H & E stain (Figure 3), cross sections of the cave WNS showed that the alveolus of the mucous acinus was wholly stained as compared with that of the house-farm birds. The parenchyma appeared foamy in cave swiftlets apparently due to the high mucous secretion and viscosity of the mucous cells, whereas the cells of house-farm birds could clearly be seen to be separated; it was expected there would be less mucous secretion and viscosity. The mucous acini were well positioned around the cell itself and were compartmentalised into several lobules by connective tissue. The nucleus could be seen at the base attached to the branch of the connective tissue. The irregular loose connective tissue was present between and encapsulating the glands, whereas the blood vessel was present in the middle and outside the cells (Figures 3a and 3d). There was no observable difference in mucous secretion between these two WNS populations (Figures 3b and 3e). The mucous acini cells could be seen to be separated from one another, but there was a clear difference in terms of density; the samples from the caves were compacted within these cells (Figures 3c and 3f). This was believed to be the cause of the foamy appearance of the gland tissues. The epithelial cells surrounding the gland tissues of the birds from cave populations (Figures 3d-3f) were observed, but the simple connecting ducts or the excretory ducts in both samples could not be observed. The presence of numerous acinus cells in both samples was weakly stained by the H & E stain; this could indicate that the sublingual glands of the WNS were only present within the mucous acini.

The sublingual gland tissue of both samples stained with AB-PAS (pH of the AB was 2.5) showed that the gland tissue was full of mucin secretion (Figure 4). Both samples contained mixtures of acidic and neutral glycoprotein-containing structures (mucin) because, histochemically, both showed positive reactivity to staining using a combination of AB and PAS stains. The staining area of the mixture of mucin and neutral mucin tissue was roughly the same in total area. However, it was apparent that samples from the cave also exhibited dominant blue staining, which indicated the occurrence of acidic mucins; this was not presented in the samples from the house-farm. Figure 4 shows the magnification of different levels of AB-PAS stain from both population samples. The samples from the house-farm population (Figures 4a-4c) clearly expressed different colours of staining, showing a mixture of mucin stained purple violet and the neutral mucin stained magenta. Meanwhile, as indicated by the intense colour stained, it was clear that mucous cells were abundant, the secretion was high in the cave samples and the cells were not well separated (Figures 4d-4f).

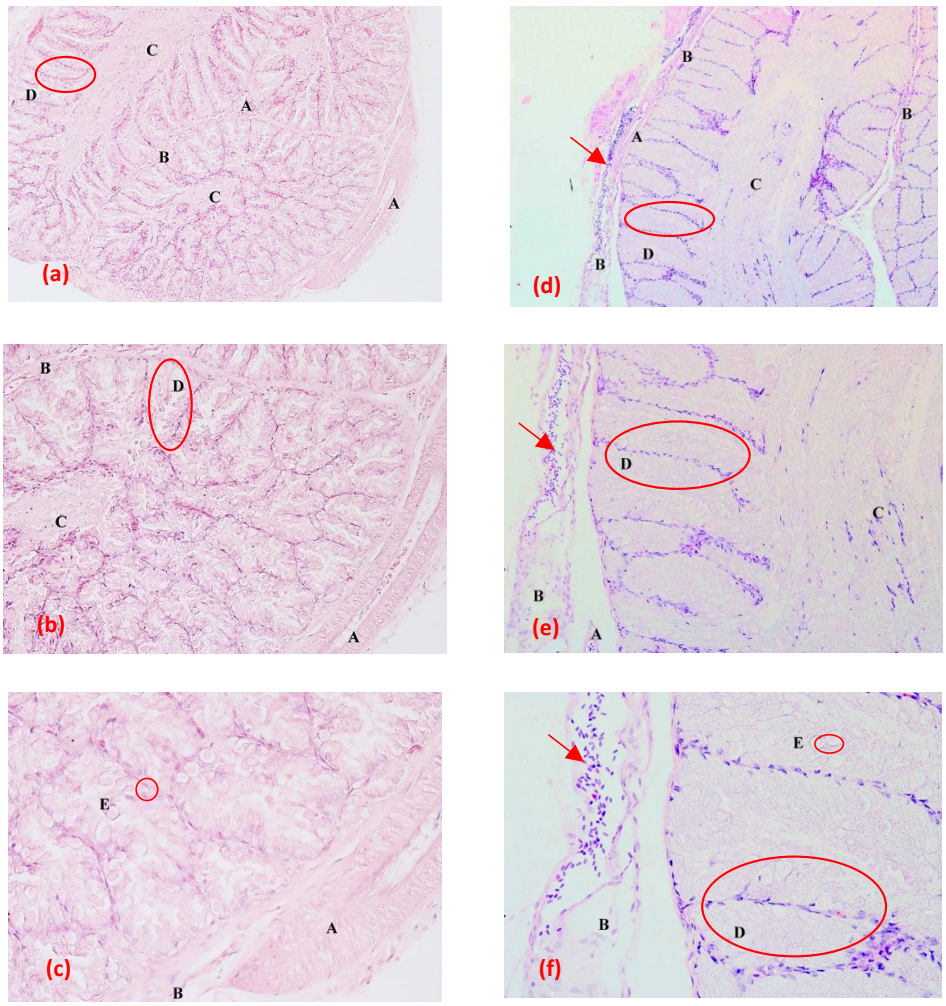

Figure 3. Photomicrographs of the cross-section of the sublingual glands of house-farm and cave WNS. Samples were stained with H & E at different levels of magnification. Cross-sections from (a-c) the house-farm and (d-f) the cave samples are shown. The magnification levels were set starting at (a and d) µ100, (b and e) µ200 and (c and d) µ400. The circles labelled D and E are the lobule of a gland cell and a mucous cell. The arrows show the epithelial cells present around the gland. A=blood vessel; B=loose connective tissue; C=mucous secretion

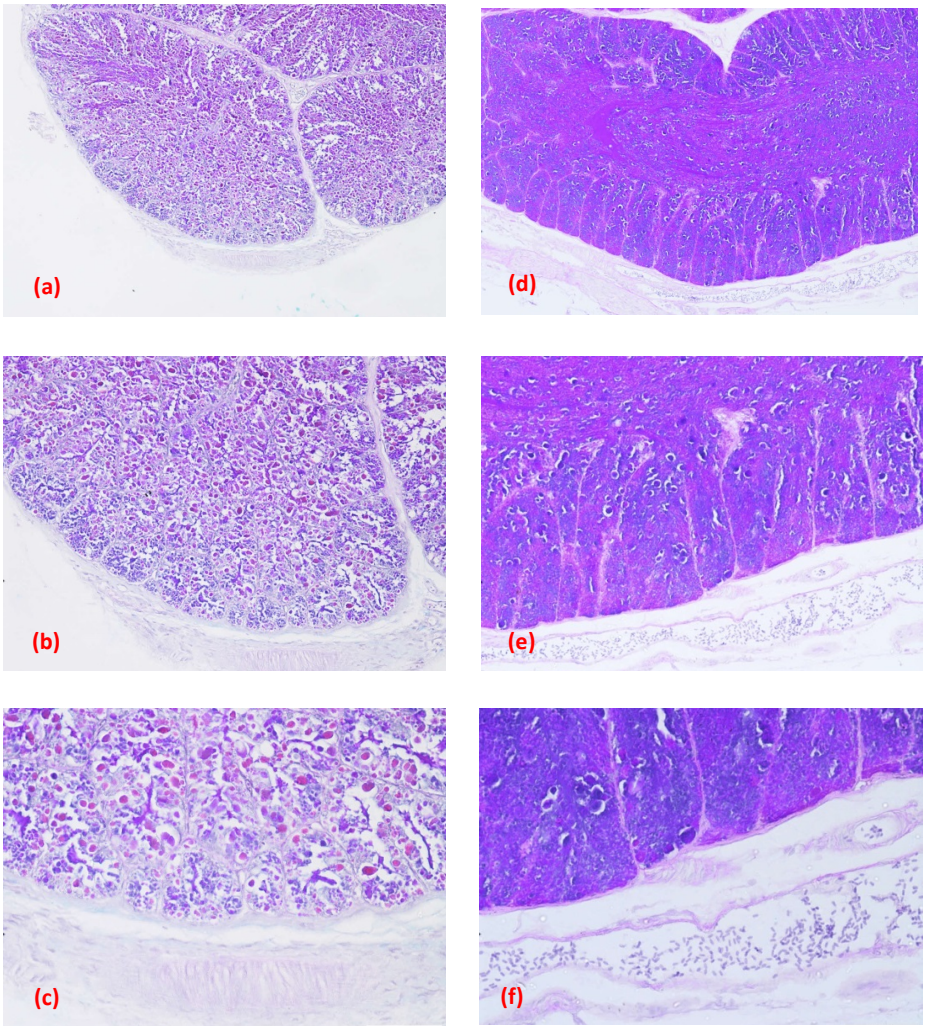

Figure 4. AB-PAS staining of the cross-sectional view of (a-c) house-farm and (d-f) cave WNS using the following magnification levels: (a and d) x100, (b and d) x200 and (c and f) x400

DISCUSSION

The EBN is wholly secreted by a pair of sublingual glands (Marshall & Folley, 1956) and this study found that the largest visible salivary gland present in WNS of both localities was the sublingual gland. The gland is a simple tissue mass with a soft to white pinkish appearance. According to Mese and Matsuo (2007), larger glands have more cells that will contribute to a higher production of saliva in either the stimulated stage or the unstimulated stage (resting saliva). The swiftlets were captured in January and December, which coincided with the active breeding season when the salivary glands expand (Lim & Cranbrook, 2014). Mating occurs throughout, but breeding is concentrated in the period from October to February (Langham, 1980). The sublingual glands appear to be the largest salivary gland; hence, the main source of saliva production in the WNS. This is in contrast with the salivary gland in humans, where the largest major salivary gland is the parotid gland (Ono et al., 2006).

Swiftlets are aerial insectivores that prefer foraging habitat across the tropical forest canopy (Waugh & Hails, 1983). It was hypothesised that the glands of cave WNS had more mass compared with the house-farm species due to its habitat preference and the high abundance and diversity of insect sources in the tropical forest. The hypothesis was derived based on food availability in the habitat as the size of the organ is most likely influenced by diet. Lewis et al. (1985) and Stoltzner (1977) stated that organ weight is often greatly reduced by dietary restriction. In addition, Lourie and Tompkins (2000) reported that the glossy swiftlets (*Collocalia esculenta*) live on forest feeds that have a higher percentage of Hymenoptera (i.e. bees and ants) (42% of total prey) and Coleoptera (i.e. beetles) (21% of total prey), whereas those that live in the urban areas preferred Diptera (i.e. flies) (71% of total prey). The percentage of crude proteins is 21.0% from Hymenoptera, 26%-30% from Coleoptera (Banjo et al., 2006) and 48% from Diptera (Odesanya et al., 2011). This shows that the glossy swiftlets from urban areas have more protein intake. In this study, none of the birds was subjected to a controlled diet, and the limited source of food intake could not be neglected. Table 1 shows that there was no significant difference in the mean weight and relative weight of sublingual glands between the two populations ($p>0.05$). This might be because of the small sample size of the study due to sampling limitation. Difference in the size of the glands between both specimens might as well influence the analysis. Lim and Cranbrook (2014) stated that the salivary glands of swiftlets expand in the breeding season. This difference is because of their different breeding seasons; harvesting might affect the breeding season of the swiftlets (Tompkins, 1999).

Based on the H & E staining, there was no visible serous cells in the swiftlets' sublingual glands, unlike in humans (Myers & Ferris, 2007) and rats (Miclaus et al., 2009). The only cells present in the swiftlets' sublingual glands were the mucous cells, as demonstrated by the pale-stained cytoplasm with flattened nuclei at the base of the

cell. Serous acini cells are darkly stained and generally spherical in shape (Ross & Pawlina, 2011). Mucous cells are associated with the secretion of viscous mucins stored in vacuoles (Ekstrom et al., 2012) and possessed a mixture of glycoconjugates with different nature (Arthitvong et al., 1999). EBN contains a high amount of glycoconjugates beneficial to humans (Nakagawa et al., 2007). Compared with the serous secretory granules, it contains less glycoconjugates and has a large amount of water and ions. The glycoconjugates of serous granules are acidic and termed 'seromucous' (Kademani & Tiwana, 2015). However, this mucous cell might not be the same as that found in other organisms. In rats, the serous acini of the submandibular gland are not identical with the serous acini that is present in the parotid gland (Miclaus et al., 2009). Other study showed that salivary glands are present with lumens that act as a passage for gland secretion to the oral cavity (Wells & Patel, 2010) and ducts (either in the form of intercalated, striated, excretory and main excretory ducts), which will modify the secretion of the acinar cells. However, it can only be observed under electron microscope observation (Amano et al., 2012) and is rarely observed in H & E stains. This sublingual glands need to be further studied with regards to its ultrastructure to observe the type of myoepithelium, secretory granules, plasma cells etc. Although the parotid glands mainly have serous acini, as in humans, the sublingual glands are mucous, whereas the submandibular glands are a mixture of the two, yet these acinar cells do not relate to all species. As diets vary from one species to another, in the same way, salivary glands vary from one another, as they are specialised mainly for diet (Tandler & Philips, 1998). The current results also showed that the sublingual glands of the WNS had a spherical outer layer and there was no demilunar structure as in cats and dogs (Shackleford, 1962). The sublingual glands might be suggested to be classified as mucous glands due to the absence of serous cells.

A combination of an AB-PAS stain can be used to differentiate neutral mucins from acidic mucins within a tissue section (Mowry, 1963), where the differentiation is based on the net charge of the molecule (Filipe, 1979). Mucins are the determinants of the functional and physical properties of mucous, which is highly glycosylated and has high molecular weight proteins (Forstner & Forstner, 1994). WNS from the cave population have higher concentrations of the acidic, neutral and mucins mixture compared with those from the house-farm population, and this probably will affect the composition of the nest. Squires (1953) revealed that the contents and varieties of salivary secretion are mostly related to the eating habits of the birds. However, it is unclear as to what extent the diet will influence the size of the glands and the secretion of the salivary glands. More than that, the different contents of secretion are due to the preference of habitat as a small change in the ambient temperature (by 2ºC) is enough to inversely affect the

flow rate of the salivary gland secretion (Kariyawasam & Dawes, 2005). Other than that, the secretion contents are also possibly related to the age of the swiftlets because the components of the salivary gland acini decrease with ageing (Drummond & Chisholm, 1984; Scott, 1986). This hypothesis remains unchallenged as there are currently no available data or studies on age determination of swiftlets. Because the nests (EBN) are abundant with glycoprotein (Wu et al., 2010), this study has proven that the sublingual glands of EBN swiftlets are full of mucin secretion, which is essential as the main source of nest production.

CONCLUSION

In conclusion, the sublingual glands appeared as the largest gland structure present in the salivary glands of the WNS. There was no significant difference in terms of the weight/relative weight of the sublingual glands between the two populations and the H0 was accepted. The only cells that could be observed under a light microscope (0µ400 magnification) were the mucous cells that were attached to a loose connective tissue forming a lobule. On the other hand, this gland holds a rich mixture of neutral and acidic mucin, which serves as the most nutritious compound in the edible nest. This study also indicated that the WNS from the cave population had a higher concentration of secretion compared to the house-farm population. However, detailed information about the types of mucin compound (glycoprotein-containing structure) in the saliva is still lacking. Further study is needed to analyse the glycoprotein content of the salivary glands, which is the source of the nutritious compound found in EBN. Other than that, it is recommended to increase the sample size for future studies concerning sexual dimorphism of the species.

REFERENCES

Adnyane, I. K. M., Zuki, A. B. Z., Noordin, M. M., Wahyuni, S., & Agungpriyono, S. (2011). Morphological study of the infraorbital gland of the male barking deer, Muntiacus muntjak. *African Journal of Biotechnology, 10*(77), 17891–17897.

Amano, O., Mizobe, K., Bando, Y., & Sakiyama, K. (2012). Anatomy and histology of rodent and human major salivary glands. *Acta Histochemica et Cytochemica, 45*(5), 241–250.

Arthitvong, S., Makmee, N., & Suprasert, A. (1999). Histochemical detection of glycoconjugates in the anterior lingual salivary glands of the domestic fowl. *Kasetsart Journal (Natural Science), 33*, 243–250.

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques* (5th Ed.). New York, NY: Churchill Livingstone.

Banjo, A. D., Lawal, O. A., & Songonuga, E. A. (2006). The nutritional value of fourteen species of edible insects in southwestern Nigeria. *African Journal of Biotechnology, 5*(3), 298–301.

Banks, W. J. (1993). Applied veterinary histology (3rd Ed.). *Missouri* (pp. 446–465). United States of America, USA: Mosby-Year Book, Inc.

Close, B., Banister, K., Baumans, V., Bernoth, E. M., Bromage, N., Bunyan, J., …Warwick, C. (1996). Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals, 30*(4), 293–316.

Cranbrook, E. O., Lim, G. W., Koon, L. C., & Abdulrahman, M. (2013). The species of white-nest swiftlets (Apodidae, Collocaliini) of Malaysia and the origins of house-farmbirds: morphometric and genetic evidence. *Forktail, 29*, 107-119.

Drummond, J. R., & Chisholm, D. M. (1984). A qualitative and quantitative study of the ageing human labial salivary glands. *Archives of Oral Biology, 29*(2), 151–155.

Ekström, J., Khosravani, N., Castagnola, M., & Messana, I. (2011). Saliva and the control of its secretion. In O. Ekberg (Ed.), *Dysphagia* (pp. 19-47). Germany: Springer Berlin Heidelberg.

Filipe, M. I. (1979). Mucins in the human gastrointestinal epithelium: A review. *Investigative and Cell Pathology, 2*(3), 195–216.

Forstner, J., & Forstner, G. (1994). Gastrointestinal mucous. In L. Johnson (Ed.), *Physiology of gastrointestinal tract* (3rd Ed.) (pp. 1255–1283). New York, NY: Raven Press.

Goh, D. L. M., Chua, K. Y., Seow, T. K., Ou, K. L., Yi, F. C., & Lee, B. W. (2001). Immunochemical characterization of edible bird's nest allergens. *The Journal of Allergy and Clinical Immunology, 107*(6), 1082–1088.

Harris, H. F. (1900). On the rapid conversion of haematoxylin into haematein in staining reactions. *Journal of Applied Microscopic Laboratory Methods, 3*(3), 777-780.

Jerret, S. A., & Goodge, W. R. (1973). Evidence for amylase in avian salivary glands. *Journal of Morphology, 137*(1), 27–45.

Kademani, D., & Tiwana, P. (2015). *Atlas of oral maxillofacial surgery: The salivary glands* (1st Ed.) (pp. 50–56). Philadelphia, USA: Elsevier - Health Sciences Division.

Kakehi, K., Susami, A., Taga, A., Suzuki, S., & Honda, S. (1994). High-performance capillary electrophoresis of O-glycosidically linked sialic acid-containing oligosaccharides in glycoproteins as their alditol derivatives with low-wavelength UV monitoring. *Journal of Chromatography A, 680*(1), 209-215.

Kariyawasam, A. P., & Dawes, C. (2005). A circannual rhythm in unstimulated salivary flow rate when the ambient temperature varies by only about 2° C. *Archives of Oral Biology, 50*(10), 919-922.

Kathan, R. H., & Weeks, D. I. (1969). Structure studies of collocalia mucoid: I. Carbohydrate and amino acid composition. *Archives of Biochemistry and Biophysics, 134*(2), 572-576.

King, A. S., & McLelland, J. (1984). *Birds: Their structure and function* (2nd Ed.). London: Bailliere and Tindall.

Koller, M. M., Cowman, R. A., Humphreys-Beher, M. G., & Scarpace, P. J. (2000). An analysis of submandibular salivary gland function with desipramine and age in female NIA Fischer 344 rats. *Mechanisms and Ageing Development, 119*(3), 131–147.

Kong, Y. C., Keung, W. M., Yip, T. T., Ko, K. M., Tsao, S. W., & Ng, M. H. (1987). Evidence that epidermal growth factor is present in swiftlet's (Collocalia) nest. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 87*(2), 221-226.

Koon, L. C. (2000). Features–Bird's nest soup–Market demand for this expensive gastronomic delicacy threatens the aptly named edible-nest Swiftlets with extinction in the east. *Wildlife Conservation, 103*(1), 30–35.

Langham, N. (1980). Breeding biology of the edible-nest swiftlet Aerodramus fuciphagus. *Ibis, 122*(4), 447-461.

Lewis, S. E., Goldspink, D. F., Phillips, J. G., Merry, B. J., & Holehan, A. M. (1985). The effects of aging and chronic dietary restriction on whole body growth and protein turnover in the rat. *Experimental Gerontology, 20*(5), 253-263.

Lim, C. K., & Cranbrook, Earl of (2002). *Swiftlets of Borneo–Builders of edible nests* (pp. 1–171). Sabah, Malaysia: Natural History Publication (Borneo) Sdn. Bhd.

Lim, C. K., & Cranbrook, Earl of (2014). Swiftlets of Borneo: Builders of edible nests (2nd Ed.). Kota Kinabalu, Malaysia: Natural History Publications (Borneo).

Lourie, S. A., & Tompkins, D. M. (2000). The diets of Malaysian swiftlets. *Ibis, 142*(4), 596–602.

Marcone, M. F. (2005). Characterization of the edible bird's nest the "Caviar of the East". *Food Research International, 38*(10), 1125-1134.

Marshall, A. J., & Folley, S. J. (1956). The origin of nest-cement in edible nest swiftlets (*Colocallia spp*). *Proceedings of the Zoological Society of London, 126*(3), 383–389.

Martin, J. E., Tanenbaum, S. W., & Flashner, M. (1977). A facile procedure for the isolation of N-acetylneuramic acid from edible bird's-nest. *Carbohydrate Research, 56*(2), 423-425.

Matsukawa, N., Matsumoto, M., Bukawa, W., Chiji, H., Nakayama, K., Hara, H., & Tsukahara, T. (2011). Improvement of bone strength and dermal thickness due to dietary edible bird's nest extract in ovariectomized rats. *Bioscience, Biotechnology and Biochemistry, 75*(3), 590–592.

Medway, L. (1962). The relation between the reproductive cycle, moult and changes in the sublingual salivary glands of the swiftlet *Collocalia maxima* Hume. *Journal of Zoology, 138*(2), 305-315.

Medway, L. (1966, July). Field characters as a guide to the specific relations of swiftlets. In *Proceedings of the Linnean Society of London* (Vol. 177, No. 2, pp. 151-172). Blackwell Publishing Ltd.

Mese, H., & Matsuo, R. (2007). Invited review: Salivary secretion, taste and hyposalivation. *Journal of Oral Rehabilitation, 34*(10), 711–723.

Miclaus, V., Oana, L., Ober, C., Rus, V., & Pestean, C. (2009). Observations concerning features of submandibular gland secretion in rats. *Lucari Stiintifice Medicina Veterinara, 42*(2), 382-386.

Mowry, R. W. (1963). The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins, with revised directions for the colloidal iron stain, and the use of Alcian blue 8GX and their combinations with the periodic acid-Schiff reaction. *Annals of the New York Academy of Sciences, 106*(2), 402–423.

Myers, E. N., & Ferris, R. L. (2007). S*alivary gland disorders*. New York, NY: Springer-Verlag Berlin Heidelberg.

Nakagawa, H., Hama, Y., Sumi, T., Li, S. C., Maskos, K., Kalayanamitra, K., & Li, Y. T. (2007). Occurrence of a nonsulfated chondroitin proteoglycan in the dried saliva of *Collocalia* swiftlets (edible bird's-nest). *Glycobiology, 17*(2), 157–164.

Ng, M. H., Chan, K. H., & Kong, Y. C. (1986). Potentiation of mitogenic response by extracts of the swiftlet's (Collocalia) nest. *Biochemistry International, 13*(3), 521-531.

Odesanya, B. O., Ajayi, S. O., Agbaogun, B. K. O., & Okuneye, B. (2011). Comparative evaluation of nutritive value of maggots. *International Journal of Science Engineering Research, 2*(11), 2229–5518.

Ono, K., Morimoto, Y., Inoue, H., Masuda, W., Tanaka, T., & Inenaga, K. (2006). Relationship of the unstimulated whole saliva flow rate and salivary gland size estimated by magnetic resonance image in healthy young humans. *Archives of Oral Biology, 51*(4), 345-349.

Pozsgay, V., Jennings, H., & Kasper, D. L. (1987). 4, 8-Anhydro-N-acetylneuraminic acid. Isolation from edible bird's nest and structure determination. *European Journal of Biochemistry, 162*(2), 445–450.

Reuter, G., Schauer, R., Szeiki, C., Kamerling, J. P., & Vliegenthart, J. F. (1989). A detailed study of the periodate oxidation of sialic acids in glycoproteins. *Glycoconjugate Journal, 6*(1), 35-44.

Ross, M. H., & Pawlina, W. (2011). *Histology: A text and atlas with correlated cell and molecular biology* (6th Ed.) (pp. 562–563). Baltimore: Wolters Kluwer Health.

Scott, J. (1986). Structure and function in aging human salivary glands. *Gerodontology, 5*(3), 149-158.

Shackleford, J. M. (1963). Histochemical comparison of mucous secretions in rodent, carnivore, ungulate, and primate major salivary glands. *Annals of the New York Academy of Sciences, 106*(2), 572-582.

Shah, S. W., & Aziz, N. A. (2014). Morphology of the lingual apparatus of the Swiftlet, Aerodramus fuciphagus (*Aves, Apodiformes, Apodidae*). *Journal of Microscopy and Ultrastructure, 2*(2), 100-103.

Spector, D. L., & Goldman, R. D. (2006). *Basic methods in microscopy protocols and concepts from cells: A laboratory manual*. United States of America, USA: Cold Spring Harbor Laboratory Press.

Spicer, S. S., & Meyer, D. B. (1960). Histochemical differentiation of acid mucopolysaccharides by means of combined aldehyde fuchsin-Alcian blue stain. *Technical Bulletin of the Registry of Medical Technologies, 30*, 453–460.

Squires, B. T. (1953). Human salivary amylase secretion in relation to diet. *The Journal of Physiology, 119*(2-3), 153.

Stoltzner, G. (1977). Effects of life-long dietary protein restriction on mortality, growth, organ weights, blood counts, liver aldolase and kidney catalase in Balb/C mice. *Growth, 41*(4), 337-348.

Stresemann, E. (1931). Notes on the systematics and distribution of some swiftlets (*Collocalia*) of Malaysia and adjacent subregions. *Bulletin of the Raffles Museum, 6*, 83–101.

Taib, N. T., & Jarrar, B. M. (1998). Histological and histochemical characterization of the lingual salivary glands of the quail, *Coturnix coturnix*. *Saudi Journal of Biological Sciences, 5*(2), 33–41.

Taib, N. T., & Jarrar, B. M. (2001). Histochemical characterization of the lingual salivary glands of the Eurasian collared dove, Streptopelia decaocta. *Pakistan Journal of Biological Sciences, 4*(11), 1425-1428.

Tandler, B., & Philips, C. J. (1998). Microstructure of mammalian salivary glands and its relationship to diet. In J. R. Garret, J. Ekstroms, & L. C. Anderson (Eds.), *Glandular mechanism of salivary secretion* (pp. 21–35). Basel: Karger.

Tomasi, T. B., & Plaut, A. G. (1985). Humoral aspects of mucosal immunity. In J. I. Gallin & A. S. Fauci (Eds.), *Advances in host defense mechanisms* (Vol. 4, pp. 31–61). New York, NY: Raven Press.

Tompkins, D. M. (1999). *Impact of nest-harvesting on the reproductive success of black-nest swiftlet Aerodramus maximus* (pp. 33–36). London: Department of Zoology, University of Oxford.

Waugh, D. R., & Hails, C. J. (1983). Foraging ecology of a tropical aerial feeding bird guild. *Ibis, 125*(2), 200-217.

Wells, K. L., & Patel, N. (2010). Lumen formation in salivary gland development. In A. S. Tucker & I. Miletich (Eds.), *Salivary Glands: Development, Adaptations and Disease* (pp. 78-89). Basel, Switzerland: Karger Publishers.

Wieruszeski, J. M., Michalski, J. C., Montreuil, J., Strecker, G., Peter-Katalinic, J., Egge, H., & Vliegenthart, J. F. (1987). Structure of the monosialyl oligosaccharides derived from salivary glandmucin glycoproteins of the Chinese swiftlet (genus *Collocalia*). *Journal of Biological Chemistry, 262*(14), 6650–6657.

Wu, Y., Chen, Y., Wang, B., Bai, L., Ge, Y., & Yuan, F. (2010). Application of SYBRgreen PCR and 2DGE methods to authenticate edible bird's nest food. *Food Research International, 43*(8), 2020-2026.

Yu-Qin, Y., Liang, X., Hua, W., Hui-Xing, Z., Xin-Fang, Z., & Bu-Sen, L. (2000). Determination of edible bird's nest and its products by gas chromatography. *Journal of Chromatographic Science, 38*(1), 27-32.