UNIVERSITI PUTRA MALAYSIA

FORMATION OF SECRETORY GRANULES AND MODE OF SECRETION IN THE SALIVARY GLANDS OF SWIFTLET- AN ULTRASTRUCTURAL STUDY

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CERTIFICATION

I hereby certify that I have read this project paper entitled "Formation of Secretory Granules and Mode of Secretion in the Salivary Glands of Swiflets - An Ultrastructural Study", by Ainul Riza Binti Abu Seman and in my opinion it is satisfactory in term of the scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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DEDICATIONS

This project paper is dedicated to Allah S.W.T., who had created me and made all things possible throughout this project,

To my family:

My father, Abu Seman B. Umar

My mother, Ruzaimah Bt. Rahmat

My brother, Mohd. Jamuri B. Abu Seman

My friends

And to all my teachers who have committed themselves towards the noble cause of education. Thank you for your continuous support and care.

May this be your inspiration and motivation for your future endeavours

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LIST OF ABBREBIATIONS

%	Percent
nm	Nanometer
µm	Micrometer
˚C	Degree Celsius
Å	Angstrom
EBN	Edible bird nest
ER	Endoplasmic Reticulum
L	Lumen
M	Molar
MV	Microvilli
N	Nucleus
rER	Rough Endoplasmic Reticulum
RNA	Ribonucleic acid
SG	Secretory granule
TEM	Transmission electron microscope

ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

PEMBENTUKAN TITISAN REMBESAN DAN MOD REMBESAN DALAM KELENJAR LIUR BURUNG WALIT- KAJIAN ULTRASTRUKTUR

Oleh

Ainul Riza Binti Abu Seman

2016

Penyelia: YBhg. Dato' Dr.Tengku Azmi bin Tengku Ibrahim

Dua spesies burung walit - *Aerodramus fuciphagus* dan *Aerodramus maximus* merupakan spesies spesies burung yang membina sarang daripada rembesan kelenjar liur. Pada musim pembiakan, terutamanya semasa membuat sarang, kelenjar liur jelas kelihatan membesar dan membengkak untuk menghasilkan rembesan air liur yang banyak yang akan mengeras menjadi sarang yang juga merupakan sarang burung boleh dimakan.

Sarang burung walit telah dilaporkan mengandungi banyak khasiat dan diperkaya dengan ciri-ciri keimunan dan antivirus terhadap penyakit. Kajian ini meneliti di peringkat ultrastruktur dengan tumpuan diberikan kepada pembentukan granul rembesan dan kaedah rembesan kelenjar liur *A. fuciphagus*. Kajian ini juga bertujuan untuk memberi bukti dari segi struktur untuk menjelaskan kemungkinan sumber keimunan dan antivirus pada sarang burung sesuai dimakan.

Untuk kajian ini, sampel kelenjar liur telah diteliti di bawah mikroskop elektron transmisi (TEM). Pembentukan granul rembesan kelenjar liur berasal daripada penghujung retikulum endoplasma kasar yang terasing dan yang bercantum antara satu sama lain. Seterusnya, granul rembesan ini dibawa kebahagian sitoplasma apikal dimana ianya juga bergabung antara satu sama lain untuk membentuk granul rembesan yang lebih besar.

Isi kandungan granul rembesan dibebaskan kedalam lumen asinus kelenjar melalui ruptur membran granul yang menkelaskan kaedah rembesan kelenjar liur burung walit sebagai rembesan merokrin. Pada peringkat ultrastruktur, bukti struktur menunjukan bahawa ciri keimunan dan antivruspada sarang burung walit berkemungkinan disumbangkan oleh ribosom sitoplasma sel asinus.

Katakunci: *burung walit, ultrastruktur, pembentuka titisan rembesan, rembesan merokrin, ribosom*

ABSTRACT

Abstract of project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Final Year Project

FORMATION OF SECRETORY GRANULES AND MODE OF SECRETION IN THE SALIVARY GLAND OF SWIFTLETS – AN ULTRASTRUCTURAL STUDY

By

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2016

Supervisor: YBhg. Dato' Dr.Tengku Azmi bin Tengku Ibrahim

Two swiftlets species, the *Aerodramus fuciphagus* and *Aerodramus maximus* are the only avian species which are known to build their nest from the secretion of their salivary glands. During the breeding season especially during nesting the salivary gland was markedly enlarged and hypertrophied to produce the salivary secretion which hardened to become the nest which is the edible bird nest (EBN).

EBN has been reported to be highly nutritious and fortified with immuno-competent and antiviral properties. The present study examined this unique gland at the ultrastructural level focusing on the formation of secretory granules and mode of secretion in the salivary gland of *A. fuciphagus*. The study was also intended to provide some plausible explanation on the origin or source of the immuno-competent and antiviral properties of the EBN.

For the above study, samples of swiftlet salivary glands were processed and examined under the transmission electron microscope. Formation of the secretory granules appeared to originate from coalescence of pinched off dilated ends of the rough endoplasmic reticulum. These were carried to the apical cytoplasm where they again coalesced to form larger secretory granules.

The content of the secretory granule appeared to be released into the lumen of acinus by rupture of the apical cell membrane which classify the mode of secretion in the salivary gland of the swiftlet as the merocrine type. Ultrastructural evidences indicated that the immuno-competent and antiviral properties of the EBN could be contributed by cytoplasmic ribosomes of the acinar cell.

Keywords: *swiftlet, ultrastructure, secretory granules formation, merocrine secretion, ribosomes.*

1.0 INTRODUCTION

Edible bird nests (EBN) made from hardened saliva of cave nesting swiftlets have long been a sought-after delicacy amongst the Chinese population and are among the most expensive animal products consumed by humans (Thorburn, 2015). According to Lau and Melville (1994) bird nest trade is of considerable antiquity as far back as the T'ang Dynasty.

There are more than 24 species of insectivorous, eco-locating swiftlets distributed around the world and only a few produce nests that are deemed "edible" (Koon, 2000). The majority of EBN traded worldwide comes from two heavily exploited species, the White nest swiftlet - *Aerodramus fuciphagus* - and the Black nest swiftlet - *Aerodramus maximus*- (Babji, 2011). White nest swiftlets are normally resident birds on islands, however lately they have been found to be distributed on mainland in large numbers (Tan, 2001).

The five common species of swiftlets found in Malaysia and Borneo Island are *Hydrochus gigas, Collocalia esculent* (White Belly Swifts), *Crypsiurus balasiensis* (Asian Palm Swift), *Aerodramus maximus* and *Aerodramus fuciphagus* (Ibrahim *et al*., 2009). The EBN is built entirely from salivary secretion (Mardiastuti and Soehartono, 1996; Ibrahim *et al*., 2009; Iswanto, 2002) and highly sought after for both cuisine and medicine. It is an exotic item for delicacies and used as medications to improve physical strength (Oktorina *et al*, 2005). Norhayati *et al*. (2010) states that the EBN is highly nutritious and reported to possess immuno-competent and antiviral properties.

With current research on swiftlet being directed on the commercial importance of EBN very limited research however have been focused on the various body systems of the swiftlet. Thus, there is a need to understand the anatomy, physiology and nutrition of this avian species if the swiftlet industry is to be fully develop in Malaysia. In the same perspective and in view of the uniqueness of the salivary gland in the swiftet which produce the highly sought-after EBN there is a need to undertake a study on the formation and secretion of the secretory granules in the swiftlet salivary gland.

1.1 OBJECTIVE

To study the formation of secretory granules and mode of secretion in swiftlet salivary glands. It is also to provide plausible explanations on the origin of the immuno-competent and anti-viral properties in the EBN.

1.2 JUSTIFICATION

A study on the formation of secretory granules and mode of secretion in the salivary gland involved the cytoplasmic organelles of the acinar cells. Cytoplasmic organelles could only be seen under electron microscope which necessitate that this study to be carried out at the ultrastructural level.

1.3 HYPOTHESIS

The substance produced by the salivary glands in the swiftlet to build its nest which is highly nutritive and fortified with immuno-competent and antiviral properties involve a production mechanism which is different from that in conventional gland.

2.0 LITERATURE REVIEW

2.1 EDIBLE BIRD NEST

EBN is bird nest made from the saliva of swiftlet. In Chinese culture, EBN is particularly prized due to their rarity and exquisite flavour ("Rashid & Syauqi", n.d.).Moreover, EBN is renowned Chinese delicacy which has been around since the ancient Chinese dynasties (Rebecca, 2013).

The saliva of certain types of swiftlets that produce the EBN belongs to a family of birds called Apodidae under genus *Collocalia.* They are small sized birds that feed on insects which mainly found from the Indian Ocean to the South Pacific in the Australasian region (Price *et al*., 2005).

Besides, several species of the Aerodramus genus also produce EBN. In Malaysia, the swiftlets that produce the EBN belong to the genus *Aerodramus fuciphagus* and *Aerodramus maximus* where they are insectivorous within the swiftlet family and known to use their saliva as the nest building material during the breeding season (Helen *et al.,* 2013). EBN has been traded for the past 500 years between the Malay archipelago and mainland China since the 16^{th} century (Sankaran, 2001).

The swiftlets built their nests from the secretion of their salivary glands located under their tongue. Three main breeding seasons: (1) from December to March; (2) April to July; and (3) August to November have been reported (Norhayati *et al.,* 2010). According to Hobbs (2003) the salivary secretion solidifies into a kind of cement in the shape of a cup which is strong enough to hold the swiftlet eggs and growing hatchlings.

2.2 ECONOMIC AND COMMERCIAL IMPORTANCE

EBN is commonly referred to as the 'Caviar of the East' as it fetches a premium price for centuries in China. Malaysia is located right at the heart of the 'golden triangle' of swiftlet bird nest production making it one of the top producers of this commodity. In view of its high demand and premium price in the global market EBN cultivation has increased dramatically in EBN producing countries including Malaysia (Hobbs, 2004).

The EBN industry has a potential to grow into a multi-million dollar industry due to its profitable return and continuous growing demand by wealthy overseas customer (Merican, 2007). Kuan and Lee (2005) state that Malaysia is the third largest producer of EBN (7% of gross supply value) in the world after Indonesia (60%) and Thailand (20%). Meanwhile, according to the Malaysian Federation of Bird's Nest Merchants Association, annual production of bird's nests in Malaysia has reached 1 billion ringgit in value.

The elixir of EBN is widely known to possess tonic properties that nourish and tone up the organ system of the body, help to dissolve phlegm, relieve gastric problems, assist in kidney function, improve complexion of the skin, alleviate asthma, supress cough, cure tuberculosis, improve the immune system, increase energy and metabolism, speedy recovery from illness and surgery as well as improve concentration (Francis, 1987; Leh, 1993; Lim and Earl of Cranbrook, 2002; Marcone, 2005).

Colombo *et al*. (2003) states that one of the nutrient component of EBN is sialic acid (9%) where this sialic acid is beneficial to the neurological and intellectual advantages in infants (Chau *et al*., 2003). EBN consists of high valued glycoprotein rich with amino acids, carbohydrate, calcium, sodium and potassium (Norhayati *et al*., 2010).

EBN is also known to exerted strong inhibitory effect on influenza viruses in a host range independent manner (Guo *et al*., 2006). Besides, the EBN is currently of considerable economic importance because of it highly nutritious and with anti-viral properties made the bird nest more valuable food commodity (Ibrahim *et al*, 2009). More recently, bird nests have been used as a component in cosmetic products (Zainab et al., 2013).

2.3 SWIFLET SALIVARY GLANDS

In Malaysia, EBN of white-nest swiftlets (*Aerodramus fuciphagus*) and black-nest swiftlet (*Aerodramus maximus*) are solely made out from saliva and are harvested for commercial purpose (Viruhpuntu, Thirakhupt, Pradatsundarasar and Poonswad, 2002). The source of the salivary secretion is believed from the paired sublingual gland of the swiftlets (Goh *et al.,* 2001; Marshal and Folley, 1956).

According to Syed and Norazlin (2014), theswiftlet's salivary glands are located in the oropharyngeal region which reflects their relative capacity to produce a massive amount of salivary secretion required for nest building. It also has been reported that the size of the salivary gland of swiftlet is influenced by its reproductive cycle (Medway, 1962).

Ibrahim *et al.* (2013) reported that the size of the submandibular salivary glands was not influenced by the size of the swiftlet body. These authors also mentioned that well developed submandibular glands can be seen clearly under the lower mandible and were large and lobulated. Meanwhile, undeveloped submandibular glands were small in size and less lobulated. Juvenile birds or where there is no nesting activity (inactive season), the submandibular glands are undeveloped (Ibrahim *et al*., 2013).

2.4 FORMATION OF SECRETORY GRANULES MODE OF SECRETION IN EXOCRINE GLAND

A diagrammatic representation on the formation and secretion of secretory granules in exocrine pancreas is presented in Figure 3. Each acinar cell is shape like a truncated pyramid with basally located nucleus and cell apex facing the lumen of the acinus, The cytoplasm is filled with pro-enzyme-containing secretory granules (Gartner and Hiatt, 2007).

The formation of secretory granules in exocrine gland began in the rough endoplasmic reticulum (rER) where the secretory products are synthesised by the ribosomes of the rER which is then transferred into the cisternae of the rER. Cisternae are stacks of flattened, crescent-shape tubes (Coville and Bassert, 2002). From the rER the secretory product is then transferred into the Golgi apparatus and gradual accumulation of the secretory product led to the ends of Golgi apparatus to become dilated and later pinched off to form the secretory or zymogen granules. These secretory granules will rupture to release their secretory products into the acinar lumen.

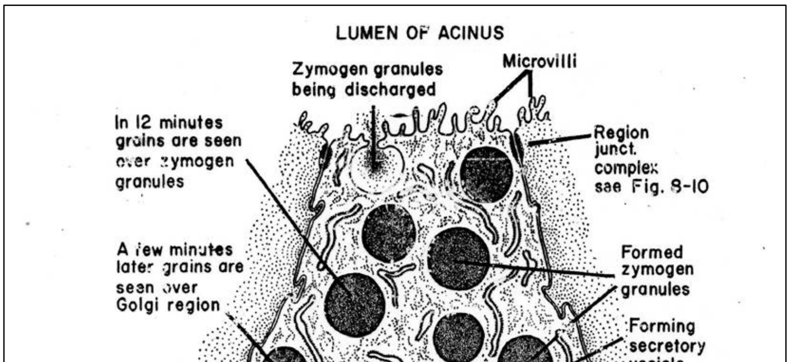

Figure 1: Diagrammatic representation of the ultrastructure of pancreatic acinar cell showing the organelles involve in the formation of secretory granules and release of its secretion (Courtesy from: L.P. Leblond).

There are three modes of secretion of the secretory granules namely, A: merocrine, B: apocrine and C: holocrine (Figure 2). According to Dellmann and Eurell (2007) in merocrine mode of secretion the content of secretory granules enclosed within a membrane. When the secretory granules reaches the cell surface, the secretory products is discharge via exocytosis as exemplified by the pancreas. In apocrine mode of secretion such as in sweat glands part of the apical cytoplasm is discharged along with the secretory granules (Samuelson, 2007). Holocrine secretion glands as in sebaceous glands involve cell detachment and subsequent death resulting in the entire cell constituting to the secretory products of the gland (Samuelson, 2007).

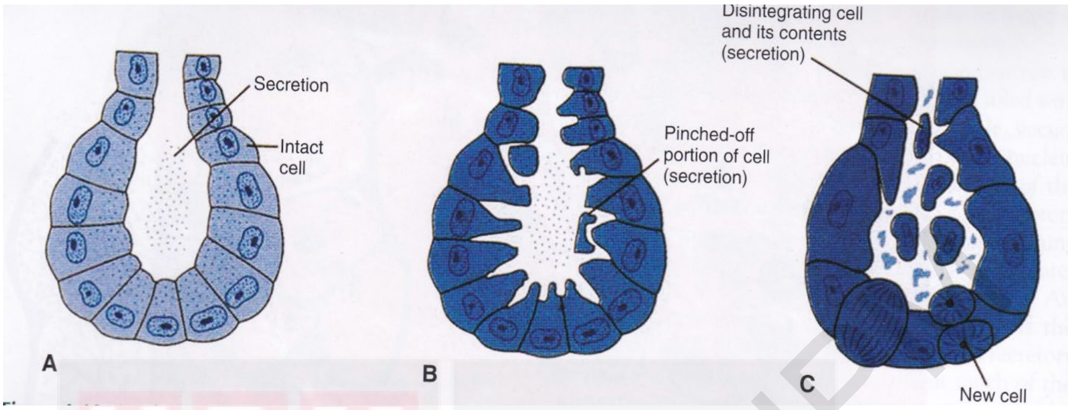

Figure 2: Mode of secretion of exocrine glands. A, Merocrine secretion B. Apocrine secretion and C: Holocrine secretion *(From Bergman RA, Afifi AK, Heidger PM Jr: Histology, Philadelphia, 1996, Saunders).*

3.0 MATERIALS AND METHODS

The swiftlets *(Aerodramus fuciphagus)* were obtained from bird houses in Kluang district and in the town of Kota Bharu. Following Muslim slaughter, the samples of the salivary glands were fixed in 4% gluteraldehyde in 0.1M sodium cacodylate buffer and post fixed in 1% Osmium tetroxide for 2 hours at 4^{o}C.

Samples were then dehydrated in graded series of acetone concentration 30%, 50%, 75%, 95% (10 minutes each change) and 100% for 15 minutes thrice. The samples were infiltrated with equal mixture of acetone and resin (50:50) embedded in prefilled beam capsule containing resin and polymerisation at 60^{o}C for 48 hours. Ultrathin section at 0.1nm/ 1Å were obtained using the ultramicrotome and stained with uranyl acetate and lead citrate and stained for 15 minutes and washed with double distilled water. Lastly, the stained sections were examined under the transmission electron microscope (TEM).

4.0 RESULTS

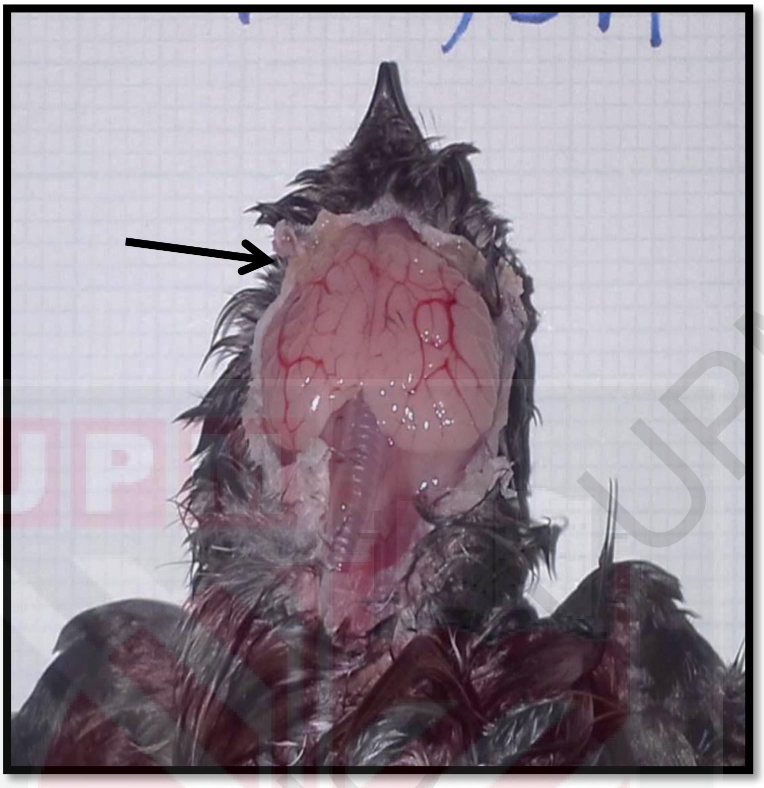

Figure 3: Gross appearance of the swiftlet salivary gland during the breeding season

The salivary gland during the breeding season especially during nesting is markedly enlarged, hyperthropied and highly vascular during the (Fig. 3). All electron micrographs shown and described in this section are from the salivary gland of swiftlets during the breeding season.

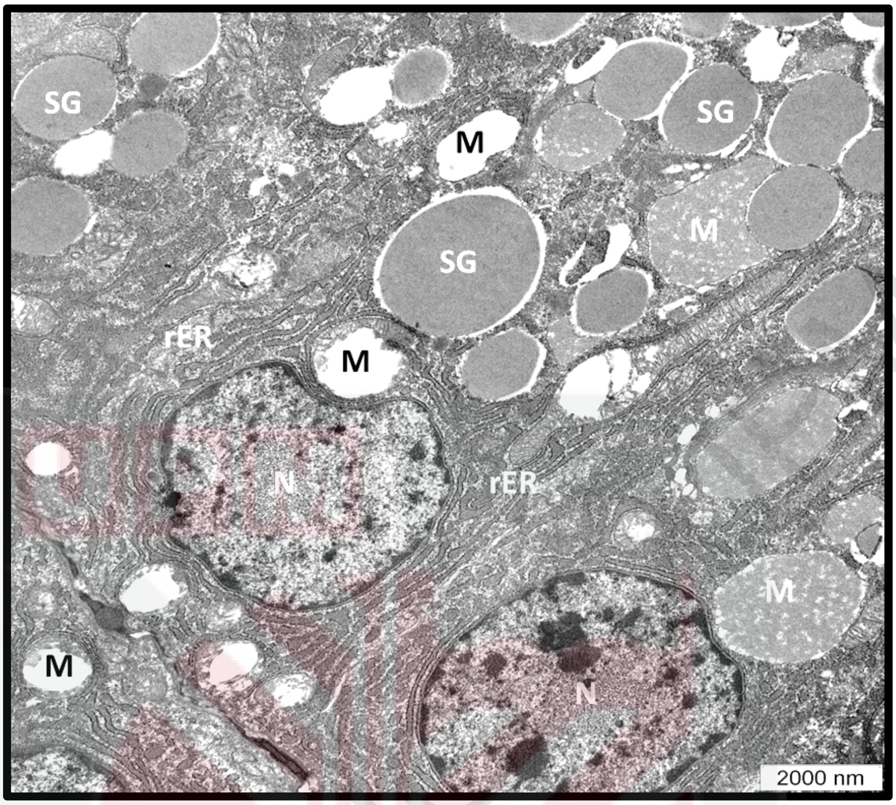

Figure 4: The acinar cells of swiftlet salivary gland showing the organelles which include an extensive rough endoplasmic reticulum (rER), numerous secretory granules (SG) and mitochondria (M). N = nucleus

Under the transmission electron microscope, major part of the acinar cell is occupied by an extensive rER numerous secretory granules and mitochondria. The rER are located infranuclearly and perinuclearly while the secretory granules are located supranuclearly (Fig. 4).

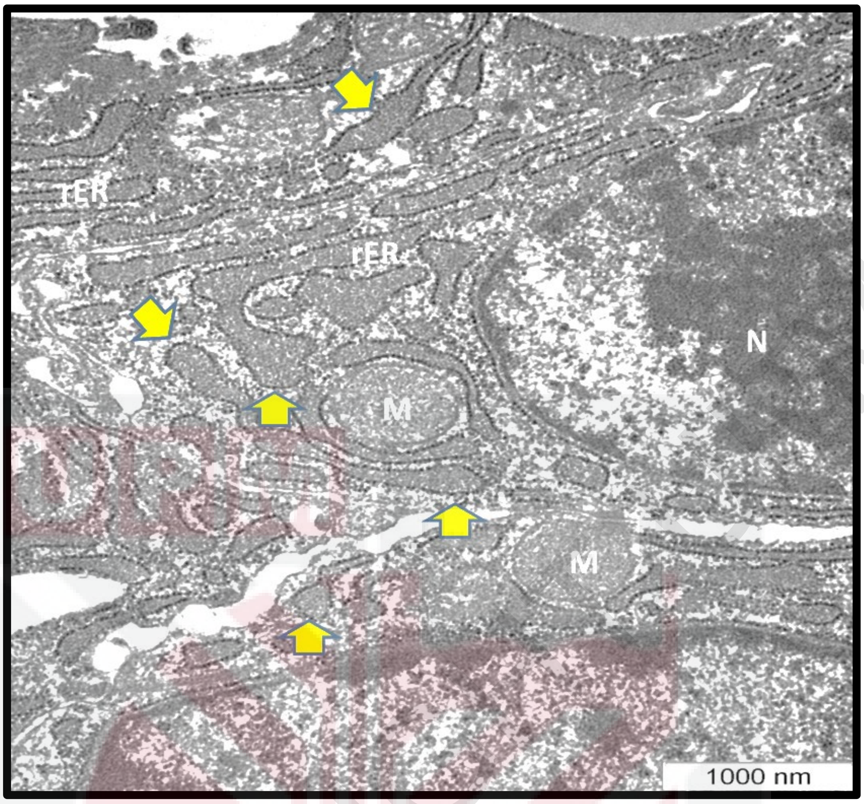

Figure 5: Ultrastructure of cytoplasmic organelles during the breeding season showing the swollen rER with dilated ends (yellow arrows). M = mitochondria; N= nucleus.

In the supranuclear cytoplasm the rER appeared swollen with dilated ends (Fig. 5). Amongst the swollen rER were a number of vesicular and irregular-shaped rER. Some of these vesicular and irregular-shaped rER appeared to coalesce with one another (Fig. 6).

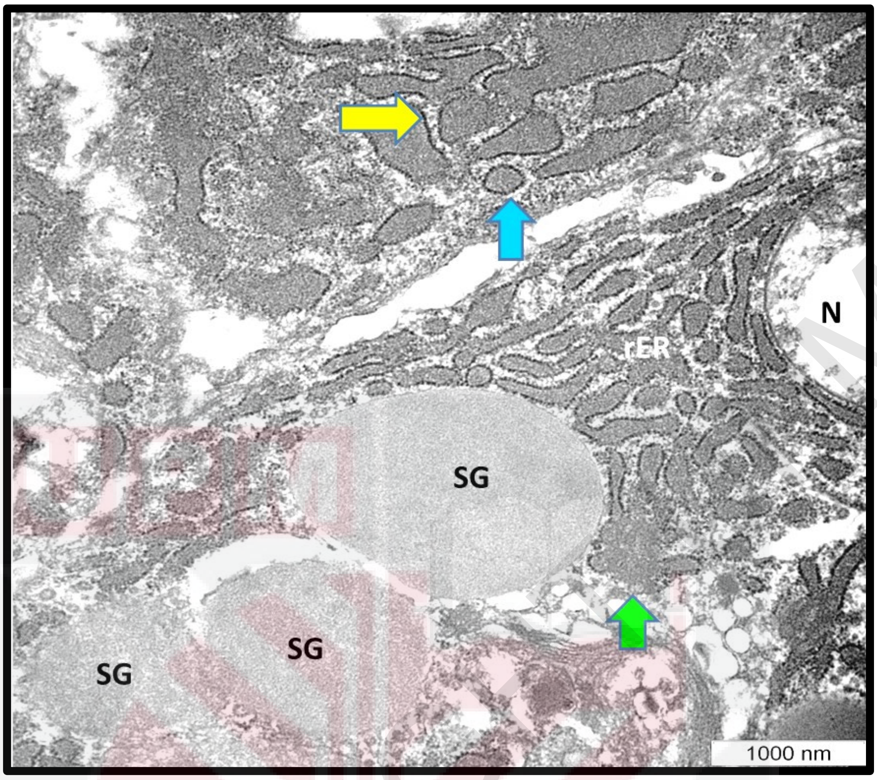

Figure 6: Electron micrograph showing vesicular and irregular shaped rER (yellow and blue arrow). Some of these bodies appeared coalesced (green arrow). SG= secretory granule, N= nucleus

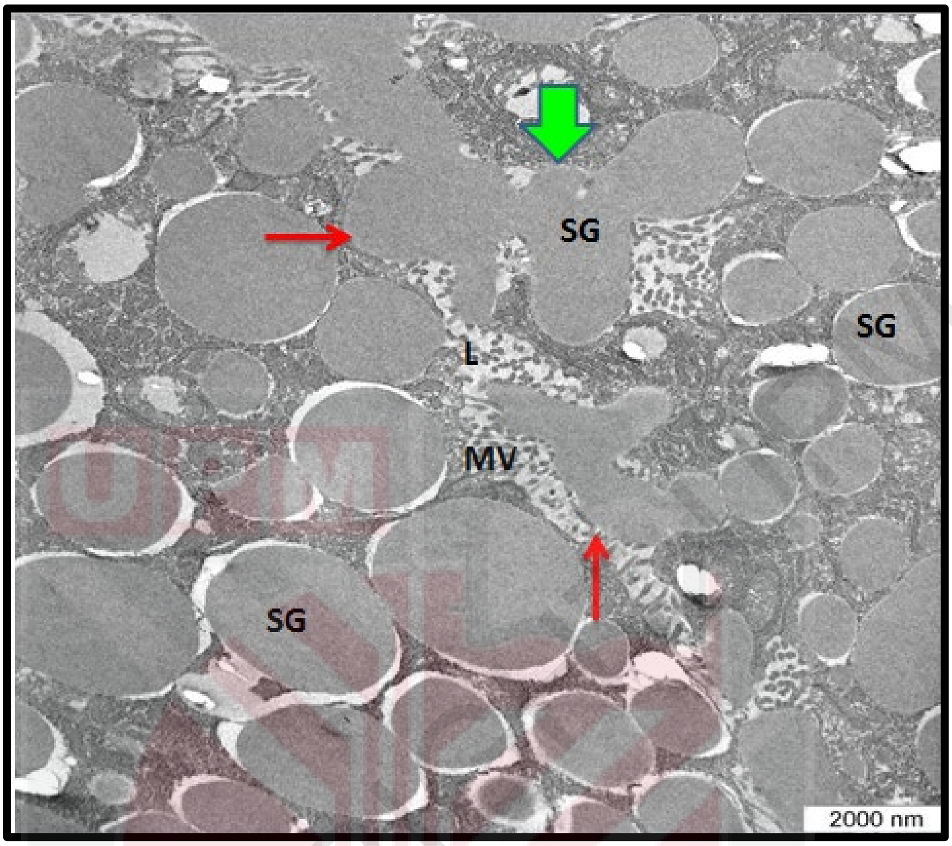

Figure 7: Electron micrograph showing a large number of secretory granules in the apical cytoplasm of the acinar cell. Some secretory granules in the apical cytoplasm and lumen acinus (L) appeared coalesce forming larger secretory granule (red arrows). MV= microvilli.

In the apical cytoplasm there were numerous secretory granules which coalesced with one another forming larger secretory granules (Fig. 7). Coalesced secretory granules were also present in the lumina of the acinus (Fig. 8).

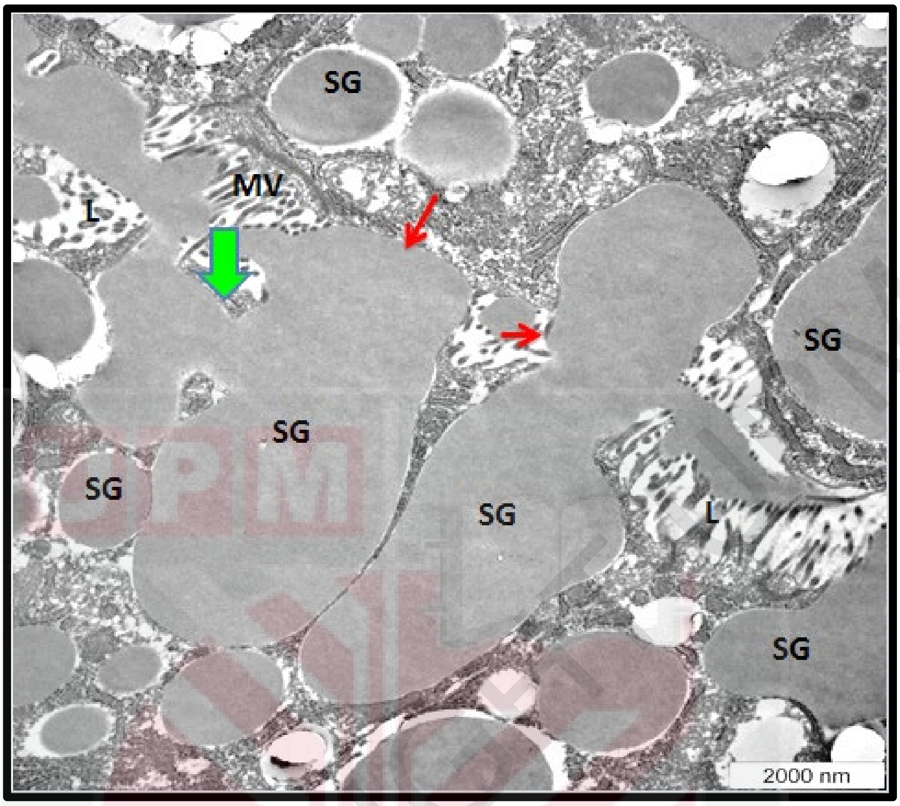

Figure 8: Coalesced secretory granules (SG) forming bigger granules (green arrow) indicating a copius amount of secretory products being secreted (red arrows) into the acinar lumen (L). MV= microvilli.

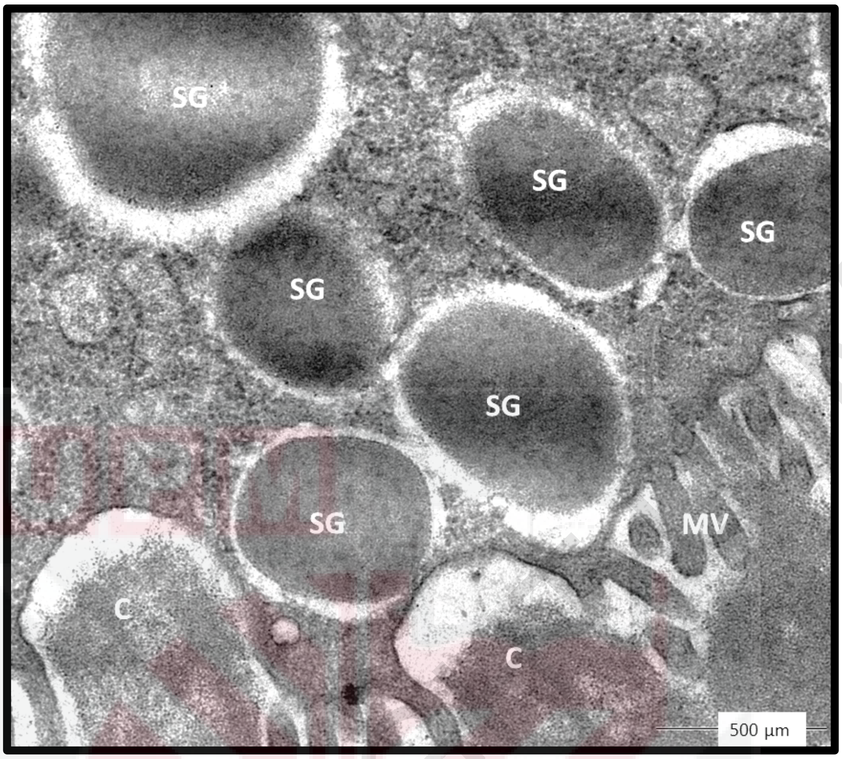

Figure 9: Rupture of the secretory granules to discharge its content (C) into the acinar lumen. SG = secretory granules; MV microvilli.

Some granules bordering the acinar lumen appear to discharge its content through rupture of the apical cell membrane (Fig. 9). Under higher magnification it was observed that there was a discontinuity of the apical cell membrane and there appeared to be a "flow" of cytoplasmic materials through this break in the cell membrane (Fig 10).

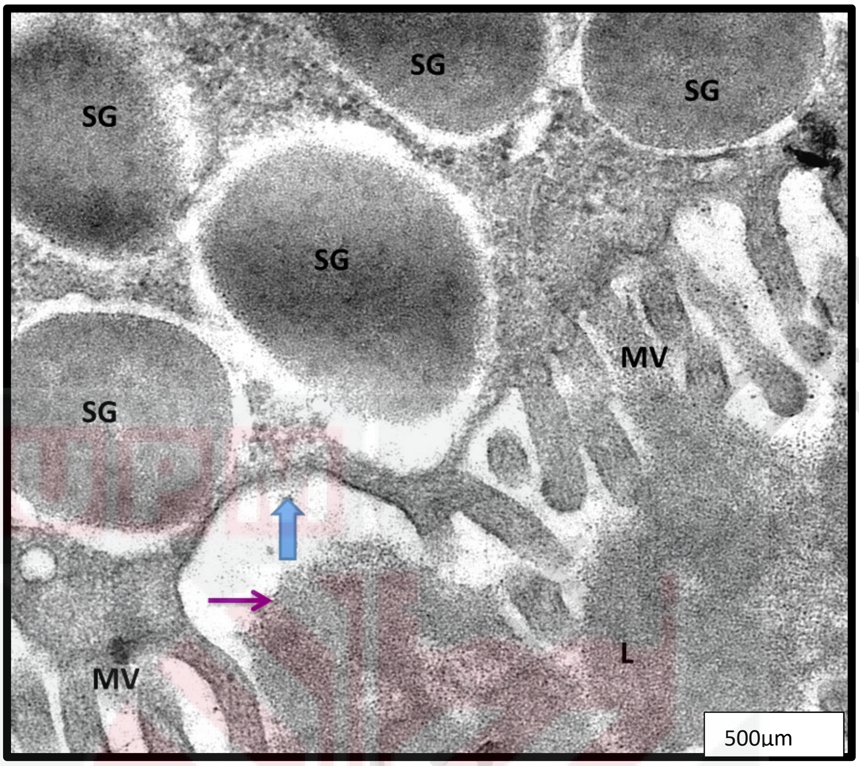

Figure 10: Higher magnification of the apical cell membrane showing a discontinuity of the cell membrane (blue arrow). Cytoplasmic materials appear to ‘flow” through this discontinuity into the lumen of the acinus.

5.0 DISCUSSION

From the present study a summary on the formation of the secretory granules and the mode of secretion in the salivary gland of the swiftlet is depicted in Fig. 11.

Figure 11: Semi diagramatic represention depicting the sequence of formation of the secretory granules and discharge of its content into acinar lumen. N= nucleus, M= mitochondria

1. rER where the secretory product is first synthesized and transferred into the cistern of the rER.
2. Swollen rER with dilated ends brought about by continuous accumulation of secretory product.
3. Pinched off dilated ends of rER.
4. Coalesced pinched off dilated ends of rER to form secretory granules.
5. Coalesced secretory granules.
6. Discharge of secretory granular content into acinar lumen through rupture of cell membrane conforming to the merocrine type of secretion.

The formation of the secretory granules in the salivary gland of the swiftlet is as depicted in Figure 11. As in conventional glands the formation of secretory granules began with synthesis of the secretory product by ribosomes of the rER and its transfer into the cisternae of the rER. Accumulation of the secretory product led to a swelling of the rER and dilatations at both ends of the organelle. Continuous synthesis and accumulation of the secretory product in turn led to enlarged dilated ends of the rER which subsequently will be pinched off. Coalescence of the pinched off dilated ends of the rER constitute the secretory granule in the acinar cell of the swiftlet salivary gland.

The formation of the secretory granules in the salivary gland in this avian species appeared to be somewhat different from that in conventional glands. In the latter following synthesis and its transfer into the cisternae of the rER the secretory product is carried into the Golgi apparatus. The vesicular part of the Golgi containing the secretory product is pinched off to constitute the secretory unit of the acinus cell in conventional glands.

From the above it appears that the formation of the secretory granules in the salivary gland of the swiftlet did not involve the Golgi apparatus. Should this be the case we then have the morphological evidence to report for the first time that the formation of secretory granules need not necessarily involve the Golgi apparatus. The non-involvement of the Golgi apparatus in the swiftlet salivary gland in all probability could be attributed to the very rapid rate of formation of secretory as evidenced the massive number of secretory granules in the supranuclear cytoplasm of the acinar cell. Bordering the lumen of the acinus some secretory granules appeared coalesced; coalesced secretory product were also observed in the lumen of the acini giving the impression of a very copious secretion by the salivary acini which is required to build the nest.

From the present study some secretory granules bordering the lumen acinus were observed to be ruptured to discharge their contents into the acinar lumen. Rupture of the secretory granules provides the structural evidence that the mode of secretion in the swiftlet salivary gland in all probability is the merocrine type. According to Samuelson (2007), the salivary glands have the merocrine type mode of secretion.

A number of reports have been published to claim that the edible bird nest contain immune-competent and antiviral properties (Norhayati *et al*., 2010). However no reports have been published on the origin or source of these properties. In the present study it was observed there was a discontinuity of the acinar cell membrane through which cytoplasmic materials, containing granular structure which could be ribosomes, are discharged into the acinar lumen. Ribosomes being ribonucleic acid could be the source or origin of the immune-competent and antiviral properties of the EBN.

6.0 CONCLUSION

The following conclusions could be drawn from the present study:

1. We have the structural evidence to report for the first time that the formation of secretory granules need not necessarily involve the Golgi apparatus. The very rapid rate of formation of the secretory granules to produce a massive amount of salivary secretion to build the nest, which is the EBN, in all probability necessitate that secretory granules arise directly from the rER.
2. The mode of secretion in the swiftlet salivary gland is the merocrine type.
3. The immune-competent and antiviral properties of EBN could possibly arise or originate from the cytoplasmic ribosomes.

REFERENCES

Chau, Q., Cantor, S.B., Caramel, E., Hicks, M., Kurtin, D., Grover, T. and Elting, L.S. (2003). Cost-Effectiveness of The Bird's Nest Filter for Preventing Pulmonary Embolism Among Patient With Malignant Brain Tumours and Deep Venous Thrombosis of the Lover Extremities. Support Care Cancer, 11: 795-799

Colombo, J.P., Garcia-Rodenas, C., Guesry, P.R. and Rey, J. (2003). Potential Effects of Supplementation with Amino Acids, Choline or Sialic Acid on Cognitive Development in Young Infants. Acta Paediatrica Supplement, 92: 42-46

Gartner L.P., Hiatt J.J. (2007). *Colour Textbook of Histology* (3rd ed.). Philadelphia: Saunders Elsevier, pp.437-462

Colville T. (2002). The Urinary System. In T. Colville & J. M. Bassert, *Clinical Anatomy & Physiology for Veterinary Technicians* (pp. 306-317). Missouri: Mosby Inc.

Guo, C.T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., Kato, K., Hidari, K.I.P.J., Miyamoto, D., Suzuki, D. and Suzuki, Y. (2006). Edible Bird's Nest Extract Inhibits Influenza Virus Infection. Antivira Research 70:140-146

Helen, M., Intan-Shameha, A. R., Kamaruddin, M.I. and Zuki, A. B. Z. (2013). Morphological Evaluation of Submandibular Salivary Glands of White *(Aerodramus fuciphagus)* and Black *(Aerodramus maximus)* Edible Bird's Nest Swiftlet: *Proceeding of WPSA (Malaysia Branch) and WVPA (Malaysia Branch) ScientificConference*, 2013. Universiti Putra Malaysia, Serdang, Selangor: Syarikat Perniagaan Weng Sing

Hobbs, J. J. (2004). Problem in The Harvest of of Edible Birds' Nests in Sarawak and Sabah Malaysian Borneo. *Biodeversity Conservation*, 13: 2209-2226

Ibrahim, S.H., Teo, W.C.and Baharun, A. (2009). A Study on Suitable Habitat for Swiftet Farming. UNIMAS E-J. Civil Eng., 1:1-7

Iswanto, H. (2002). Kiat Mengatasi Permasalahan Praktis Walet. *PT AgroMedia Pustaka* , pp:41-50

Koon, L.C. and Weeks, D.I. (2000). Features-Bird's Nest Soup- Market Demand for This Expensive Gastronomic Delicacy Threatens the Aptly Named Edible-Nest Swiftlet with Extinction in the East. *Wildlife Conservation*, 103 (1): 30-35

Kuan, H. and Lee, J. (2005). Swiftlet Farming. *The Complete Introductory Guide to Swiftlet Farming*. Penang: Struan Inc. Sdn. Bhd

Lau, S.M. and Melville, D.S. (1994). International Trade in Swiftlet Nests (with Special Reference to Hong Kong). Cambridge : TRAFFIC International

Lim, C. K. and Cranbrook, G. G. H. E. (2002). Swiftlet of Borneo: Builders of Edible Nest.Kota Kinabalu: Natural History Publication (Borneo), pp: 171

Lim, C. K. and Cranbrook, E (2014). Swiftlet of Borneo: Builders of Edible Nest, 2nd ed.Kota Kinabalu: Natural History Publication (Borneo)

Looi Q. H. & Abdul Rahman Omar. (2016). Swiftlets and Edible Bird's Nest Industry in Asia. *Pertanika Journal of Scholarly Research Reviews,* 2 (1): 32-48

Mardiastuti, A. and Soehartono, T. (1996). Current Situation of The Edible Bird-Nest Swiftlet in Indonesia.: A Rivision. Surabaya, Indonesia

Medway, L. (1962). The Relation Between the Reproductive Cycle, Moult and Changes in the Sublingual Salivary Glands of the Swiftlet *Collocalia maxima.* Hume. Proc Zool Soc Lond, 14:75-81

Merican, H.S. (2007). The 2007 Malaysian Swiftlet Farming Industry Report. *SMI Association of Penang*, Penang, Malaysia

Norazlin, A.A. and Waliullah, S.S. (2014). Morphology of the Lingual Apparatus of the Swiftlet, *Aerodramus fuciphagus* (Aves, Apodiformes, Apodidae). Journal of Microscopy and Ultrastructure 2 (2014): 100-103

Norhayati M.K., Azman O. & Wan Nazaimoon W.M. (2010). Preliminary Study of the Nutritional Content of Malaysian Edible Bird's Nest. Malaysian Journal of Nutrition, 16 (3): 389-396

Oktorina, R., Indarjulianto, S., Widyarni, S. and Wasito, R. (2005). The Detection of *Staphylococcus aureus* in Swiftlets' Nest Using Immunohistochemistry (*Streptavidin Biotin*). *Folia Med. Indonesiana*, 41: 266-269

Raihan Adnin (2015). *Histology of the Swiftlet Kidney* (Degree of Doctor of Veterinary Medicine). Universiti Putra Malaysia

Rashid, M.R. and Syauqi, M.M.N. (n.d.). Malaysia's Edible Bird Nest (EBN) Industry

Samuelson, D.A. (2007). Textbook of Veterinary Histology. Missouri: Mosby Inc., pp: 60-71

Sankaran, R. (2001). The Status and Conservation of the Edible- Nests Swiftlet *(Collocia fuciphaga).* In: The Andaman and Nicobar Islands, Biological Conservation 7, pp: 283-294

Tan, S. P. (2001). Problems in Breeding the White-Nest Swiftlets. In: Proceeding of the Commercial Viability of Bird Houses to *Aerodramus fuciphagus,* the Edible White-Nest Swiftlet. Penang: 37-53

Thorburn, C.C., (2015). The Edible Nest Swiftlet Industry in Southeast Asia: Capitalism Meets Commensalism. Hum Ecol, 43: 179-184

Wong, R.S.Y. (2013). Edible Bird's Nest: Food or Medicine?. *Chin J Integr Med,* Sep: 19 (9): 643-649

Zainab, H., Sarojini, J., Nur Hulwani, I., Kamarudin, H., Lee, B.B. and Othman, H. (2013). Commercial Potential of Refined Nutrient-Rich Wasre of Edible Bird

Nest (EBN). 24th International Invention Innovation Technology Exhibition 2013. KLCC, Kuala Lumpur, Malaysia