UNIVERSITI MALAYSIA KELANTAN

Comparison of Nitrate and Nitrite Contents in Edible Bird's Nest (EBN) obtained from Natural (Cave) and Modified (House) Habitats of Swiftlet Bird (Species: *Aerodramus)*

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for higher degree to any universities or institutions.

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I certify that the report of this final year project entitled " Comparison of Nitrate and Nitrite Contents in Edible Bird's Nest (EBN) obtained from Natural and Modified Habitats of Swiflet Bird (Species: *Aerodramus*)" by Alvin Amos Anak Adrian Susin, matric number F14A0014 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan

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LIST OF ABBREVIATION AND SYMBOLS

EBN	Edible Bird Nest
G	Gram
MG/KG	Milligram/Kilogram
ML	Milliliter
PPM	Parts-Per Million

Comparison of Nitrate and Nitrite Contents in Edible Bird's Nest (EBN) Obtained from Natural (Cave) and Modified (House) Habitats of Swiflet Bird(Species:*Aerodramus*)

ABSTRACT

The edible bird's nest (EBN) is an expensive animal by-product from glutinous saliva secreted by Swiftlet bird (species: *Aerodramus*). High contents of nitrate and nitrite in EBN could bring negative health impact on consumer. The aim of this study was to investigate the nitrate and nitrite contents in EBN, which was obtained from natural habitat (cave) and modified habitat (house) sources. It was hypothesized that EBN from natural habitat (cave) would contribute more nitrate and nitrite contents compared to modified habitat (house). Commercial kit and ion chromatography were used to detect the nitrate and nitrite contents in EBN. Using commercial kit, it was observed that there was no difference on nitrate and nitrite contents in EBN obtained from cave and house. Using ion chromatography, however, nitrate and nitrite contents in EBN obtained from cave were shown higher numerical values compared to the nitrate and nitrite contents in EBN obtained from house, although the number of sample was very few. In conclusion, more research should be carried out on nutritional and anti-nutritional properties of EBN obtained from these two different habitats with appropriate number of samples.

Keywords: Nitrate, Nitrite, Edible Bird Nest, Swiftlets

Perbandingan Kandungan Nitrat dan Nitrit dalam Sarang Burung Walit (SBW) yang diperolehi dari Habitats Semulajadi (Gua) dan Modifikasi (Rumah walit) Burung Walit (Spesis: *Aerodramus*)

ABSTRAK

Sarang burung Walit (SBW) adalah produk dari air liur berglutinous yang dihasilkan oleh spesies Swiftlet Apodidae. SBW dikenali sebagai produk sampingan yang lazimnya digunakan sebagai produk perubatan, makanan dan minuman dan farmaseutikal. Walau bagaimanapun, pemberian paras nitrat dan nitrit yang melebihi paras keselamatan pengguna telah membawa kesan besar kepada kesihatan pengguna dan ekonomi negara. Pengguna SBW kebanyakannya lebih suka di SBW habitat semulajadi (gua) berbanding dengan (rumah walit) SBW kerana kaya dengan khasiat tanpa menyedari kandungan kompaun nitrat dan nitrit boleh membawa kesan kesihatan. Tahap nitrat dan nitrit antara habitat semulajadi (gua) SBW dan habitat modifikasi (rumah walit) SBW adalah berbeza kerana habitat alam sekitarnya yang berbeza. Projek penyelidikan telah buktikan bahawa SBW dari habitat semula jadi (gua) akan menyumbang lebih banyak kandungan nitrat dan nitrit berbanding dengan habitat yang diubah suai (rumah). Ion Kromatografi dan kit komersial digunakan untuk mengesan kandungan nitrat dan nitrit dalam SBW. Menggunakan kit komersil, diperhatikan bahawa tidak terdapat perbezaan pada kandungan nitrat dan nitrit dalam SBW yang diperolehi dari gua dan rumah. Menggunakan kromatografi ion, bagaimanapun, kandungan nitrat dan nitrit dalam SBW yang diperoleh dari gua ditunjukkan nilai berangka yang lebih tinggi berbanding kandungan nitrat dan nitrit dalam SBW yang diperolehi dari rumah, walaupun jumlah sampel adalah sangat sedikit. Sebagai kesimpulan, lebih banyak penyelidikan harus dilakukan terhadap sifat nutrisi dan anti-pemakanan SBW yang diperoleh dari dua habitat ini dengan bilangan sampel yang sesuai.

Katakunci: Nitrat, Nitrite, Sarang Burung Walit, Burung Walit

CHAPTER 1

INTRODUCTION

1.1. Background of Study

Edible bird's nest (EBN) is considered as the most valuable human food product in Asia due to the excellent nutritional properties. The EBN is also possessed mysterious medical properties which had been practiced since during the ancient time until it has high demand in modern time. The EBN forms into gelatinous food product which can either become desserts or beverage. The EBN product is widely used for medical purpose as it can strengthen the immune system. The EBN is made from saliva, which is secreted by Swiftlet bird belongs to the genus of *Aerodramus*. This bird usually rears in modern swiftlet farming to produce commercial EBN and also is available in natural habitats (may be termed as "cave").

The EBN are contributes beneficially to market value for EBN producer. The price of EBN are high due to import by main consumer countries like China and Hong Kong. Its price depends on the EBN's nutritional quality as well as its appearance. For example, grade A EBN price per kilogram is RM 8,000 (Rashid and Nazmi, 2015). In 2009, EBN production in Malaysia had received income worth of RM 500 million after producing about 100 metric tons of EBN. The Malaysian government has set a target of EBN production to reach up to 860 metric tons in year 2020.

However, the price for EBN fluctuates due to high nitrate and nitrite contents in EBN, which results the restriction of EBN export to China (Looi et al., 2016). The EBN industry in Malaysia received many difficulties as the Chinese government has banned the EBN product due to the presence of nitrate and nitrite compounds. If the EBN product exceeds these compounds than the safety limit, it causes toxic to the consumers' health. The high contents of nitrate and nitrite are mostly found in EBN of natural habitats cave especially the coloured EBN due to the longer exposure of nitrate and nitrite. The nitrate and nitrite tests in EBN samples usually analyse by ion chromatography (IC) and also can be carried out by analysing with commercial kits.

1.2. Problem statement

The contents of nitrate and nitrite in EBN depend on the source of different habitats environment. The location of edible bird's nest focuses on the natural and modified habitats of cave and house of swiftlet bird, respectively. The environment of the habitat influences the nitrate and nitrite contents in EBN. The nitrate and nitrite contents in EBN are influenced by the duration of exposure emitted by bird soil (Guano), which had given health concern to the consumer. The dosage of nitrate and nitrite found EBN had exceeded the safety dosage for human consumption. The consumption of high level of nitrate and nitrite had brought toxic effect onto the consumers' health. The present study was carried out to investigate which EBN source of habitats has minimal nitrate and nitrite contents to ensure food safety.

1.3. Objective of Study

The aim of the study was to investigate the contents of nitrate and nitrite in EBN obtained from natural and modified habitats environment of swiftlet bird.

1.4. Hypothesis

The contents of nitrate and nitrite from EBN obtained from natural habitat (cave) is higher than the EBN obtained from modified habitat (house) of swiftlet bird.

1.5. Significant of Study

The study of nitrate and nitrite determination in EBN obtained from natural habitat (cave) and modified habitat (house) of swiflet bird will provide some comparative information such as nitrate and nitrite contents. To understand how the nitrate and nitrite content affect the EBN quality, the current study was initiated that would provide information for future reference of food safety and security. This is to ensure the swiftlet industry would provide more concern on high nitrate and nitrite contents in EBN in order to require more processing on natural habitat's (cave) nest. The understanding of nitrate and nitrite contents in EBN for food safety and security which will restore the economic value of EBN market from low to higher price value.

1.6. Scope of Study

The method of the study will be analysing of nitrate and nitrite contents in EBN samples, which will be from natural habitat (cave) and modified habitat (house) of swiftlet bird. The analysis of EBN will either undergo dried powder samples and supernatant samples to determine the nitrate and nitrite compound. The weights of solid and supernatant samples for determination of nitrate and nitrite were constant. The independent variable was the different habitats source of EBN, which was from natural habitat (cave) and modified habitat (house) of swiftlet bird and the dependent variable was the contents of nitrate and nitrite. Determination of nitrate and nitrite contents were tested by ion chromatography in solid EBN samples and commercial kits for EBN supernatants samples.

1.8. Expected Outcome

The result from this project is that the nitrate and nitrite content in natural habitat (cave) nest is greater than modified habitat (house) nest, because it may due to the exposure of extreme climate inside the cave environment. The nitrate and nitrite contents in house nest would be less since the swiftlet ranching farmers perform good animal husbandry practices (GAHP) in the swiftlet house by performing regular removal of guano to reduce the chance of nitrate and nitrite gas emission.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Edible Bird's Nest

2.1.1 History

During the years of 618-907 A.D, the Tang Dynasty started the trading of EBN. In years 1368-1644, the imperial court of Ming dynasty was enlightened with the deliciousness of EBN which introduced by admiral Cheng He (Babji et al., 2015). The Chinese dynasty usually traded the edible bird nest from Borneo (Ann, 2013). The EBN were usually harvested from Niah cave in early year of Tang Dynasty (Amy and David, n.d). In recent year, China are currently contributed high demand of EBN from Southeast Asian countries (Thiha, 2016). The most producer of EBN are Indonesia, Thailand, and Malaysia which Malaysia are currently third producer (Babji et al., 2015).

2.1.2 Price of Edible Bird Nest

The price of edible bird nests is fluctuating every month which is influence by the demand of the main consumer countries. In 2009, the retail price of edible bird nest was sold at RM 20, 000 per Kilogram in China. The Malaysian government had established the price for producing edible bird nest according to the grade of the nest with the range of RM 5,000-RM 8,000 per Kilogram (Rashid and Nazmi, n.d). The price of edible bird nest will become high which rely on the quality of edible bird nest base on the size, cleanliness, shape and colour (Tan et al., 2014).

2.1.4 Constrained of Edible Bird Nest Production

The production of EBN was drop due to the over harvesting of edible bird nest from cave resulting population of EBN drop. Deforestation also contribute the reduction of swiftlet population while usage of pesticide destroyed the food chain of swiftlet. China had banned the EBN exportation due to high nitrate and nitrite content (Qi Hao et al., 2016). The banning importation of EBN by the Chinese government is to ensure food safety for the consumer. According to the Chinese Ministry of Health,4400 mg/kg was found in the EBN which had exceeding the 30 mg/kg maximum level of safety (Thorburn, 2015). The restriction of EBN exportation from Malaysia resulting the drop of price of EBN per kilogram from RM 4,800 drop to RM 4,000 (Noriah and Saiful, 2012). However, the Chinese government restore the importation from Malaysian EBN production which are well-established and recognized company with the panel in 2014 (Alif et al, 2016).

2.2 Swiftlet Description

2.2.1 Distribution of Swiftlet

The swiftlets have widely distributed around the west Indian Ocean at the southern part of the Asian continental. The swiftlets are mostly found at the south-east Asian regions which they highly reproduce at these countries like Indonesian archipelago, Southeast Asian peninsular and Borneo or southern part of Philippines Archipelago. The swiftlet is derived from a species of Apodidae who feed on insect that commonly found within these region which are commonly found inside the cave habitat or environment which similar with cave.

2.2.2 Overview of Swiftlet Edible Bird Nest

The swiftlet of Apodidae also consist of sub-species of Aerodramus which usually live inside the cave and build their glutinous nest from saliva secretion (Hamzah et al., 2013). Swiftlet species produce an expensive edible bird nest due to the nutritional and medical properties (Craig, 2015). The *Aerodramus*swiftlet produce abundant of glutinous saliva which are usually rely on swiftlet EBN ranching. The Aerodramus specie with genus of AerodramusFuciphagus (White-Nest) andAerodramus Maximus (Black nest) are currently used for commercial ranching in artificial cave-like house to produce the valuable edible bird nest (Hao et al., 2015). the Aerodramus species are dark-dull blackish brown on the upperpart of the body along the tail while have grey brown colour at the lowerpart of the body. The swiftlets characteristic have narrow pointed wing and have sharp claws in order for them to easily attach to vertical wall surface. The swiftlets daily routine are mostly flying around the roosting area for food. During roosting time, they spend their time at their roosting site for nest construction.

2.2.1 Nest Building Behavior of Swiftlet

The swiftlets activity for nest construction usually occur at night during the roosting season. The nest construction site are attach vertically on the wall surface. The nest are also located at the small hole chamber or concave wall surface beside on the straight vertical wall. The beginning of nest construction started with the edible salivary materials which make it as cement or glue before attaching other material as the reinforce for the nest. The behavior of nest building usually involving chewing together with the movement. The soft saliva will harden once it get dry after exposing to the air. The nest construction will continues which take about 30 days until the edible nest were becoming angular shape. The swiftlets will lay their eggs which take about 7-10 days on the nest when the nest are stronger attached.

2.3 Edible Bird Nest Characteristic

2.3.1 The Edible Bird Nest

The term of edible in bird nest referring to the swiftlets by product that is consumable by people. The swiftlets species like Aerodramus are among the species that produce edible bird nest for the swiftlet ranching. The swiftlets product of edible bird nest that derives from white-nest (*Aerodramusfuciphagus*) and black-nest (*Aerodramus* *maximus*) are common swiftlet species that heavily traded. The edible bird nest is made up of glutinous saliva used as cement that laminae other material which include leaf or feather as reinforce until the nest are moult into angular shape. The swiftlet species which both male and female were cooperating together to constructing their nest that form the valuable commercial animal by product. The expensive value of EBN was due to it special content on medical and therapeutic properties which still undergoes further research on medical and pharmaceutical field.

2.3.2 The Edible Bird Nest (EBN) Salivary Substance

The unique salivary liquid from the swiftlets acts as the cement to support the structure of the nest between other materials. The sticky substance of the saliva is important and acts as glue to help the EBN attached on the surface of vertical wall. The swiftlet secreted their saliva from the sublingual glands below the tongue. The size of the glands fluctuates from time to time and the size enlarges as the saliva store excessively during nesting building period.

2.3.3 Edible Bird Nest (EBN) Nutrient content

The basic nutrient overview is important in EBN content. The protein content in EBN was 59.8 %- 65.8 % by analysing with automatic CHNS/O analyser. The carbohydrate content in edible bird nest after analysed with phenol sulphuric acid was 18 % for clean raw EBN. Fat content is the lowest found in EBN with the amount of 0.07. The percentage of ash and moisture of EBN was 8 % and 13 % respectively (Hamzah et al., 2013). The EBN also contain specific nutrient like glycoprotein; 9 % sialic acid, 7.2 % galactosamine, 5.3% glucosamine, and 0.7 % frucose. (Babji et al., 2015) Amino acid content in EBN has essential amino acid: histidine (1.4%), methionine (2.2%), leucine (5.3%), and valine (3.3%), non-essential amino acid: alanine (3.9%), arginine (3.8%), glycine (2.5%) and serine (2.4%) (Nurfatin et al., 2014). The sialic acid content in EBN shows the sialidase activity and haemagglutination inhibition that fight against influenza virus.

2.3.4 Edible Bird Nest (EBN) Colour

Edible bird's nest from swiftlet house are usually dull white or dull yellowish while from the cave are dull orange red (Paul, 2012). The colour of EBN was influenced by the exposure of vapour produced by the bird soil in hot and humid condition (Paydar et al., 2013; Quek, 2015). They stated that the investigation of EBN colour was carried out by exposing the EBN bird soil and hydrochloric acid + sodium nitrite in a sealed glass jar at room temperature for longer period. The colour of EBN was changed from lighter colour into darker for 48 hours.

2.3.5 Nitrate and Nitrite in Edible Bird Nest (EBN)

The EBN are originally white colour, which will turn into dull brownish colour due to the exposure of bird soil which is also known as guano, since it is emitting vapour of the compound gases due to the chemical reaction within the swiftlet deposit. The environment habitat for swiftlet adapts at the humid environment. The water vapour from the hot and humid environment contacts with the bird soil causing high water content (Ismail et al. n.d.). The compounds were produced due to an anaerobic microbial fermentation that produced ammonia, which converted to nitrate and nitrite (Paydar et al., n.d.). The EBN from the cave has the highest nitrate and nitrite levels compared to EBN from house. The nitrate and nitrite contents were 349.3 ppm and 39.6 ppm, respectively (Zainab et al., 2013). The reducing amount of nitrate and nitrite compound from the EBN can be done by proper handling which must be undergoes cleaning process of EBN (Rebecca, 2013).

2.4 Swiftlet Habitat

2.4.1 Swiftlet of Natural Habitat (Cave)

Swiftlet (*Aerodramus*) is an insectivorous species of *Apodidae* which mostly lives inside the cave and builds their glutinous nest from saliva secretion. (Zainab et al., 2013). This species known to echolocate which relies on echo to move around in the dark cave. (Patricia et al., 1996) The most swiftlet genus that found in cave is *Aerodramus maximus* (Black EBN). The swiftlet in the cave will rely sound of the surrounding for echolocation to navigate the dark environment (Novick, 1959).

2.4.2 Swiftlet of Modified Habitat (House)

The swiftlet ranching was available in this modern ranching by constructing a cave-like environment known as modified habitat called swiftlet house. The swiftlet genus of *AerodramusFuciphagus* (white-nest swiftlet) lives in a swiftlet house. These species usually produce a valuable EBN product. (Looi et al., 2015) The building of swiftlet house was beneficial to maintain the swiftlet population as well as protecting them from over exploitation and other predators (Ibrahim et al., 2009).

2.5 Harmful of Nitrate and Nitrite

Nitrate and nitrite are an inorganic compound that usually derived from ammonium (Maayah, 2016). High dosage of nitrate and ingestion is hazardous health due to the conversion of nitrate to nitrite in human body, which led to toxicity (Ward, 2011). The levels of 80–800 mg/kg for nitrate and 33–250 mg/kg for nitrite are considered as lethal (Chamandoost et al., 2015). The main health hazard of nitrate and nitrite contributed the disease condition in human body called methemoglobinemia.

The nitrate and nitrite compound do not cause any sign of harm until they undergone endogenous process during the dietary reaction. The endogenous reaction of nitrate and nitrite were finally forms into hazardous toxic product. The compound product is called nitric oxide which interrupts the normal function of the healthy human body. The nitric oxide had cause a severe side effect which is mainly resulting in abnormality function of hemoglobin called methemoglobinemmia. The methemoglobinemmia disease is a condition where the hemoglobin (red blood cells) is unable to transporting oxygen to whole body system. The methmoglobinemmia could become responsible for metabolical disorder within the body tissues caused by the restriction retrieve of an oxygen due to the carrying of oxygen by red blood cells which is no longer for function. The conversion nitrate and nitrite into nitric oxide were caused by the bacteria that had already present inside the human gut causing chemical reaction between the microorganism and the compound. The gram-negative bacteria that converts the nitrate and nitrite compound inside the gut usually in high in pH condition.

The harmfulness of nitrate and nitrite causes the imbalance of metabolism activity in human bodies which would affect the blood pressure, immune system, and neurologic function (Douglas, 2002). The inorganic compound is common threat to the cardiovascular system due to the oxidative stress by which could resulting hypertension. This problem had caused imbalance of blood pressure as it could bring high risk of cardiovascular diseases (Mattias and Filip, 2010).

2.6 Nitrate and Nitrite Analysis

2.6.1 Nitrate and Nitrite Extraction

The nitrate and nitrite compound can be extracted from the samples into the solution. The extraction of nitrate and nitrite can be done through the soaking of samples in distilled water for few minutes. For example, extraction of nitrate and nitrite from EBN can be done by heating in water bath with temperature of 90°C and with the duration of 15 minutes. The samples solution then undergoes centrifuge process to at the speed of 5 000 rpm for 10 minutes (Malaysian Standard, 2015).

2.6.2 Using Ion Chromatography (IC)

Before testing by ion chromatography, 100 mL of water were mixed with EBN into beaker and heating in water bath. The mixture was stirred occasionally while heating to make sure it is homogenized (Hamzah et al., 2013). The ion chromatography (IC) is convenient for testing of nitrate and nitrite in the samples. The ion chromatography is capable to analyse the multiple compounds like nitrate and nitrite in one injection (Wurttemberg, 1991).

2.6.3 Using Commercial Kits

The nitrate and nitrite water test kit is commercial kits which used to test nitrate and nitrite in aquarium water. The test required 5 mL of sample solution with a 5 drops of nitrate/nitrite reagents. The mixture was shake for 5 seconds and will change colour result in 5 minutes. (Determining the Amount of Nitrate in Water, n.d) The analysing of nitrate and nitrite using nitrate and nitrite water test kits is very simple procedure and convenient to determine the levels of nitrate and nitrite compound in the samples. The result of nitrate and nitrite amount in the samples will be given with a unit of mg/kg or mg/ml (Isa, 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Place of study

This study was conducted at Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli, Kelantan.

3.2 Sample Collection

The EBN samples from modified habitat (house) were collected randomly from kampong BatuBedang, Serian district, Sarawak, Malaysia (0°58'11.4"N 110°32'03.4"E). Similarly, the EBN samples from natural habitat (cave) were collected randomly from Silabur, Serian district, Sarawak, Malaysia (0°57'16.5"N 110°30'08.2"E). The climate during the sample collection was hot (28℃) and humid (84%).

3.3 Sample Preparation

For test of EBN supernatants, the EBN from natural (cave) and modified habitats (house) with 5 replicates for each were tested with commercial Kits (API Nitrate and Nitrite test, USA). For the test of solid EBN, the 5 grams of EBN from cave and house with 1 replicate for each were prepared using Ion chromatography (IC).

The supernatants preparation for EBN of natural habitat (cave) and modified habitat (house) were prepared. The nest samples were ground into small powder and then filtered with sieve (1 mm). The each EBN powder samples were weighted for 1 g and then were mixed into 40 mL of distilled water in falcon tube, and then repeated the step to get 5 replicates for each EBN habitat. The supernatants were heated in water bath for 90°C in 15 minutes to extract the nitrate and nitrite. The supernatants samples were filtered with filter paper (Whatman, Maidstone, UK)to get the pure clean supernatants. The 5 mL EBN supernatant samples from each replicate were collected into falcon tube for analysis of nitrate and nitrite using commercial test kits. The supernatants samples were left cool down for 1 hour. The supernatants of 40 mL each were tested by using 5 mL each to get the ppm unit for EBN sample with the weight of 0.125 grams using following formula:

$$\frac{40ml}{5ml} = \frac{1gram}{x}$$

$$x = \frac{5ml}{40ml} g$$

$$x = 0.125gram$$

3.4 Chemical Analysis

3.4.1 Using Commercial Kits

The supernatant samples of EBN from both habitats were tested for nitrate and nitrite contents using commercial kit. The samples (5 mL) were prepared into test tube. Then, 5 drops of nitrate reagent were dropped vertically (up-side-down) into the test tubes containing samples. The test tube was shaken for 5 seconds to ensure the sample and the reagent completely mixed. The test tube was left for 5 minutes to show the result. Thus, the whole process was repeated with another supernatant samples and repeated again with other samples (eg. Cave nest solution). The nitrite tests in all supernatants were repeated with the same technique following the method of commercial kit. Each replicate was repeated 3 times to obtain the average value. The data was recorded as shown in Table 1 for natural habitat (Cave) EBN and Table 2 for modified habitat (house) EBN. The statistical analysis was done by using significant to differentiate the result and standard deviation was used to find the mean of repeated test result for each replication. The graph was created and sketched into two categories which were nitrate and nitrite as shown in Figure 1 and Figure 2, respectively (based on the results from Tables 1 and 2).

3.4.2 Using Ion Chromatography (IC)

The EBN solid samples (5 gram) from natural habitat (cave) and modified habitat (house) with 1 replicate was analysed for nitrate and nitrite contents using ion chromatography with the help of laboratory of Permulab SDN. BHD. (Selangor, Malaysia).

CHAPTER 4

RESULT

4.1. Nitrate and nitrite contents in EBN supernatants using commercial kits

Nitrate and nitrite contents in the EBN supernatants using commercial kit are shown in Tables 1 and 2. The commercial kit for nitrate and nitrite were able to detect the sensitivity of 5 ppm and 0.25 ppm, respectively. Table 1 showed that there were no significant difference on nitrate content between EBN of cave and house, except replications 3 and 5. However, Table 2 showed that there was significant difference on nitrite content for EBN of natural habitat (cave) and modified habitat (house) for replication 4. There was a trend that natural habitat (cave) EBN supernatants showed higher numerical values for nitrate and nitrite contents compared to the modified habitat (house) EBN, except for nitrate level in replications 3 and 4. The average of nitrate content in natural habitat (cave) EBN was higher (32.5 ppm) compared to modified habitat (house) EBN (6.54 ppm) (Table 1), and the average of nitrite content in natural habitat (cave) EBN was higher (1.27 ppm) than modified habitat (house) EBN (0.50 ppm) (Table 2), although it was not found significant difference between treatments.

Table 1: The nitrate content (ppm) of EBN supernatant using commercial kits

Number of replication	EBN Natural Habitat (Cave)	EBN Modified Habitat (House)	Significant Level
Replication 1¶	5.00 ± 0.00	0.00	-
Replication 2¶	0.00	0.00	-
Replication 3	0.00	5.00 ± 0.00	***
Replication 4	13.30 ± 5.77	18.30 ± 18.90	NS
Replication 5	133.30 ± 46.20	6.67 ± 2.89	***
Overall Mean	32.5 ± 57.80	6.54 ± 10.70	NS

NS = Non significant (P>0.05); *** = P< 0.01. ¶ = only two tests

Table 2: The nitrite content (ppm) of EBN supernatant using commercial kits

Number of replication	EBN Natural Habitat (Cave)	EBN Modified Habitat (House)	Significant Level
Replication 1¶	0.00	0.00	-
Replication 2¶	0.00	0.00	-
Replication 3¶	0.00	0.00	-
Replication 4	5.00 ± 0.00	2.00 ± 0.00	***
Replication 5	0.50 ± 0.00	0.33 ± 0.14	NS
Overall Mean	1.27 ± 2.14	0.50 ± 0.83	NS

NS = Non significant (P>0.05); *** = P<0.01. ¶ = only two tests

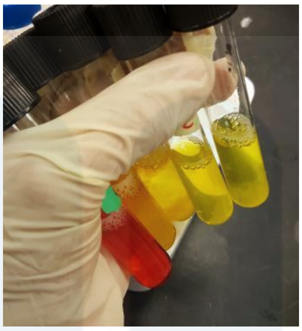

Plate I : Nitrate result of EBN supernatant samples for EBN natural habitat (cave) and modified habitat (house) by commercials kits (Nitrite water test kit)

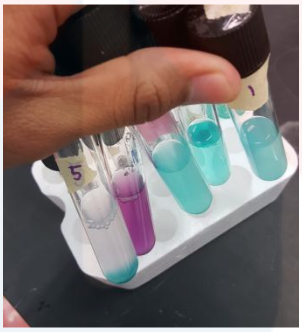

Plate II : Nitrite result of EBN supernatant samples for EBN natural habitat (cave) and modified habitat (house) by commercials kits (Nitrite water test kit).

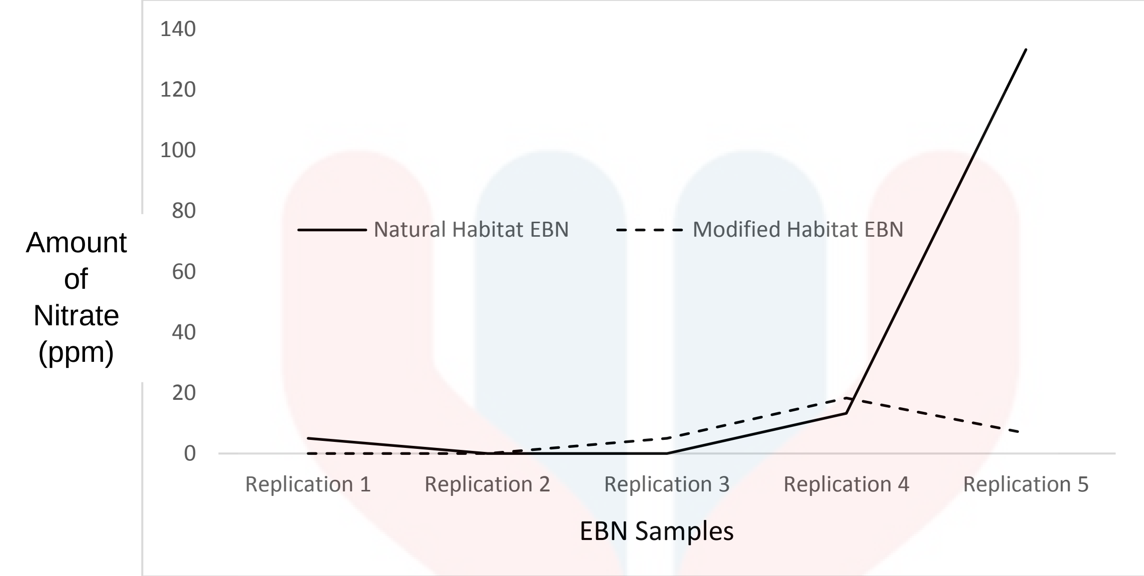

Figure 1: Nitrate content (ppm) in edible bird's nest (EBN) between natural habitat EBN and modified habitat EBN of swiftlet bird.

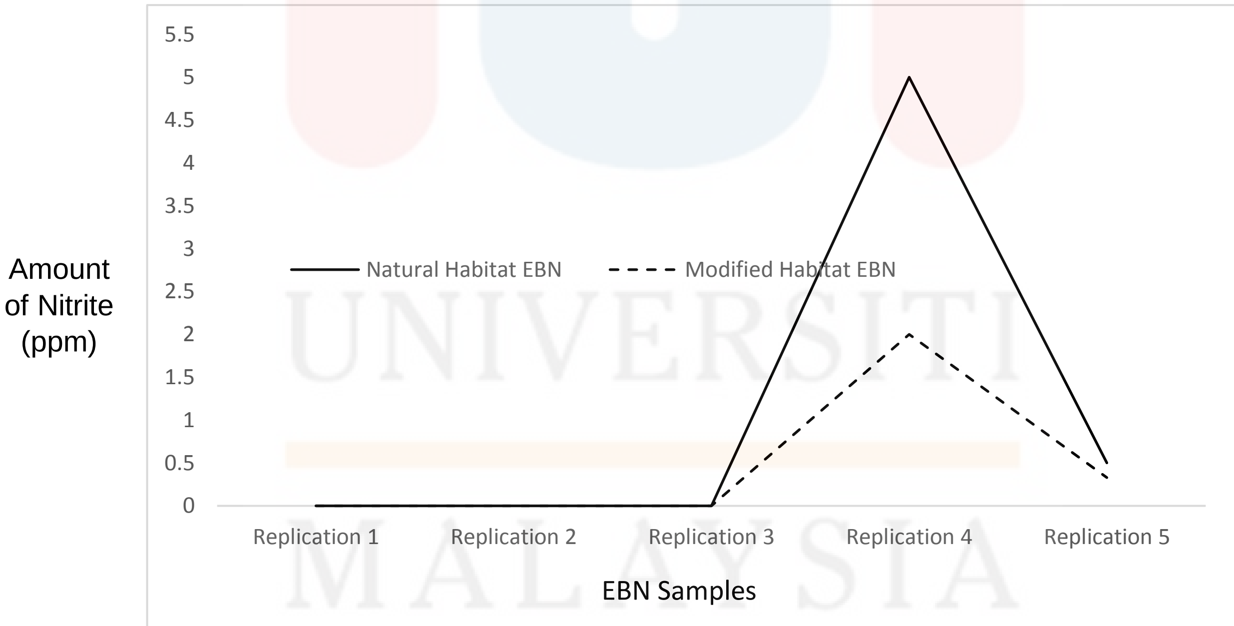

Figure 2: Nitrite content (ppm) in edible bird's nest (EBN) between natural habitat EBN and modified habitat EBN of swiftlet bird.

4.2. Nitrate and nitrite contents of solid EBN samples using Ion Chromatography (IC)

Nitrate and nitrite contents of solid EBN samples using Ion Chromatography are shown in Table 3. Sensitivity using Ion chromatography was below 0.01 ppm. Both of the nitrate and nitrite contents in EBN from natural habitat (cave) were numerically higher value than the contents in EBN from modified habitat (house), although statistical analysis was not performed due to limited number of sample analysis.

Table 3. Results for analysis of nitrate and nitrite found in EBN cave and EBN House

Parameter	Treatment	
	EBN from natural habitat (cave)	EBN from modified habitat (house)
Nitrate (mg/kg)	1411	39.1
Nitrite (mg/kg)	145	4.12

CHAPTER 5

DISCUSSION

5.1 Nitrate Content in Edible Bird's Nest (EBN)

There was no difference on nitrate content between natural habitat (cave) EBN and modified habitat (house) EBN using commercial test kit. This may be due to the samples collection of old and fresh EBN from habitats. During the sample collection, EBN was randomly selected without identifying the fresh and old nest. The replications which were tested with zero result might be from the fresh EBN samples compared to old EBN which were detected. The results from replications 3 and 4 had gave opposite result to the hypothesis. The reasons for this error were may be due to the EBN from natural habitat, which was fresh compared to the EBN from the modified habitat. The used of commercial kit had failed to prove that the results for this study to be same with the expectation from the study hypothesis.

In this study, nitrate content in EBN from natural habitat and modified habitat using ion chromatography had shown different results which were successful based on the study hypothesis. The reason that the nitrate content in EBN from natural habitat was higher compared to the modified habitat and it might be due to the environmental condition. The environmental conditions in natural habitat of swiftlet bird were dirty, while the modified habitat was mostly clean. The management routine in these habitats may influence the environmental condition inside the habitat. For example, higher level of dirt inside the environment resulted to increase the nitrate content in the EBN. This result is in line with the previous study of Hamzah et. al. (2013).

5.2 Nitrite Content in Edible Bird's Nest (EBN)

There was difference on nitrite content between natural habitat (cave) EBN and modified habitat (house) EBN during the test analysis using commercial test kit. However, the detection of nitrite was only shown on replications 4 and 5 compared to the rest of the replications. The possible cause of no detection occurred in these 3 replications may due to the samples collection of old and fresh EBN. During the sample, the collecting of EBN was randomly selected without identifying the fresh and old nest. The replications which were tested with zero result might be obtained from the fresh EBN samples compared to the old EBN which were detected. The results from replications 4 and 5, which was in line with our study hypothesis. The used of commercial kit had shown limitation to detecting the fresh EBN to prove that the results for this study would be the same with the expectation from the study hypothesis

The nitrite content in EBN from natural habitat and modified habitat using ion chromatography had shown in different results, which was expected based on the study hypothesis. According to Malaysian Standard (2015), The reason that the higher nitrite content in EBN from natural habitat compared to the modified habitat may be due to the environmental condition. The environmental condition in natural habitat of swiftlet bird was dirty, while the modified habitat was mostly clean. The management routine in these habitats may influence the environmental condition inside the habitat. For example, higher level of dirt inside the environment resulted to increase in nitrite content in the EBN.

5.3 Source of Nitrate and Nitrite in EBN

The presence of nitrate and nitrite found in EBN was due to the air pollutant inside the environmental surrounding. The nitrate and nitrite emission were emitted from the swiftlet bird dropping which is known as bird soil or guano. The bird soil guano consists of decompose organic materials and also contains bacteria that make chemical reaction in humid condition. The waste product formed from the swiftlet bird dropping was mainly composed of ammonia NH_3.

The fermentation occurs in bird dropping produce high amount of ammonia reduction which carried out by varieties of different endogenous intestinal gram-negative anaerobic bacterial. The presence of NH_3 formation firstly was derived inside the bird intestinal organ before it was secreted. The anaerobic bacteria were responsible to the formation of ammonia during the breakdown of the organic materials that digested inside the bird stomach to form waste product. The NH_3 production by the bacteria forms during low pH within the surrounding. The formation of ammonia is from protein source which is derived from the insect that rich in protein source as the diet of the swiftlet.

Moreover, the formation of ammonia from degraded protein are influences by the amount of carbohydrate content in swiftlet diet as the carbohydrate could breakdown into simple sugar like lactose to form source of energy for ammonia bacterium production (Vince and Burridge, 1980). The ammonia inside the bird dropping (guano) caused biological nitrogen cycle by conversion of nitrogen oxidation to form nitrate and then convert to nitrite. The process of biological reaction involves the bacterial metabolic activity to form nitrate and nitrite from the ammonia.

The explanation for the nitrate and nitrite sources from this study in EBN was originated from the environmental habitat of the swiftlet bird which shows that the amount of bird dropping (guano) were found abundantly from the natural habitat (cave) of swiftlet bird. The high content of bird dropping (cave) inside the natural habitat (cave) could result the EBN from the environment received high number of nitrate and nitrite. The small content of nitrate and nitrite in EBN from modified habitat (cave) of swiftlet may be due to less amount of bird dropping (guano) inside the modified habitat (house) as it would be less chance for ammonia conversion to nitrate and nitrite based on the study from Vince and Burridge (1980).

5.4 Impact of Nitrate and Nitrite on Environment

The nitrate and nitrite compound had causing air pollutant inside the environmental habitat of swiftlet ranching area. The nitrate and nitrite gases form during the chemical reaction inside the bird dropping in acidic condition. The nitrate and nitrite gases could deposit into the EBN surface in short period of time and then increase the rate of deposition on the EBN within longer period. The bird dropping (guano) are responsible in the nitrate and nitrite pollution inside the environmental habitat of swiftlet ranching.

The study of nitrate and nitrite pollution occurred inside of the natural habitat (cave) and modified habitat (house), which were similar to the research done by Paul et. al. (2013). The contents of nitrate and nitrite deposited on EBN were influenced by the duration of exposure to the bird dropping (guano) inside the environmental habitat. In this study, the contents of nitrate and nitrite pollutant may be influenced the content between fresh and old EBN. The old EBN were had been longer exposure to the bird dropping (guano) compared to the fresh EBN. The analyzing of the content of nitrate and nitrite by using commercial kit was failed to show different in fresh and old EBN from natural habitat (cave) and modified habitat (house).

5.5. Health Issues of Nitrate and Nitrite

The nitrate and nitrite compound were main concerned among EBN consumers. The health issues had been concerned in the ingestion of nitrate and nitrite. However, the nitrate and nitrite level that below the safety level (80 mg/kg for nitrate and 33 mg/kg for nitrite) considered to safe for consumption unless the level were exceeded the safety level (Chamandoost et al., 2015).

Based on this result of study from Table 3, the nitrate and nitrite contents in EBN from natural habitat (cave) had failed to achieve the safety requirement, which the content had exceeding the permitted level. Meanwhile, the nitrite content in modified habitat (house) EBN has shown the safety limit, which below the permitted level (80 and 33 mg/kg respectively) as reported by Chamandoost et al. (2015). The high content of nitrate and nitrite in EBN either from natural habitat (cave) and modified habitat (house) may be harmful to EBN consumers as it could give health impact based on the study carried by Mattias and Filip (2010).

5.6. Removal or Reduce Technique of Nitrate and Nitrite

In this study, the removal entire amount of nitrate and nitrite from EBN for dietary safety is considered a big challenge and require more study. However, the reduction of nitrate and nitrite in EBN can be done during the processing of raw-unclean EBN to raw-clean EBN. The method is used by submerging the EBN into water or running water. The nitrate and nitrite were easily washed and removed from the EBN since the nitrate and nitrite are soluble inorganic compound (Maayah, 2016). From the nitrate result of modified habitat (house) EBN that had exceeded the safety limit, the method used for reduction of nitrate and nitrite emission from the EBN were also done by good swiftlet ranching management like swiftlet house.

The swiftlet ranching farmers perform good animal husbandry practise (GAHP) by regular removal of guano. The regularly removal of guano reduce the chances to emitting nitrate and nitrite gases around the surrounding environment. The cave EBN usually content high in nitrate and nitrite compound due to exposure of guano for long period inside and absorption of nitrate and nitrite from the limestone wall of the natural habitat (cave). The removal of guano from the cave would reduce the nitrate and nitrite emission. The cave with less guano is as a result from the guano harvesting business which the guano were harvested and sell as agricultural fertilizers. This practices would reduce the content of nitrate and nitrite from the EBN test result from Table 3.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In this study, there was no difference on nitrate and nitrite contents between the EBN from natural habitat (cave) and modified (house) of swiftlet bird using commercial kit. However, using ion chromatography, it was observed that nitrate and nitrite contents in EBN of natural habitat were showed higher numerical values compared to the nitrate and nitrite contents in EBN of modified habitats of swiftlet bird, although the number of samples were very few (only one for each parameter).

For the next research regarding the comparison of nitrate and nitrite contents in EBN between natural habitat (cave) and modified habitat (house) of swiftlet bird, the research should be carried out by choosing the appropriate number of samples of EBN like the fresh and the old EBN. I would also suggest that further research should be conducted by comparing the nutritional properties of EBN (e.g., protein, fat, ash, carbohydrate) from these two difference habitats and also study the morphologies between these two samples.

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APPENDIX A

Figure A.1: Sample of EBN that obtained from modified habitat (House).

Figure A.2: Sample of EBN that obtained from natural habitat (Cave).