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"UTILIZATION OF BIODIVERSITY FOR THE GREATER BENEFIT OF MANKIND"

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"Utilization of Biodiversity for the Greater Benefit of Mankind"

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PREFACE

This book is a compilation of research papers which had been successfully presented at the Malaysia International Biology Symposium 2019 (*i*-SIMBIOMAS 2019). The symposium had been organized by the Department of Biology, Faculty of Science, Universiti Putra Malaysia on August 23-24, 2019. Twenty-four manuscripts had been received from participants and were categorized into three subfields in biological sciences. Chapter 1 presented the multidisciplinary fields in Ecology, Chapter 2 contained studies on Animal Physiology, and Chapter 3 presented research works on Plant Physiology.

Last but not least, we are thankful to the huge support by all authors by supporting us in every step of our journey towards the success completion of this book. It is hoped that the book will benefit a lot of people including academic scientists, research students, industry researchers, and the public.

Thank you.

THE EDITORIAL TEAM

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CHAPTER 1: ECOLOGY

1.1 Conflict between the ecological impacts and socio-economic gains of alien fishes: a sustainability concerns

Abdulwakil Olawale Saba, Mohammad Noor Azmai Amal, Ismail Ahmad
and Syaizwan Zahmir Zulkifli

Introduction

The world population is growing at an alarming rate making the need for increased food production indispensable (Singh, 2019). Countries of the world have engaged in the culture of certain introduced fish species due to their desirable characteristics (Diana, 2009). Some of these introduced species have helped to boost fish production in many folds thereby contributing to food security and economic development (Ellender and Weyl, 2014). Besides, other ones have been introduced through the aquarium industry and they also help to improve human well-being (CBD, 2010; Strecker et al., 2011). Apart from the Antarctic region which lacks information on invasive alien fishes, the highest number of alien fish species are attributed to north America with Australia having the lowest (Figure 1). Asian aquaculture, as with global aquaculture, is dependent to a significant extent on alien fish production (De Silva *et al.*, 2009). Furthermore, in Brazil, alien fish contributed about $US 250 million for the year 2008 and this comprises more than half of the total value of freshwater fish aquaculture (Briton and Orsi, 2012). However, in many cases, cultured alien fish species may end up in local ecosystems where they threaten biodiversity. In fact, High levels of species extinction have occurred the world over due to loss of biodiversity and species invasion has been identified as one of the major causes (Reid et al., 2019). For freshwater ecosystems, this has been linked with dwindling fish productivity (Chong et al., 2010). In fact, about 37% and 49% of all introduced fish species in South Africa and Okinawa-jima Island, China are known to have fully established breeding populations, respectively (Ellender and Weyl, 2014; Ishikawa and Tachihara, 2014). Although some other challenges to ecosystems' biodiversity and health which can also facilitate invasion have been identified and these include habitat degradation, over-exploitation, pollution, endemism and anthropogenic factors (Chong et al., 2010), the impacts of invasive or potentially invasive fish species should not be underestimated. Amidst this conflict, sustainability is essential to help generate maximum benefits and minimal impacts on the ecosystem (Cucherousset and Olden, 2011).

Assessing the impacts of alien fish species

Perception of Invasive Alien Fish (IAF) impacts is influenced by the viewpoint which may be *'ecocentric'* or *'anthropocentric'*. The latter will attach value to an improvement in human welfare which emanates from IAF. This, however, would be viewed as counter

ecological by the more ecocentric opinion (Vellend et al., 2007). Any assessment of alien fish species should consider all the advantages and disadvantages of the species in longitudinal and time-based scales (Bartley and Casal, 1998; Leal-Flórez, 2007) since it would facilitate the cause of sustainability. Alien fishes are, for the most part, intentionally introduced for reasons including aquaculture, fisheries improvement (stocking), aquarium trade, sport fishing, and biological control (Esmaeili *et al.*, 2014; Chong et al., 2010; Khairul-Adha et al., 2013). These introductions have produced several benefits from reducing the pressure on wild stocks, to income and employment generation (Aqmal-naser and Ahmad, 2019; Diana, 2009). As time goes on, some of these beneficial alien fishes somehow get into the local waters (Salam and Gopinath, 2006; Chong et al., 2010) where they run riots by predating on, competing with, and probably altering the ecosystem or wiping out the native species.

Monitoring fish invasion

Introduction (intentional or unintentional) of alien fish species for various reasons paves the way for invasion in a gradual process which may go unnoticed at the early stages. For introduced fish species to invade, they go through about five stages (Figure 2). If the process is unchecked, they become more challenging and even impossible to eradicate as the stages culminate. Early detection of Invasive Alien Fishes (IAF) is the best approach so that eradication still has a chance (Kiruba-Sankar et al., 2018). In cases where invasion had finally occurred, opportunities to exploit the species and minimize their impact on the ecosystem and socio-economics should be sought. Early detection of Invasive Alien Fishes (IAF) is the best approach so that eradication still has a chance (Kiruba-Sankar et al., 2018).

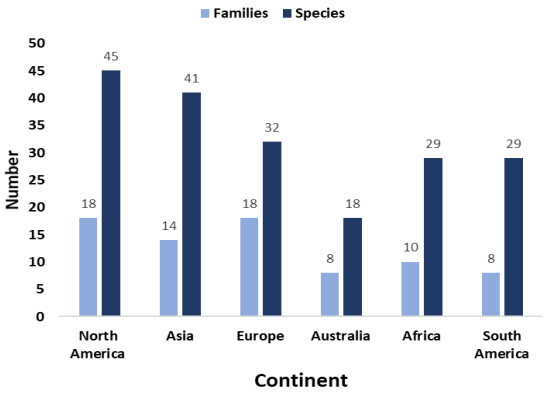

Figure 1: Number of invasive alien fish by species and families across the continents (Adapted from Sultana and Hashim, 2015)

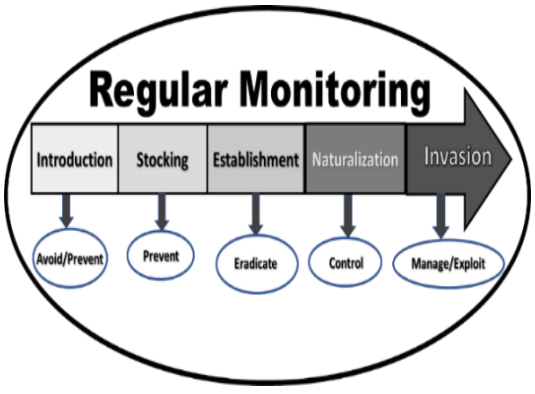

Figure 2: Stages of fish invasion with required measures (Modified from Gozlan et al., 2010; Kiruba-Sankar et al., 2018)

Striking a balance for sustainability

Sustainability, with its environmental, social and economic components requires that the benefits and risks posed by existing and potential alien fish species be analysed to ascertain holistically the *pros* and *cons* of fish introduction into any new environment (Ellender and Weyl, 2014). In effect, proper monitoring and management measures should be intensified to minimize the possible negative impacts, maximize the positive impacts and ensure sustainability. Certification and monitoring systems should be put in place to ensure sustainable practices (Diana, 2009). In addition, merits should be weighed against demerits in the short, medium and long terms.

Conclusion

Despite identifying huge ecological impacts of alien fish species in some cases, analysis of the socio-economic dynamics of alien fishes for present and the future pains and gains should be carried out. For local management to be effective, more appropriate native or less invasive candidates should be researched and promoted for use in aquaculture after adequate risk assessment and relevant policies are put in place. These should be monitored and communicated on a regular basis to ensure the participation of all stakeholders.

Acknowledgement

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1.2 Prediction 2035 of the landscape changes in Central Forest Spine 2, Peninsular Malaysia

Saiful Arif Abdullah, Amal Najihah Muhamad Nor and Nur Hairunnisa Rafaai

Introduction

In peninsular Malaysia, land use planning significantly influenced the ecological landscape of wildlife habitats, mainly forested areas. A wide variety of modelling approaches have been tested to simulate land use changes (Nor et al., 2017a); however, the effectiveness of simulations of the spatial structure of land use change in the forest area remains unexplored. This study aims to model and predict landscape changes in the Central Forest Spine 2 (CFS2) area which cover the southern part of peninsular Malaysia and to identify which are the main drivers, including spatial planning, in the resulting spatial changes.

Materials and methods

This study focused on the Central Forest Spine 2 (CFS2) area in the state of Pahang which cover the southern part of peninsular Malaysia. The geo-coded Landsat ETM+ 30 m resolution images of the year 1988, 1996, 2005 and 2017 were processed using ERDAS Imagine software to develop the land use map. After the raster land use maps were converted to vector form using ArcGIS 9.3 (ESRI, New York Street, Redlands, CA, USA), Land Change Modeler (LCM)-Markov Chain models were used, parameterised on changes observed between 1988 and 1996 and verified with the land use form observed for 2017. These models were then used to simulate land use change for the year 2035. The spatial changes of the simulated 2035 land use were then compared with land use area in 1988, 1996 and 2017 using ArcGis.

Results and discussion

In this study, the 80% percentage of agreement and persistence showed that the LCM model verification for 2015 was reliable and therefore, the model is appropriate for predicting future transitions. Differences between the modelled spatial changes and that observed in 2014 supplied evidence for successful planning interventions. Previous research shows that data generated using LCM is more accurate when the per transition susceptibilities are combined to compose an overall potential change map because neural network outputs can express the simultaneous potential change for various LULC types more adequately than the individual probabilities obtained (Pérez-Vega et al., 2012; Triantakonstantis et al., 2015). These predictive capacities allow models to be useful tools for local stakeholders involved in urban change decision making. The results from these models obtained in this study would suggest that a verified LCM-Markov Chain model is a useful tool to simulate future urban expansion.

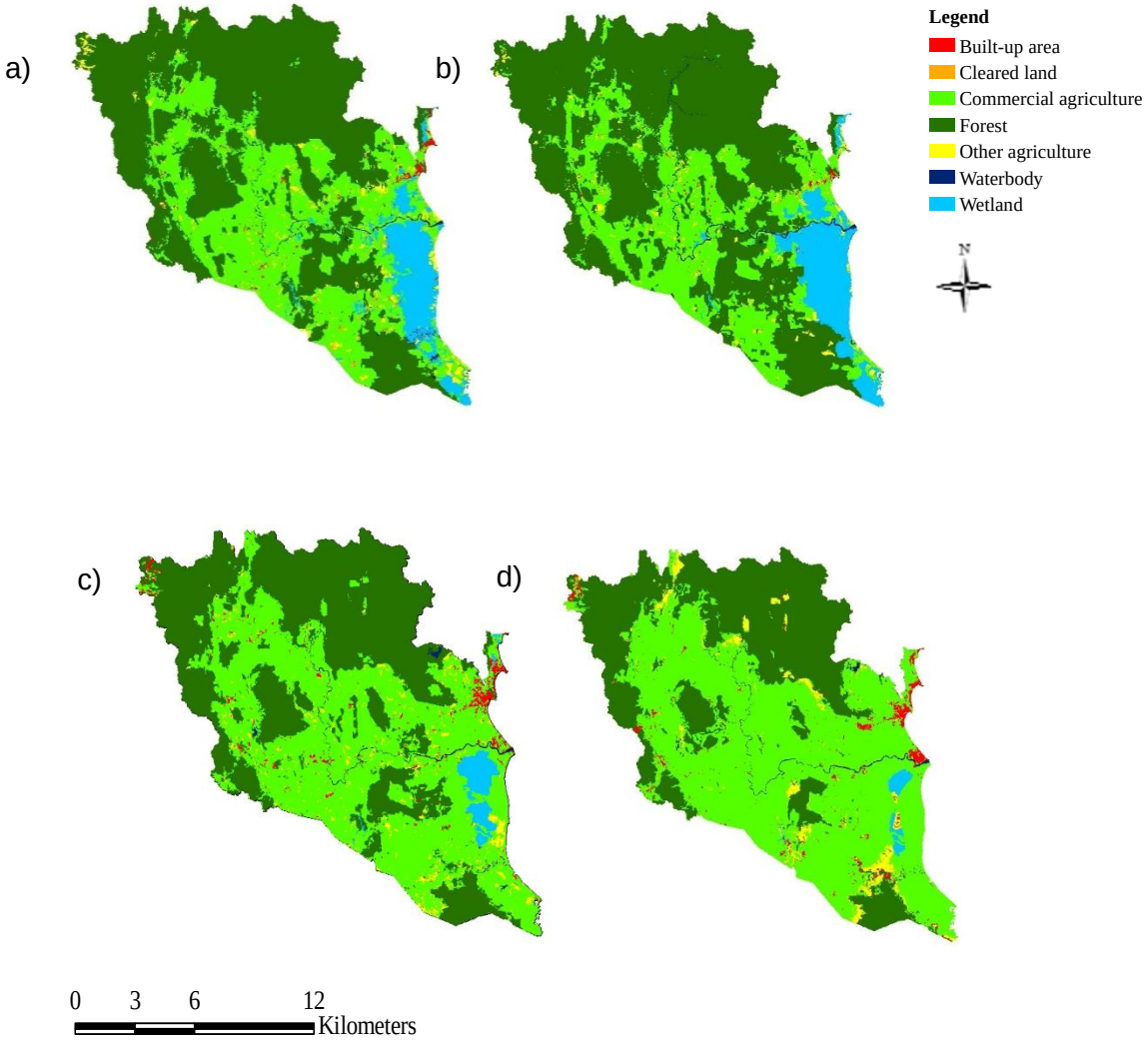

Figure 1: Land use land cover distribution in Pahang in a) 1988, b) 1996 c) 2017 d) 2035.

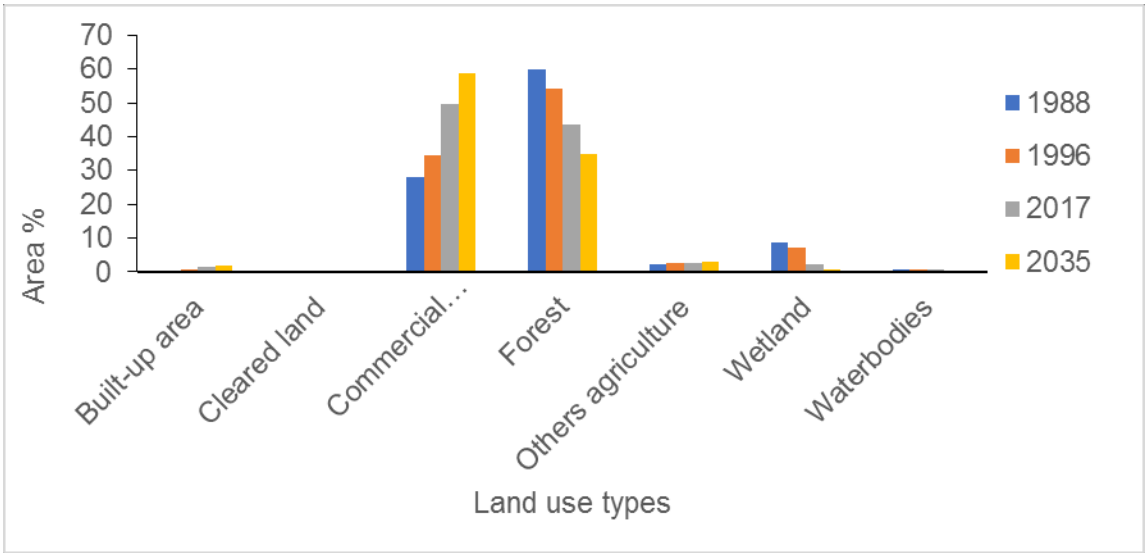

Figure 2: The area proportion (%) of land use distribution in Pahang in 1988, 1996, 2017 and 2035.

Over the 29 years, Pahang has experienced a decrease in green space and an increase in the built-up area (Fig. 1 and 2). The predictions indicate a further increase in a built-up area and decrease in green space by 2035 (Fig. 1 and 2). The results further suggest that built-up area expansion and the location of the variables affecting the model outputs are the significant drivers of green space change. The projected Markov Chain conditional probability matrices for 2035 revealed that the growth of built-up areas in this area showed a multidirectional urban expansion growth pattern, tending to occur in areas of better road accessibility, near the green space edge, on higher elevations and steep slopes where there is a low risk of flooding. These results agree with the findings of other studies, in which the distance from main roads is linked to the degree of landscape changes (Mishra et al., 2014; Nor et al., 2017a; Nor et al., 2019). The combined fragmentation and barrier effects of road networks considerably degrade landscape connectivity and ecological processes in the landscape (Nor et al., 2017b, Nor et al., 2018). Inherently, green space edge has a high probability of being decreased and in the results from this study show that development changes tend to start from the edge of existing green space. Evidence suggests that these spatial changes are influenced by the forms of land use activities experienced in the study area, the historical spatial changes, and uncontrolled land use activities.

Conclusion

LCM-Markov Chain was proven to be suitable for simulation of the future Central Forest Spine 2 area. Human land use activities affect the landscape change, has experienced green area loss over the study period. The planning authorities could design interventions which support planning at the landscape level. This study is designed to

provide the novel integrated approach for predicting landscape changes for the forest area to provide the initial guideline for sustainable conservation planning of wildlife habitat CFS2 area.

Acknowledgement

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1.3 Stomach content of tiny scale barb, *Thynnichthys thynnoides* at Perak River, Malaysia

Siti Nursyakinah Muhamad, Mohd Yusoff Ishak, Farhana Ahmad Affandi and Kartina Lakuli

Introduction

Habitat loss or modification, overfishing, deforestation are factors that threatened most of freshwater fish. According to Ismail et al. (2015) proper management are needed to ensure the sustainability since freshwater fish like *Thynnychthys thynnoides* are being harvest in large amount annually. In fact, the fish is targeted as it is the main ingredients for traditional fermented dish called 'pekasam' that can be produce up to 12 tonnes annually (Dolasoh, 2017). Management efforts such as the availability of feeding habits data are one of the information basis towards this efforts since there is a limited information available about *T. thynnoides* feeding habit in Malaysia. Studies on fish feeding habit are important to identify the trophic relationship in aquatic ecosystem, identifying feeding composition, structure and stability of food webs. Therefore, this study aims to determine the composition of stomach content of *T. thynnoides* at Perak River and to compare the stomach contents between different body sizes.

Materials and methods

The study was conducted at Chenderoh Reservoir (05°01′ to 40°56′ N and 100°55′ to 101°00′ E) which is located at the middle part of Perak River basin. Total of 30 fish samples were collected from the local fisherman at the sampling site. The total length and weight were measured and recorded. The fish sample was then injected with 10% formalin into the gut area till bloated to prevent further digestion of prey. Fish samples were kept in ice-box and transported back to the laboratory in Faculty of Environmental Studies, Universiti Putra Malaysia. The gut contents analysis was done following the laboratory procedure by Gelwick and McIntyre (2017) and Manko (2016) and the gut contents were identified following Lokman (1990).

Results and discussion

Total of 63 food items were detected in *T. thynnoides* (Table 3) namely phytoplankton, plants, insects, detritus and some unidentified items. Results showed that *T. thynnoides* mainly fed on phytoplankton which was present the highest, 54% of the stomach contents in total of 30 samples. There were only three phytoplankton family recorded which is Bacillariophyceae, Chlorophyceae and Chrysophyceae. Similar result were found by Ananda et al., (2015) where phytoplankton Cyanophyceae is the highest phytoplankton

consumed by *T. thynnoides* compared to other food items. Previous study by Rainboth (1996) also reported that *T. thynnoides* in Mekong River, Cambodia mostly fed on periphyton, algae, phytoplankton, and small zooplankton. In fact most freshwater and marine organism depend on phytoplankton which serves as a base for the aquatic food chain (Begon et al., 2007). This result corresponds with the abundance distribution families of phytoplankton in Perak River (Nursuhayati et al., 2013). The study stated that there were six main family of phytoplankton in Perak estuary namely Bacillariophyceae (diatoms), Chlorophyceae (green algae), Cyanobacteria (blue-green algae), Chrysophyceae (golden-brown algae) Euglenophyceae (euglenoids) and Pyrophyceae (dinoflagellates).

Table 3: Stomach content of *Thynnichthys thynnoides*

Type of food	Percentage of food items (%)
Phytoplankton:	
Bacillariophyceae	30
Chlorophyceae	19
Chrysophyceae	5
Plants	16
Insects	13
Detritus	8
Unidentified items	9

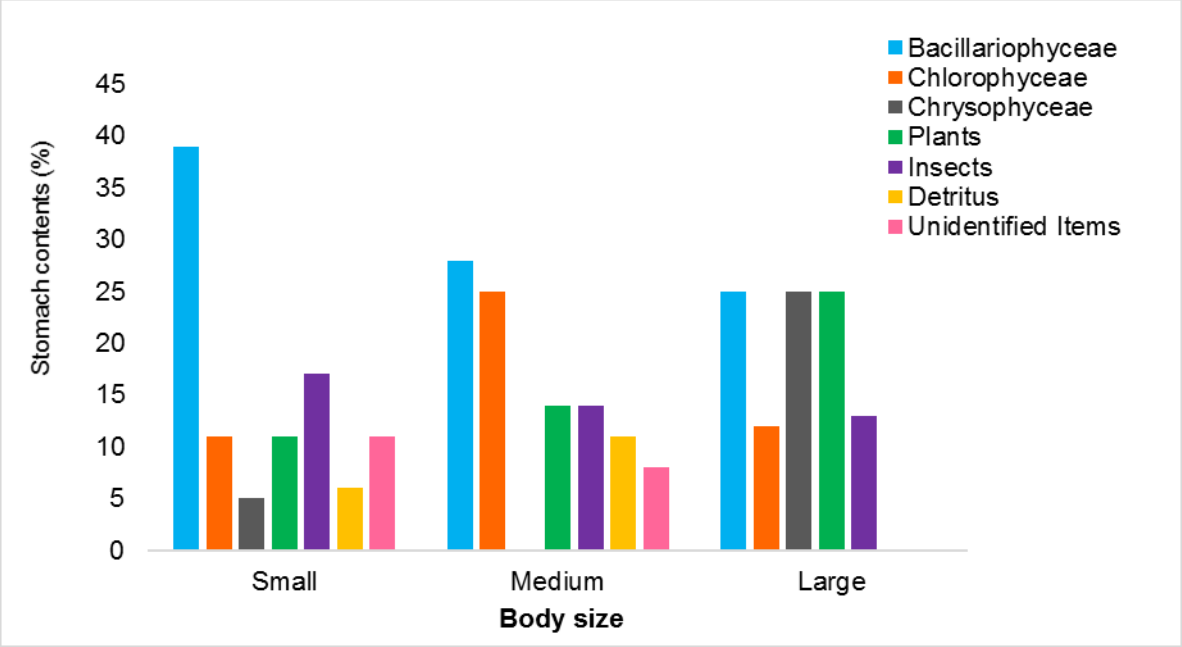

Figure 2: Stomach contents in different body size of *T. thynnoides*.

Figure 2 shows the percentage of stomach contents in different body size of *T. thynnoides*. Overall, all three categorized body size of *T. thynnoides* mostly fed on phytoplankton particularly Bacillariophyceae compared to other food items such as plants, insects, detritus and unidentified items. There is no significant different in trend of percentage between stomach content for each food items recorded among different body sizes of *T. thynnoides*. However, percentages of food items were observed decreasing with body size of *T. thynnoides* from small (20-22.0cm) to medium (22.1-24.0cm) and to large (≥24.1cm). Result shows that trends percentage of Bacillariophyceae were decreasing from small, medium and to large body sizes which were 39%, 28% to 25%, respectively. On the other hands, increasing trends percentage of plants fed increasing with body size from small, medium to large which were 11%, 14% and 25%, respectively. The trends showed an opposite result where the percentage of food item consumed increasing with body size. Therefore, it can be concluded that there is a difference between stomach content analysis among different body size of *T. thynnoides*. According to Schafer et al., (2002) the largest fish feeds more on teleost than those of smallest fish which feeds more on small zooplankton. Similar cases were also recorded by several studies where the increasing size of prey was increasing with the body size (Jennings et al., 2002; Platell et al., 2007; Park et al., 2017).

Conclusion

The tiny scale barb, *Thynnichthys thynnoides* at Perak River can be classified as a phytoplankton feeder since the phytoplankton was the primary food items consumed by the fish. Studies on natural food and feeding habits of fish is a subject of continuous research as it constituted the basis for development of a successful fisheries management plan on fish conservation and protection.

Acknowledgement

This study was funded by the Fundamental Research Grant Scheme (No: 5524759) awarded by Ministry of Higher Education Malaysia (MOHE). The authors would like to acknowledge Mohammad Noor Amal Azmai and Intan Faraha A. Ghani for their advice and technical assistance from the locals during sampling.

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1.4 Nest selection by female Baya Weaver *Ploceus philippinus* based on nest architecture in Selangor, Malaysia

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Introduction

The Baya Weaver *Ploceus philippinus* is a member of the Ploceidae family found across South and Southeast Asia. Baya Weavers are gregarious birds that roost communally throughout the year. These birds are distributed from India, Sri Lanka to South China and some parts of Southeast Asia such as Peninsular Malaysia, Singapore, Sumatra, Java and Bali (Davison and Yeap, 2012). Among five subspecies of Baya Weaver, three are known to inhabit Southeast Asia, and one subspecies, *Ploceus philippinus infortunatus* has been recorded in Malaysia (Howard, Moore and Dickinson, 2003). A unique feature of these birds is their large bell or oblique shaped nest that hangs from the edge of tree branches built by the males. Nest selection is vital with respect to reproduction of birds because it determines the survivability of the chicks (Asokan, Ali & Nagarajan 2008). Female Baya Weavers inspect nests built by males to ensure sufficient conditions are available for chicks' growth (Mainwaring, Hartley, Lambrechts & Deeming, 2014).

While some birds build nests by concealing the nests from eyes of predators, the nests of Baya Weavers are especially large and can be easily spotted. However, the nest structure does not exclude predatory possibilities. Hence, weavers build their nests higher from the ground to avoid terrestrial predators (Collias, 2016). Within the nest structure, there are different stages of the nests that are built by the male birds, namely the wad stage, the ring stage, helmet stage, and finally the completed nest with an entrance tube (Asokan, Ali, & Nagarajan, 2008). If a female accepts a helmet stage of the structure which is also known as the incomplete nest, the male and female birds will then complete the nest, and egg laying may follow immediately after the flow of the brood chamber has been woven (Quader, 2003).

The indicator that a nest at a helmet stage has been abandoned or has not been chosen by female bird is when it is brown (Quader, 2005). Various aspects of the nest architecture was chosen for this study in order to examine the nest structure that contributed to a female Baya Weaver's choice in nesting preference. Since the female's choice is based on the structure of the helmet stage nest, attributes pertaining to helmet stage nests were examined in order to determine the pertinent structure that led to female Baya Weaver's choice to complete the nest. Even though these local birds are protected under the country's Wildlife Conservation Act 2010, little study thus far has been conducted locally to

understand the unique nest structure of Baya Weavers especially with respect to nest selection. This study aimed to identify the structures of a nest that are important for a female Baya Weaver's choice to complete the nest to commence reproduction.

Materials and methods

Study area

The samplings were conducted in various locations in the Selangor state namely Universiti Putra Malaysia (Serdang), Bangi, and Puchong where colonies of Baya Weaver nests can be found.

Data collection

The sampling was carried out from August 2018 until April 2019. A study on Baya Weaver's nest architecture was conducted by measuring the following nest structures of both complete (Figure 1) and incomplete stages (Figure 2), i.e. suspension length (cm), brood chamber (cm), nest depth (cm) and height from ground (m). The parameter measurements were taken according to the methods by Quader (2006b) and Quader (2005).

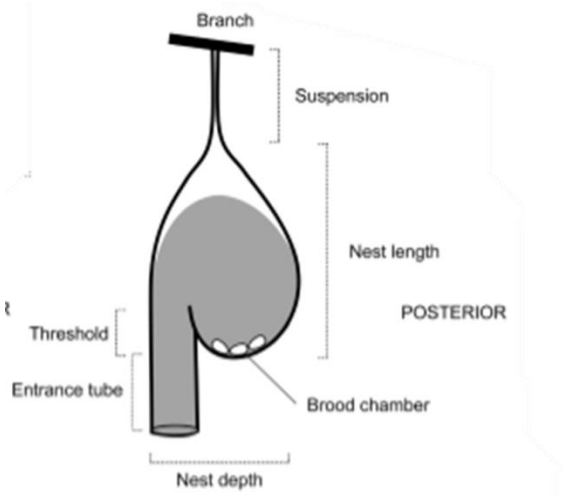

Figure 1: Nest structure of a complete nest (Quader, 2006b)

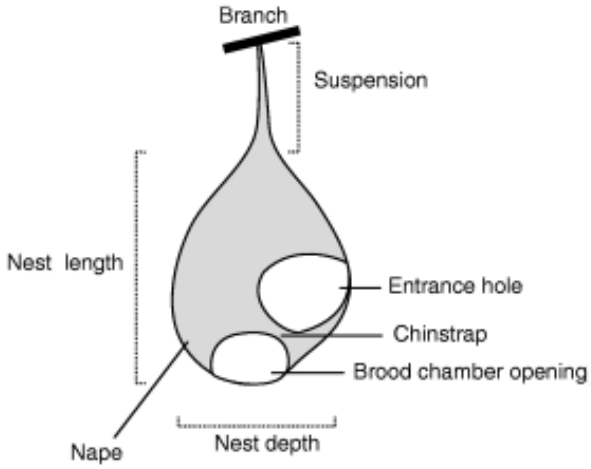

Figure 2: Nest structure of an incomplete nest (Quader, 2005)

One-way ANOVA test was performed using the SPSS software Windows version 23.0 to determine the differences in means of nest architecture variables measured between complete and incomplete nests (Ali, 2009).

Results and discussion

A total of 30 complete nests and 12 incomplete nests from 12 colonies and 11 trees from Selangor were recorded. The average values of all variables are as presented in Tables 1.

Table. 1: Mean values of measurements taken from complete and incomplete nests of Baya Weaver

Nests	Suspension (cm)	Nest Height (m)	Brood Chamber (cm)	Nest Depth (cm)	Branch Diameter (cm)
Complete Nest N = 30	31.0	3.5	11.1	13.8	4.4
Incomplete Nests N=12	26.7	3.3	8.0	18.3	2.4

The one-way Analysis of Variance (ANOVA) showed that the suspension length and height of nests from ground showed no significant difference ($p>0.05$) between complete and incomplete nests. However, for brood chamber nest depth and branch thickness, the analysis showed a significant difference ($p<0.05$) between complete and incomplete nests.

The size of brood chamber of all nests ranged from 7.0 to 15.6cm. As the average size of brood chamber in complete nests is higher than that in the incomplete nests (Tables 1), this implies that female Baya Weavers prefer a wider brood chamber which can be linked to the necessary internal climatic condition needed for incubation. As for nest depth, the range of nest depth for all nests was from 7.0 to 25.1cm. The significant difference in nest depths as well as the higher average nest depth among incomplete nests could be due to female Baya Weavers wanting a smaller nest as bigger and deeper nests are normally heavier and could easily fall down during strong winds or bad weather. The branch thickness for all nests ranged from 1.7 to 7.5cm. The significant difference in branch thickness between complete and incomplete nests showed that branch thickness plays a role in females' nest selection as nesting success is known to increase with branch thickness (Achegawe, Chavan, Patil & Tarte, 2016). Thicker branches provide a sturdy support for the large hanging nests during strong winds and prevents the nest suspension from being torn off the branch.

The range of nest height from ground for all nests was from 1.1 to 4.5cm. Though the results showed that the differences in height of nest from the ground were non-significant between complete and incomplete nests, past studies showed that the nest height has increased nesting success which is directly associated with fledging success. This is because higher nests have lower risks of predation (Quader, 2006; Achegawe et al., 2016). In this study, it is possible that for both complete and incomplete nests, the factor pertaining to nest height may have been confounded as most nests were constructed at certain height, and that there might be other parameters that play a stronger role in nest selection by females. The range of suspension length for all nests was from 8.3 to 57.5cm. Similar to nest height, the same could be said for suspension, and that all male Baya Weavers are capable of building the nest suspension of equal quality which does not contribute to the female Baya Weaver's choice.

Conclusion

In conclusion, the nest structures that contribute to the female Baya Weaver's choice in nest selection includes brood chamber, nest depth and branch thickness. This study came by with limitations such as not being able to access all nests in a tree especially those being built at unreachable heights. Further study is required to determine other parameters such as nesting location and climatic conditions to better understand the nest choice of the Baya Weaver.

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1.5 Distribution of mosquitoes in selected areas in Universiti Putra Malaysia emphasis on *Aedes* sp.

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Introduction

Dengue has created significant health and economic burden for individuals, societies and countries predominantly in tropical and subtropical regions. It is estimated that 390 million dengue infections and 96 million severe cases occur annually mainly among children (Bhatt et al., 2013). Dengue virus is transmitted through *Aedes aegypti* and *Aedes albopictus* (Gratz, 2004). Eradicating dengue vectors is the primary prevention and control measures to lower dengue infections in the absence of effective dengue vaccine (Chansang & Kittayapong, 2007). The World Health Organization (WHO) proposed entomological surveillance as part of global strategies for dengue prevention and control generally aimed at determining the density and geographical distribution of the vector and identifying the areas with high infestation density of vector (WHO, 2012). By knowing the species composition and the abundance of mosquitoes, it enables the assessment of disease risk to be performed well and provides guidance for the vector control operations (Bazin & Williams, 2018).

Mosquito trap is a trap system based on odour and heat that is being used extensively as one of the vector surveillance components (Bazin & Williams, 2018; Chaiphongpachara, Bunyuen, & Khlaeo Chansukh, 2018). There are various raps available for use, however, in deciding the types of trap to be used, factors such as cost, effectiveness and portability should be taken into consideration (Bazin & Williams, 2018). Ovitrap is a sensitive, cost-effective and passive trap used to sample the eggs and ovitrap that is modified with sticky surfaces can determine the presence of adult female (Mackay, Amador, & Barrera, 2013). A study performed by Hasnan et al. demonstrated that ovitrap is sensitive in detecting *Aedes aegypti* in Malaysia (Hasnan, Dom, Rosly, & Tiong, 2016). There is another sticky trap called MosquiTRAP designed specifically to capture gravid adult females for the purpose of identification (Gama, Silva, Silva, Resende, & Eiras, 2007). In Brazil, a cluster analysis study reported that MosquiTRAP gave better results in signalling the risk of dengue transmission both temporally and geographically (de Melo, Scherrer, & Eiras, 2012). In addition, light trap and electric trap designed with light sources are now widely used to trap insects; however, their application has been limited due to the dependence of electric power. The use of light traps may be useful as most species of mosquitoes are night insects where their eyes are sensitive to light and are attracted to light sources (Warrant & Dacke, 2011).

In this study, we have developed a few mosquito traps and have identified the effectiveness of traps in measuring the abundance of mosquitoes. Furthermore, we have determined the composition of the mosquito distribution using the most effective trap at the selected residential and non-residential areas.

Materials and methods

Study areas

Mosquito trap surveillance aimed to determine the distribution composition and mosquito species was conducted in the Universiti Putra Malaysia, Serdang campus, Selangor. Two types of study areas were selected within the campus: Serumpun College as residential area and Biology Department, Faculty of Science as non-residential area. In the present study, residential area refers to an area that provide facilities for people to live while non-residential area is an area consist of office and educational buildings.

Trap development

Vector surveillance was employed using four types of modified mosquito trap: ovitrap, MosquiTRAP, light trap and electric trap. A disposable plastic bottle was cut into two parts and the bottom part was used to make an ovitrap (Figure 1a). The ovitrap then was covered with black plastic to maximize the capture ability since the adult females prefer to lay their eggs in dark places (Farnesi, Barbosa, Araripe, & Bruno, 2018). Water is placed in the ovitrap to attract the gravid mosquitoes to lay eggs and provide moisture for the wooden paddle that serves as oviposition substrate.

The second type is MosquiTRAP, made of disposable transparent plastic bottle 2L. The bottle was cut into two parts, top and bottom. The bottom part is loaded with 200ml sugar yeast solution, a mixed of 280 g sugar, 5g of yeast and 1L of boiling water. The carbon dioxide (CO_2) produced from the yeast fermentation is an important component that serve as an attractant for the mosquitoes (Mathew, Ayyanar, Shanmugavelu, & Muthuswamy, 2013). The bottle cap of the top part was removed and some bristles were placed around the bottle neck to reduce the possibility of trapped mosquitoes to escape. The top part then was sealed with the bottom part in inverted position as shown in Figure 1b. Some space is left below the bottle neck to allow the mosquitoes hovering inside the bottle.

Next, light trap was developed which consists of power supply, blue light-emitting diode (LED) light, fan funnel and 6L bottle. Similar to previous trap, a 6L bottle was cut into two parts where a funnel that is attached to a fan was placed at the bottom while the blue LED light was attached to the top part. Blue colour is one of the colour spectrums that are

visible to mosquitoes (Ponlawat, Khongtak, Jaichapor, Pongsiri, & Evans, 2017). Then the top and bottom parts were assembled together (Figure 1c).
Lastly, an electric trap was developed, made up of a small device that consists of bug zapper and blue LED light (Figure 1d). The device was placed inside the 6L bottle to prevent it from being exposed to rain and it was connected to the wire and plug.

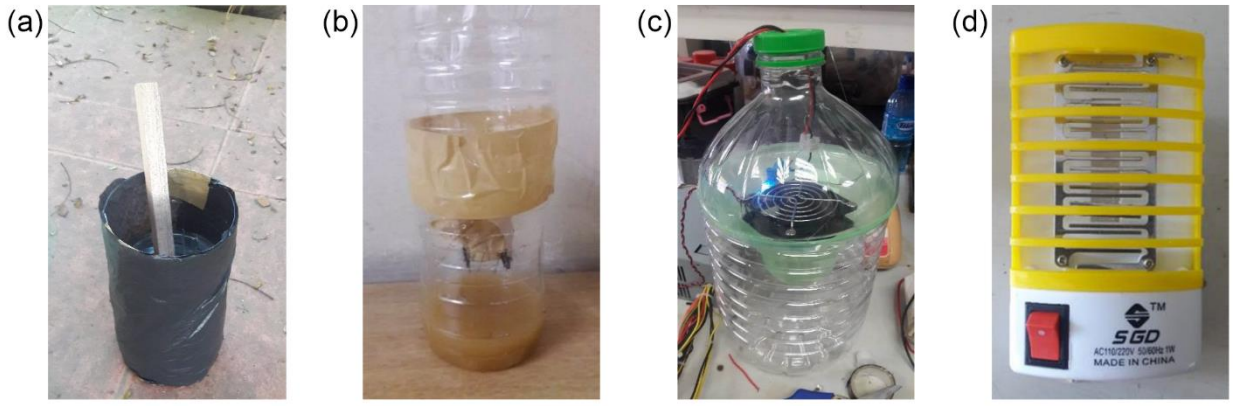

Figure 1: Different types of mosquito traps. (a) Ovitrap. (b) MosquiTRAP. (c) Light trap (d) Electric trap.

Trap setting

Four different traps were set in the same area and at the same time for 13 hours from evening until the next morning. This is because the infective biting by the mosquitoes occurs throughout the night and the peak time is after 10pm (Kabbale, Akol, Kaddu, Onapa, & Matovu, 2016). The number of mosquito larvae or adult mosquitoes was counted for each trap. The most effective trap was determined and was used for the next step.

Ovitrap setting

Ovitrap was determined as the most effective trap and was used to compare the composition of mosquitoes in two different areas. Six ovitraps were set at Serumpun College and another six ovitraps were located at the Biology Department for 5 days. The traps were placed randomly at shaded and partially shaded places to avoid from direct sunlight and heavy rain that may cause water spillage (Lim et al., 2010).

Mosquito collection, rearing and identification

The ovitraps were collected after 5 days and were brought back to the laboratory. The contents were poured into individual plastic containers filled with fresh water. The hatched larvae were reared until they become adults. Algae were added into each container as

larval food. After the pupae turned into the adults, the plastic container was put inside the box covered with net and the mosquitoes were killed inside the killing jar. Then, the mosquitoes were kept in a vial contains with 70% alcohol to preserve it. The number of larvae, pupae and adults were counted and recorded individually for each ovitrap. The adults were observed, and species identification was done using the stereomicroscope.

Statistical analysis

Descriptive statistics and independent t-test was conducted using SPSS version 23 to compare the mosquito abundance in the two different areas.

Results and discussion

Effectiveness of mosquito traps

Different types of traps were set at Serumpun College and Biology Department for 13 hours starting from 6pm to 7am the next morning. The larvae and adults collected from the traps were counted and recorded as shown in Table 1.

Table 1: The number of eggs and adult mosquitoes collected by four different types of traps.

Type of trap	Egg	Adult	Total
Ovitrap	4	-	4
MosquiTRAP	-	0	0
Light trap	-	0	0
Electric trap	-	1	1
Average temperature (°C)	34		

A total of 4 mosquitoes were collected from ovitraps, compared with 1 from electric traps and null from mosquiTRAP and light trap at the average temperature of 34°C. The results of the present study revealed that ovitrap had a higher trapping efficacy compared to others. According to Roslan, Ngui, Vythilingam, & Sulaiman, 2017, ovitrap are considered the most effective sampling tool to locate and detect the presence of adult mosquito population. Ovitrap is utilized with black colour plastic on the outside which is the highly attractive visual cues for the mosquitoes and can increase the trap efficacy (Hoel, Kline, & Allan, 2009). Although ovitrap recorded the highest number of mosquitoes collected, it has some limitation. First, the condition of local area may compete with the ovitrap and secondly, the

number of eggs deposited in the trap may be influenced by the skipping oviposition behavior of *Aedes* mosquito (Roslan et al., 2017).

Light traps were developed with blue LED lights, one of the colour spectrums that are visible to mosquitoes and are able to attract the mosquitoes (Ponlawat et al., 2017). However, the traps did not collect any mosquito in this study. According to Overgaard et al. (2012), the relative trapping efficiency of light traps are inconsistent across the seasons and location. Besides, low efficacy of light trap for some species of mosquitoes especially diurnally active mosquitoes do not respond well to light traps such as *Aedes* mosquitoes and this may influence the efficacy of light trap (Hoel et al., 2009).

In the present study, brown sugar and yeast solution was used as a source of CO_2 in the MosquiTRAP. Carbon dioxide has been shown as the most effective attractant to attract adult mosquitoes (McMeniman, Corfas, Matthews, Ritchie, & Vosshall, 2014). However, no mosquito were collected from the traps. Roey (2009) reported that yeast do not generate CO2 at a constant flow rate but at an infrequent rate and mammals release breaths in pulses and the alternations of CO_2 will attract the mosquitoes as well. This may be one of the factors which may affect the efficacy of MosquiTRAP. Besides, it was found that mosquitoes also respond to the non-olfactory cues as well such as body moisture and heat (Roey, 2009).

Another trap that is proven to be effective to catch mosquitoes are human-baited trap of human landing collection (HLC). However, this method is not appropriate and have serious ethical concerns because it exposes human to infected mosquito bites that may lead to serious disease (Hiwat, Andriessen, de Rijk, Koenraadt, & Takken, 2011). Moreover, according to Majambere et al. (2013), this method is difficult to supervise, labour intensive and requires a good and consistent skill in collecting the mosquitoes over long duration of time.

Mosquitoes population distribution

Six ovitraps were located at Biology Department and another six ovitraps were located at Serumpun College for five days. Different stage of mosquitoes including larvae, pupae and adults were found in the traps. The number of larvae, pupae and adults were counted, and the means were recorded in Table 2. The results show that Biology Department recorded the highest number of mosquitoes as compared to Serumpun Collage. Based on the observation, there are many of breeding grounds or stagnant water at Biology Department compared to Serumpun College that may be one of the factors that influence the abundance of mosquitoes.

Table 2: The mean and standard deviation of the number of larvae, pupae and adult mosquitoes collected at Serumpun College and Biology Department, UPM

Area	Mean number of Larvae	Mean number of Pupae	Mean number of Adult
Serumpun College	5.83±6.29	0.39±0.85	0.22±0.43
Biology Department	12.67±10.30	0.61±1.29	0.61±0.92

Mosquitoes species distribution

The larvae, pupae and adult mosquitoes that were collected from Biology Department and Serumpun College were observed under the stereomicroscope. Several species of adult mosquitoes that were identified which is shown in Table 3. There are only two genus of mosquitoes that were collected which are *Aedes* and *Culex*.

Table 3: Mosquito species distribution collected in Serumpun College and Biology Department, Universiti Putra Malaysia.

Mosquito species	Serumpun College	Biology department	Total (%)
Aedes aegypti	5	2	28
Aedes albopictus	4	2	24
Culex quinquefasciatus	0	4	16
Culex fuscocephala	3	4	28
Culex hutchinsoni	0	1	4

The species of the mosquitoes that were collected were *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, *Culex fuscocephala* and *Culex hutchinsoni*. At Serumpun College, the number of *Aedes* mosquitoes is greater compared to Biology Department. Meanwhile, *Culex* mosquitoes are more abundant in Biology Department compared to Serumpun College. According to Yee, Kneitel, and Juliano (2010), the hatching stimuli can affect the abundance of mosquito population. *Aedes* sp. lay eggs above the water line, so if the place where they hatch got flooded, the eggs will hatch. Meanwhile for *Culex* sp., they lay their eggs on the water's surface and this will cause their eggs to hatch faster compared to *Aedes* mosquito's eggs.

There are two types of Aedes sp. that were collected during the experiment which are *Aedes aegypti* and *Aedes albopictus*. *Aedes albopictus* is an aggressive feeder and able to breed in both natural and artificial container and this makes it more difficult to handle than any other mosquitoes (Hoel et al., 2009). The composition and abundance of mosquito

species are important to target disease prevention efforts because it provides information about the spatial distribution of vector species (Brown et al., 2008).

Conclusion

Ovitrap has been found as the most effective mosquito trap in this study. Ovitrap recorded highest number of mosquitoes collected compared to other traps. The advantages of using ovitrap is it is low cost and very practical to use. Ovitrap can be used as a mosquito surveillance tool to determine the mosquito population. This study also emphasizes the importance of using the best trapping options available for mosquito surveillance and control. From this study, Biology Department was found to have more number of mosquitoes compared to Serumpun College. However, Serumpun College had higher number of Aedes mosquitoes compared to Biology Department. This shows that residential area in UPM have low number of mosquitoes but the species that occupy the area is dangerous to humans. Meanwhile for non-residential area especially in Biology Department, the number of mosquitoes is higher, but the species is less dangerous as compared to residential area.

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1.6 Knowledge and awareness level of local community towards wildlife trade of Sumatran serow and Malayan porcupine in Perak, Malaysia

Siti Aisah Dahlan, Marina Mohd. Top @ Mohd. Tah and Shamarina Shohaimi

Introduction

Malaysia is acknowledged for their high number of biodiversity. However, many of the species are under threat currently. Most of these wildlife species are threatened and vulnerable where one of its factors is due to commercial wildlife trade in our country. This trade becomes more widespread because of high economic demand (Hinsley & Roberts, 2018). Therefore, many traders in this kind of market are taking a short cut to gain profit by involving themselves in 'black market' that is done illegally without a license (Symes et al., 2017). Black market which is also known as "underground economy" or "shadow economy" is defined as the informal trade sector that would be difficult to trace including smuggling, illegal cash transaction, and unlicensed gambling (Davidson et al., 2007; Goel et al., 2019). This caused a number of wildlife species such as Sumatran serow and Malayan porcupine that have high commercial value to decrease in numbers which could lead to their extinction in future. In relation to this, IUCN Red List reported by Lunde et al., in 2008 that Malayan porcupine is declining even though it is in the Least Concern status and there is no current population status reported until 2019. This is also the same for the Sumatran serow as there were no status reported from 2008 until 2019 on their population as they were also reported as declining even they were in Vulnerable status by IUCN Red List (Duckworth et al., 2008).

On January 2018, PERHILITAN had arrested two men in Alor Setar Kedah for keeping wildlife body parts illegally and their failure on showing special permits from PERHILITAN that would otherwise allow them to keep it. The parts that were believed to be Sumatran serow's head were placed in a plastic bag together with some oil. They also believed that this serow's head was being used for traditional dispensation and ointment (Malaysia Gazette, 2018). A similar case also occurred on Jun 2017 where two local men for kept serow's head illegally and were imprisoned for two years because they failed to pay a fine not less than RM100,000 (Malaysia Gazette, 2017). In Seremban, PERHILITAN managed to find two heads, 12 legs and a piece of serow's skin during their raid to a targeted house at Jalan Pengkalan Pauh, Lubok China. All of these body parts could cost almost RM100,000 in the market (Yahya, 2016).

Malayan porcupines are also not exempted from this illegal hunting threat even when PERHILITAN had approved 42 permits for commercial breeding (Ramli, 2015). This might be due to the wild porcupine meat that is believed by consumers to have higher quality and

nutrient than captive porcupines (Norsuhana et. al, 2012). According to Norsuhana et. al, (2012), their research stated that more than (55.6 %) of people that live in urban areas took alternative meat such as rabbit, deer, mousedeer, and also porcupine. Unfortunately, 54.7% of the consumers preferred to buy wild meats in which 33.3 % of the meats are obtained by hunters. They also stated that the Malayan porcupine had higher nutritional value than another alternatives meat that they eat (Norsuhana et. al, 2012). If this situation continuous to persist, the population of Malayan porcupine in the wild will increasingly decline due to the highest demand in the commercial market.

As this illegal hunting has become one of the main reasons for wildlife trafficking, the Ministry of Natural Resources and Environment and PERHILITAN has taken serious action to overcome this problem (Sharma, 2017). Perak is one of the states in Malaysia that has already taken this matter seriously and is committed to their aim which is to achieve zero poaching by 2020 (Sharma, 2017). As they were trying to achieve that goal, it is necessary to know the level of awareness and the attitude of community perceived in Perak especially for high commercial value animals such as Sumatran serow and Malayan porcupine. Hence, the community should also be aware and help the government at the same time. In concern to this problem, many researchers have studied the roles and threats of the high commercial value of wildlife in Malaysia, but only a few of them are focusing on human awareness itself. Some researchers also studied on the way to overcome this matter, but most of them were not focusing more on the attitude and practices that had been done by Malaysians regardless of their rank, age, and gender.

Therefore, this study is focusing on measuring the local community knowledge on wildlife trade of Sumatran Serow and Malayan Porcupine and determine the level of awareness of local communities in Perak towards the importance of wildlife and biodiversity.

Methodology

Study site

This project took place in the vicinity of Perak. The two cities involved in this study are Taiping and Ipoh. From both cities, a few villagers that participated were from the district of Matang and Ipoh Kinta. There were eight villages involved in this study that are Kampung Batu Tegoh, Kampung Jelapang Jaya, Taman Sri Larut, Kampung Manjoi, Kampung Tengku Hussein, Kampung Tersusun Sungai Tapah Seberang, Kampung Sungai Tapah Tambahan and Kampung Dato' Ahmad Said Tambahan 1 dan 3.

Research framework model

As can be seen below, biodiversity awareness is affected by people's knowledge, their attitude and their practice which refers to their action to conserve this biodiversity (Carrasco et al., 2017; Venuste et al., 2017; Walpole & Leader-Williams, 2002).

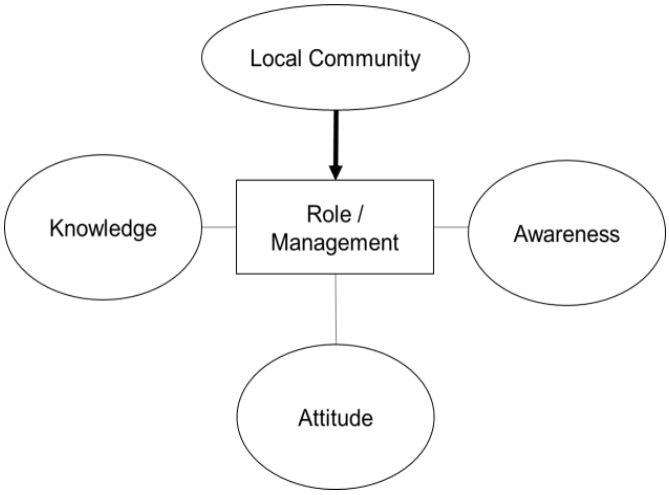

Figure 1: Research framework model of the local community role and management that could influence awareness on wildlife trade adapted and modified from Ajzen-Fishbein's Theory of Planned Behaviour (Ajzen, 1991).

The public plays an important role in conservation as they could give a hand to the government in conservation. In economic view, the public usually creates the highest demand for any trading of animals in terms of food, medicinal purposes, pet and production of accessories even if it would cost a lot of money (Pires & Moreto, 2016; Nielsen et al., 2017). This could bring profit to some party, but if this kind of business is not being controlled, it would lead to the illegal trade of some animals especially animals that are under threat (Reality & Trade, 2018). Here, the community should be supportive in knowing the source of the product they consume, use, and apply in their daily life. Other than that, the community can also help by reporting any illegal activities around them as soon as possible so that the culprits can be caught (English, 2000). They can also help in supporting and joining activities being held by NGOs and governments which can at the same time expand their knowledge in biodiversity conservation issues (Zhang et al., 2008). Therefore, the relationship between governments, NGOs, private sectors and the public are very crucial in managing the biodiversity around us.

Sampling technique

For this study, a cross-sectional study was used where the qualitative survey was conducted. Here, the sample was selected by using non-random sampling. Non-random sampling was used in case if the population was not well defined as the population size was not accurate (Author, 2012). Apart from that, this non-random sampling technique method is chosen because of the convenience and the cost in conducting this study which can be minimized as it was less expensive compared to random technique. On the other hand, by using this type of sampling technique, the data collection process could be implemented quickly (Bornstein et al., 2013) and was suitable for this study as there was a limited time in conducting this research.

Apart from that, the convenience sampling technique was used where the enumerators conduct the interview themselves by asking directly to the person (Zhang et al., 2008). This also can be considered a face-to-face interview and self-administered interview method (Shuib, 2012).

Target of population

The main target while conducting this survey activity is the local community that lives in Perak areas. As had been stated before, eight villages were chosen. In obtaining the estimated population information in these villages, the head of villages or head of the community was contacted to gain confirmation and also by referring to Buku Data Asas Negeri Perak 2014 ("Data Asas Negeri Perak Darul Ridzuan - Set Data - MAMPU," n.d.) Hereby, after obtaining the estimated population, Cochran's (1977) formula was used to acquire the number of the sample that needs to be taken (Bartlett, Kotrlik, & Higgins, 2001). Only 119 samples were needed to be taken, but 124 respondents participated in this survey.

Survey instrument

As part of the survey, the main tool that was used while conducting this survey is the questionnaire (Alsanoy, et al., 2014; Shuib, et al., 2012). It is the most important part of this research. This questionnaire is divided into eight sections adapted from Shuib et al. (2012), Williams et al. (2017) and Zhang et al. (2008). For section A, the questionnaire focused on the respondent's demographic background such as their gender, occupation, name, education level, age and their ethnic. For section B, the questions focused more on their knowledge on wildlife and wildlife trade. While for Section C and D, the respondent knowledge towards Sumatran serow and Malayan porcupine wildlife trade respectively was recorded. For section E, the questions were geared towards the local community's

awareness on wild animals and biodiversity conservation. As for section F, the questions designed were focusing more on wild animal management of the local community. In section G, attitude and roles of respondents in conserving wild animals was recorded. Then, in section H, the involvement of respondents in wild animal conservation activities was focused. The questions were constructed with the combination of structural question and by using Likert's Scale orientation (Venuste et al., 2017). A 5 points Likert's Scale was used to measure their response based on the Level of Agreement.

Data analysis

In analyzing data, the Statistical Package for Social Science (SPSS) software Version 22 was used. To obtain the result, descriptive analysis was conducted. Descriptive analysis was used to sum up the socio-demographic information that was obtained based on its frequency, percentage, mean and median.

Results and discussion

Reliability test

For this study, Cronbach's Alpha score for the questionnaire used was 0.98 that was acceptable to be used for further analysis. Cronbach's Alpha is known to measure the relationship between sets of questions and its consistency. For most social science research conditions such as this research, a reliability coefficient of 0.7 or more is considered acceptable and can be explained as closely related questions (Bruin, 2016). In this research, the reliability test was conducted by using SPSS Software.

Descriptive analysis for socio-demographic background

In this study, the respondents that participated were aged from 17 to 78 with the mean of respondents aged 44 years old. More male respondents (55.6%) participated in this survey compared to female respondents (43.5%) with a variance of 0.25. This might be because most of the study was conducted at the food stall around the study area where most of the customers were male. Other than that, most of the respondents were self-employed (36.3%) where there were a few of them running their own businesses. there were even respondents from the government (8.1%) and private sector (25.0%), though their field of work was not from wild animal conservation. At the same time, as for the unemployed respondents (26.6%), most of them were already retired and available at the moments of the survey. The respondents were also comprised of students (3.2%) who participated in this study. Most of them had previously migrated to other areas of Perak and shared some information on other places where wildlife trade has taken place before. As for their

educational level background most of the respondents had attended secondary school (54.0%) followed by respondents with higher educational institutions (23.4%), which they have received their certificate, diploma, and a degree. There were also some respondents that attended only primary school (16.1%) especially among the elders aged 60 to 78 years old. For respondents with lowest educational background are from others (4.8%) such as ILSAS-TNB Integrated Learning Solution and residents without any education.

Local communities' perception towards knowledge on wildlife trade and their level of awareness on the importance of wildlife towards biodiversity

In order to determine the level of knowledge and awareness that was perceived by the local community around Perak, a mean index score was calculated such as in Table 1 below. This questionnaire was measured by using Likert's scale, then the mean index showed their level of knowledge and awareness based on their consent to agree and disagree answer. Mean index of 1 would represent a very low awareness and knowledge level, while the mean index of 5 showed a very high level of awareness and knowledge.

The result below (Table 1) also shows that the local community in Perak has moderate knowledge (with a mean of 3.68) on the wildlife trade of Sumatran serow with a variance of 1.43. They also agree that Sumatran serow was sold in the market as end product such as ointment for medicinal purposes which is quite popular among the local community. Then, it also sells in forms of meat, horn and as well as the head. Apart from that, they also had moderate knowledge on wildlife trade of Malayan porcupine (mean index of 3.85) with a variance of 1.71. According to the local community, they knew that this Malayan porcupine was sold in forms of meat and spine. The local community also agreed that it is sold with the purpose of medicinal, collection and for food.

However, both of this species was captured through forest infringement by the sellers. As can be seen in Table 1, the local community had resulted in moderate awareness (3.65) on the importance of wildlife to biodiversity. Therefore, the result explained that the local community around Perak area agrees regarding this awareness issue and they were aware of the importance of this wildlife towards the ecosystem. At the same time, the local community also strongly agrees that wildlife trade has become a threat to wildlife and its conservation efforts. They are also aware that to hunt this wildlife such as Sumatran serow and Malayan porcupine, they should apply for a permit and license to do so.

Table 1: Mean index for awareness index and knowledge of the local community awareness.

Variable Index	Mean
Awareness of local community on the importance of wildlife towards biodiversity	3.65
Knowledge of local community on the wildlife trade of Sumatran serow	3.68
Knowledge of local community on the wildlife trade of Malayan porcupine	3.85

Conclusion

In conclusion, the local community in Perak showed that they know that the wildlife trade of Sumatran serow and Malayan porcupine in Perak are still conducted and some of them also had consumed and used the products sold by the seller and most of them were using it for medicinal purpose and for consumption purpose. For Malayan porcupine, they know that there were few sellers breeding this species with a license. Unfortunately, there were still some of them who accidentally caught the Malayan porcupine while trapping for other small mammals such as rats and squirrels but did not release their actions and ended up eating the wild animals. Besides that, this study reveals that the local community in Perak is aware of the importance of wildlife to our ecosystem. They are also aware of the existence of law enforcement that had been enacted by the government in order to protect these wildlife species.

However, awareness campaigns, courses and trainings need to be done by the authority such as governments and NGOs to increase the local community knowledge. Then, this could lead to raise of awareness to the public itself. At the same time, the authority also could share information such as hotline number that can be called if there is wildlife crime happen around the local community for example illegal wildlife trade and keeping of wildlife parts without permit. Therefore, cooperation between the local community and responsible agencies are needed in order to save our wildlife for the future.

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1.7 Spatial climatic heterogeneity on the occurrence of terrestrial birds in Peninsular Malaysia

Martins Chukwumeka Onwuka, Olaniyi Oluwatobi Emmanuel and Zakaria Mohamed

Introduction

In Malaysia, multiple lands use by humans have opened the way to substantial loss of wetland ecosystem, and shrinkage of the populations, habitat and food bases of avian species. However, the study of avian population becomes eminent to understand the complexity of wetlands ecosystem structure and also develop appropriate management with robust monitoring tools to ensure their ecological sustainability.

Researchers have also begun to explore the potential influence of climate change on bird populations (Both et al. 2010; DeLeon et al. 2011; Dybala et al 2013). Understand the habitat requirements of birds and successfully manage these species, researchers are dependent on standardized collection techniques. Hence, the study aims to determine the eco-climatic factors that influences the occurrence of terrestrial birds and to develop their habitat suitability model in Paya Indah Wetland.

Materials and methods

The study was conducted in Paya Indah Wetland which in the Malay language called as "beautiful swamp". The wetland reserve is made up of about 3050ha of lands out of which 450 ha are under the management of the Department of Wildlife and National Parks, Peninsular Malaysia. (Fig 1).

Distance sampling point count technique using stratified random design was employed to survey avian (from November 2016 to January 2019) from 57-point stations established around 14 lakes in Paya Indah wetland. An automatic linear modelling algorithm (ALMA) and geographic information systems were employed to compute the importance ratios of seventeen environmental factors (hydrology, climatic, waterscape, and landscape factors) (Fig 2).

Results and discussion

We also recorded 104,872 observations of terrestrial birds belonging to 71 bird species and 30 families using point-count distance sampling techniques in Putrajaya wetland. Most of the terrestrial birds are Least concern according to IUNC.

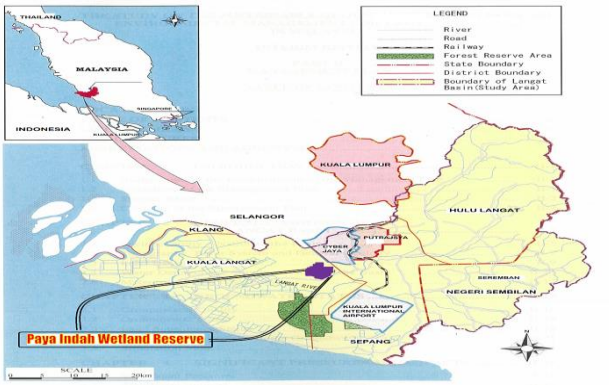

Figure 1: Location map of Paya Indah Wetlands Reserve, Peninsular Malaysia

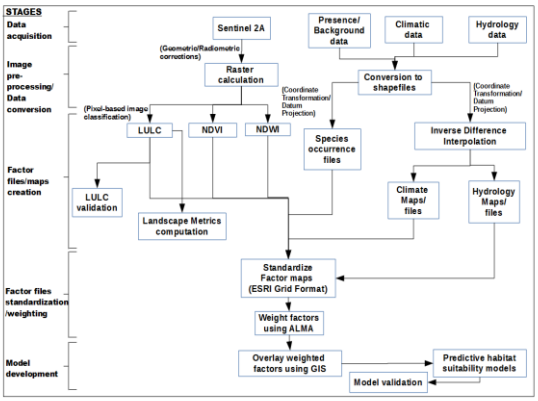

Figure 2: Framework for habitat suitability modelling of terrestrial birds in Paya Indah Wetland of Peninsular Malaysia.

Table 1: Diversity indices and densities of *Terrestrial Birds* in Paya Indah Peninsular Malaysia

Estimate	Terrestrial Birds Paya Indah Wetland
Observed birds' individuals	104,872
Shannon's diversity index(N)	7.25
Margalefs richness index(R)	132.50
Pielou's J evenness index(E)	0.92
Dominance	0.00

The Automatic linear modelling algorithm results also showed that WQI, Turbidity, salinity, pH, Pressure and Land use/land cover significantly contributed to the occurrence of terrestrial bird species in Paya Indah Wetland (Table 2). The wetland was moderately suitable for terrestrial birds (Figure 3 and Table 3).

Conclusion

The results of this study revealed that terrestrial birds had a rich diversity in Paya Indah Wetlands. This is due to the rich vegetation and suitable aquatic food present in the wetlands. In addition, the occurrence and richness of food resources such as fruits, seeds, insects (locus, moths, butterflies, crickets, flies, termites and beetles), nectar, reptiles (lizards, snakes), mammals (mice and rats), amphibians and birds is also a key factor that affects diversity and richness of bird species (van Heezik & Adams, 2016).

Factors	Paya Indah		
	Importance ratio	Weight (%)	Rank
Econd	0.000^{ns}	0.00	14
DO	0.033^{ns}	3.30	9
WQI	0.153*	15.30	2
Turbidity	0.252*	25.20	1
Temperature	0.000^{ns}	0.00	14
Salinity	0.100*	10.00	5
pH	0.090*	9.00	6
Minimum Depth	0.038^{ns}	3.80	8
Maximum Depth	0.059^{ns}	5.90	7
Relative humidity	0.004^{ns}	0.40	11
Rainfall	0.005^{ns}	0.50	10
Wind Speed	0.005^{ns}	0.50	10
Pressure	0.146*	14.60	3
Atmospheric Temperature	0.000^{ns}	0.00	14
NDWI	0.001^{ns}	0.10	13
NDVI	0.002^{ns}	0.20	12
LULC	0.112*	11.20	4

Table 2: Habitat suitability evaluation criteria importance judgment weights for terrestrial birds in Paya Indah wetlands

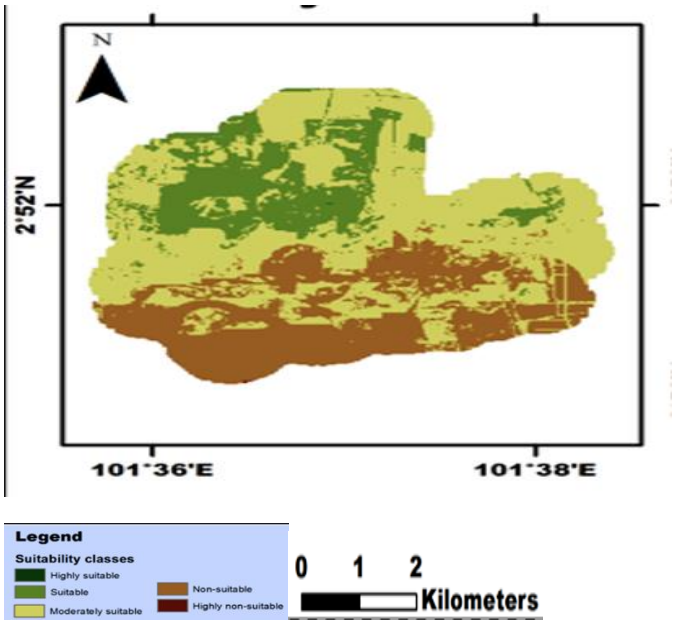

Figure 3: Habitat suitability models terrestrial birds in Paya Indah wetlands Peninsular Malaysia

Table 3: Attributes of habitat suitability models for Terrestrial in Paya Indah wetlands

Suitability classes	Area (Ha)	Proportion (%)
Highly suitable	0.10	0.01
Suitable	298.33	18.89
Moderately suitable	800.62	50.69
Non-suitable	480.25	30.40
Highly non-suitable	0.26	0.02
Total	**1,579.55**	**100.00**

This Model approach can be adopted as a management tool coupled with a robust population monitoring database to enhance management effectiveness of terrestrial bird species in the wetland. We recommend that a meteorological station should be established in this wetland in order to sample the microclimatic data such as rainfall, sunshine, relative humidity and wind speed in the area. These microclimatic data will be useful in future ecological studies.

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1.8 Canonical correspondence analysis of freshwater algae in a North Lake of Ayer Hitam Forest Reserve, Puchong, Selangor

N.N.F. Mat Lazim, S. Zulkifly, I.F. Uyob and N.S. Rosli

Introduction

Currently, environmental degradation and water pollution are environmental problems that can affect the water quality and algae diversity directly or indirectly in aquatic ecosystems. North Lake of Ayer Hitam Forest Reserve, is a freshwater lake located in Puchong Selangor. This lake is located approximately 20 kilometers from Universiti Putra Malaysia and is surrounded near to the housing area which is Kinrara Hills. The effluents discharged and the removal of wastewater into the North Lake from the nearby housing area may affect the water quality and diversity of wildlife habitat including other organisms such as algae in the lake. The study of the distribution and biodiversity of freshwater algae is important as they can be an indicator to assess water quality in the lake as well as to provide information about the current status of algae species in Malaysia and the potential for robust species to be used in the field of biotechnology.

Materials and methods

Sample collection and physico-chemical analysis

Triplicate water samples were collected at three different sites for weekly sampling in November 2013 until February 2014 by using a phytoplankton net. The physical analysis of water was measured, *in situ* by using YSI Multi-parameter probe while chemical analysis of nutrients was analysed in laboratory.

Identification, enumeration of algae and data analysis

The identification of algae species was made based on their morphological characteristics by observing under the microscope and the information from Algaebase website. The enumeration of algae was carried out by using a Sedgwick- Rafter counting chamber. Canonical correspondence analysis (CCA) was performed to determine the correlation between algae species and environmental variables in the North Lake by using Microsoft Excel Stat.

Results and discussion

There were 13 of algae species belonging to 5 phyla identified in this study as shown in Table 1 and Figure 1 showed the image of some species present in the lake. In the present study, Ochrophyta was the dominant group and among all species present,

Gonyaulax apiculata was the most abundant species in the North Lake. The diversity of algae community was different in each site and during the sampling period due to the differences of environmental variables availability in the lake in each site. The environmental variables were fluctuated during the sampling period caused by weather condition and might affect the composition of algae in the lake.

Table 1: List of freshwater algae species in North Lake of Ayer Hitam Forest Reserve, Puchong from November 2013 until February 2014.

Phylum	**Species**
Ochrophyta	*Dinobryon sertularia* *Navicula* sp. *Pinnularia* sp. *Frustulia* sp. *Gomphonema* sp.
Dinophyta	*Gymnodinium palustre* *Gonyaulax apiculata* *Massartia musei*
Charophyta	*Cylindrocystis brebissonii* *Micrasterias radiate*
Cyanophyta	*Holopedium irregulare* *Anabaena subcylindrical*
Chlorophyta	*Pandorina morum*

Figure 1: Some of species present in the North Lake Ayer Hitam, Puchong; A) *Navicula* sp., B) *Dinobryon sertularia*, C) *Anabaena subcylindrica*.

From the CCA ordination triplot (Figure 2), a pattern of the seasons, associations of the physicochemical properties and algae species list could be seen. One is the wet season sampling (November and December) and another is the dry season sampling (January and February). For the dry season sampling, Ochrophyta, are most associated with dissolved oxygen. Dissolved oxygen creates a niche for the diatoms for their biological productivity. Ochrophyta (diatoms) are also associated with silica, which they need for their cell growth. *Navicula* sp. was found to be present in all months of sampling. They required silica in the lake for their cell growth as they have a unique structure of the silicified cell wall. In freshwater environment, the diversity of pennate diatoms was diverse and can be found frequently (Basavaraja, Narayana, Puttaiah & Prakash, 2013).

Cyanophyta are positively correlated with temperature. *Anabaena subcylindrica* became dominant on February 2014 at all sites as the temperature was high in this month and a green surface layer can be seen in the lake which indicated their abundance. Cyanophyta can survive in variety of habitat and conditions such as in extreme, strongly alkaline lakes, support high metal concentrations, freezing environments and arid desserts (Rampelotto, 2013).

The high temperature and also silica are associated with the presence of Charophyta species in North Lake. The optimal growth temperature for the desmids species corresponds to a range of 25°C to 30°C. Some Charophyta also requires silica for their cell wall. *Cylindrocystis brebissonii* was found to be abundant compared to *Micrasterias radiata*. The optimal growth temperature for the desmids species corresponds to a range of 25°C to 30°C (Coesel & Wardenaar, 1990). High number of desmids species were obtained when there was a high value of temperature in the lake that range between 28.51°C to 29.21°C on January and February 2014.

For the wet season sampling, Dinophyta are positively correlated with the phosphate and nitrate. The fluctuation of these nutrients might influence species in this phylum which have a role to limit the growth of most algae species in the lake (Naqqiuddin, Kader, Muhamad, Nor, Shohaimi, & Zulkifly, 2017)

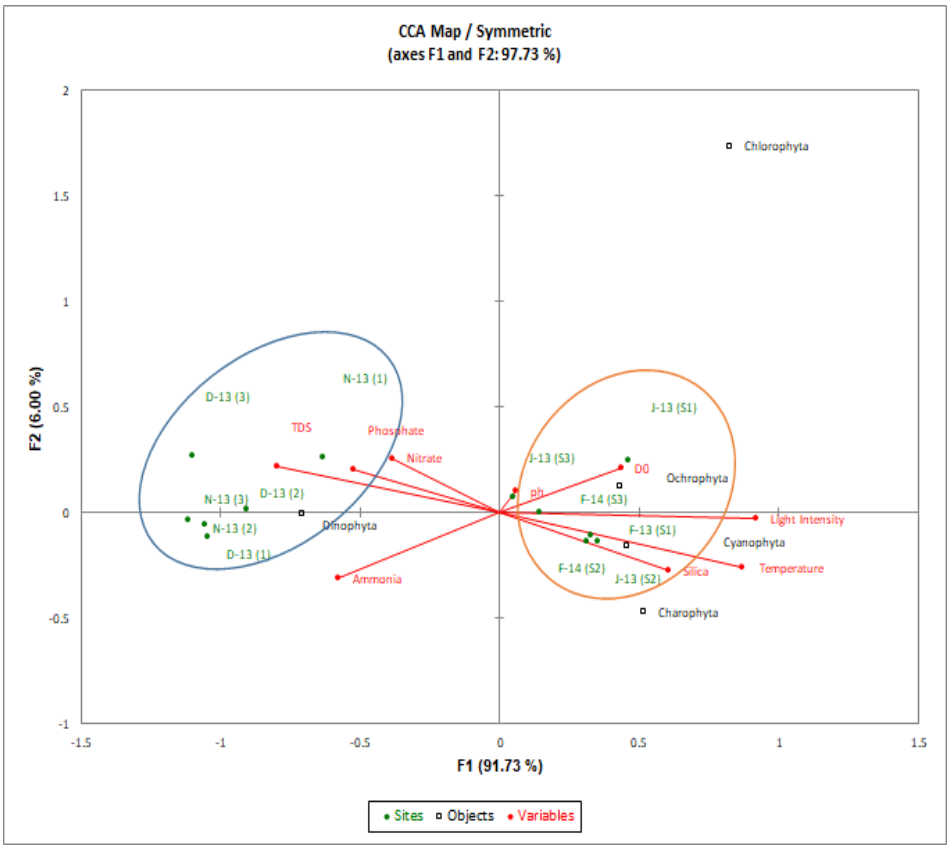

Figure 2: The ordination triplot for canonical correspondence analysis (CCA).

Conclusion

The study findings indicated that the variation of algae species in North Lake of Hutan Simpan Ayer Hitam, Puchong. As the pH level of the lake was below than 7, the lake was in acidic condition and slightly polluted. This was revealed by the presence of some species, including *Dinobryon sertularia* (Ochrophyta) which preferred to survive in basic and acidic water. This information would be useful for the public to evaluate the use of the lake as a water source for consumption and recreational use.

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1.9 Toxicity effects of nanomaterial (Quantum dot) following sub-acute and sub-chronic exposure to zebrafish

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Introduction

Quantum Dot (QD) is a nanomaterial that nowadays has been widely used in various advanced applications such as biological imaging, food science applications and electronic applications (Bonilla et al., 2016). QD exhibited many interesting properties including high photostability, narrow emission wavelength, strong luminescence intensity and broad excitation window (Yan & Chen, 2014). Interestingly, QD is also easy to prepare and modify in which by only altering its cap and size, different desirable properties of QD can be produced (Hoshino et al., 2004). Despite of the advantages, according to Hardman (2006), QD can bring harm through variety of exposure routes. Additionally, different types of cores and caps of the QD apparently can lead to different type of toxicity (Hoshino et al., 2004). The production of QD has greatly risen yearly, yet, the research elucidating the adverse effects of QD is still lacking. Thus, in this present study, we evaluated the toxicity effects of QD by assessing the morphological and behavioral changes as well as biochemical alterations using adult zebrafish as a model organism. According to McGrath and Li (2008), zebrafish nowadays is increasingly being use for drug toxicity assessment due to its small size, low maintenance cost, easily bred and single female can produce 100-200 eggs. In addition to this, drug administration to zebrafish also can be done easily as their skin and gills can absorb small molecules in the water. Importantly, zebrafish toxicity profiles are strikingly similar to the mammalian with the organs and tissues have also been shown to resemble mammals (McGrath et al., 2008).

Materials and methods

Random sex wild-type adult zebrafish were purchased from local pet shop and acclimatized accordingly with standard laboratory care procedure. The fish also were fed four times a day with live feed (*Artemia salina*) and commercial dry flakes (*Sera Vipan*) alternately. QD was obtained as a kind gift from Dr. Che Azurahanim Che Abdullah from Department of Physics, Faculty of Science, Universiti Putra Malaysia (UPM). Random sex adult zebrafish were divided into two different groups of exposure; sub-chronic (received media renewal every 24 hours) and sub-acute (no media renewal throughout 96 hours of exposure). The fish were exposed to four different concentrations of QD including control (0, 50, 100, 200 µg/L) for 96 hours in a tank with diameter 23.5 cm length X 11.5 cm width X 12.5 cm height. Both groups were dispersed with actual insoluble form of QD. Each group of exposure received five fish with all exposures were duplicated and repeated for three times (n=10). Following the

exposure, morphological and behavioral changes were observed every 24 hours until 96 hours of the exposure duration. Total percentage of mortality rate, changes in body shape, eye and fin structures, body discoloration and internal bleeding exerted by the fish were recorded. In addition, erratic swimming and swimming speed were manually observed to confirm the locomotor abnormalities. All changes were compared with the respective controls. In addition, biochemical assessment of the zebrafish gills was conducted via Fourier Transform Infrared Spectroscopy (FTIR). After the exposure, survived zebrafish with selected concentration (50 µg/L) were dissected to obtain the gills. The gills were then fixed with 4% paraformaldehyde (PFA) for 24 hour. The gills sample were rinsed three times and freeze dried. Dried samples were grounded using agate mortar and pestle to obtain its powder. 100 mg potassium bromide was mixed with the powder. Mixture was subjected to a pressure of 5t in an evacuated die for 5 minute. Clear KBr obtained were used for FTIR. Measurement of spectre were done using Thermo Nicolet Nexus, Smart Orbit Spectrometer based on KBr disc method (Bakar et al., 2017).

Results and discussion

Based on the experiment, we found that QD produced toxicity effects on adult zebrafish in which QD exposed fish exhibited morphological changes including changes in the body shape, eye structure, fin structure as well as the presence of internal bleeding in both exposure condition. In addition, the mortality rate recorded were high with 200 µg/L cause 100% mortality rate in both exposure conditions (Table 1). The tremendous mortality rate and severe morphological impairments exerted by the exposed fish had caused the anxiety-like behavioural assessment to be aborted from the experiment.

Table 1: Morphological abnormalities exerted by exposed fish following the exposure to QD.

Mode of exposure	Concentrations (µg/L)		0	50	100	200
Sub-chronic	Morphological abnormalities	Mortality rate (%)	0	90	100	100
		Average body mass (g)	0.43	0.40	0.00	0.00
		Body shape	n	n	y	y
		Eyes structure	n	n	n	y
		Fin structure	n	y	y	y
		Body discoloration	n	y	n	y
		Internal bleeding	n	y	y	y
	Behavioral abnormalities	Erratic swimming	n	y	n	n
		Swimming speed	n	y	n	n
Sub-acute	Morphological abnormalities	Mortality rate (%)	0	0	60	100
		Average body mass (g)	0.45	0.45	0.43	0.40
		Body shape	n	y	y	y
		Eyes structure	n	n	y	n
		Fin structure	n	y	y	y
		Body discoloration	n	n	n	n

	Internal bleeding	n	n	n	n
Behavioral abnormalities	Erratic swimming	n	y	y	y
	Swimming speed	n	y	y	y

Note: y indicating the presence of the selected endpoints, while n indicating the absence of selected endpoints.

In agreement with our result, a study by King-Haiden et al., (2009) showed that QD induced mortality rate and morphological alterations in both adult and developing zebrafish accordingly with the concentrations. QD possed nanocrystalline core of cadmium selenium (CdSe) that can cause adverse effects to the organisms and environment (Leigh et al., 2012). Moreover, besides Cd, Se itself also can contributed to the malformations of the organism (King-Haiden et al., 2009).

Additionally, QD also changed the biochemical constituents of the zebrafish gills exposed with 50 µg/L QD (Figure 1).

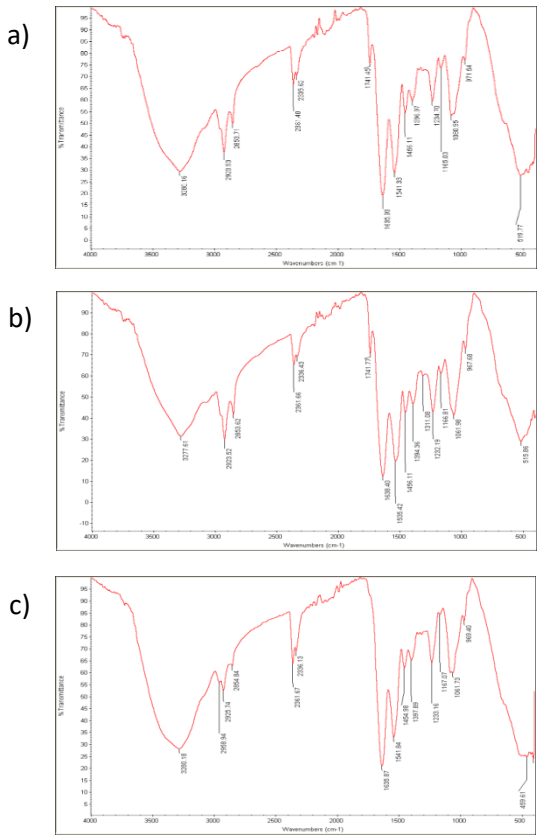

Figure 1: Average representative of FTIR spectre of normal, sub-chronic and sub-acute exposed zebrafish respectively.

In general, sub-acute exposure produced less alterations as compared to sub-chronic exposure. QD caused alterations to the peak positions and transmission intensity (Figure 1a and 1b). Moreover, existence of the newly recognized peaks and disappearance of existed peaks indicated damage of the particular constituents (Figure 1a and 1b). The most prominent effects observed in the gills include changes of the lipid, protein, glycogen and carbohydrate as well as nucleic acid.

Theoretically, the prominent effect should be seen in the gills as it is a crucial target of waterbone objects like nanoparticles. Alterations of the macromolecules (protein, lipid, glycogen and carbohydrate) may be explained by the deposition of nanomaterials on the epidermis layer which tends to occur via diffusion. Besides, accumulation of nanomaterials on the skin can lead to diffusion into the tissues, which then resulted in the impairments of the biochemical constituents on the organ (Chen et al., 2011).

Conclusion

QD resulted in distinctive adverse effects on the zebrafish in a dose dependent manner. Additionally, sub-chronic exposure produces higher toxicity compared to sub-acute exposure. Taken all together, exposure to QD both sub-chronic and sub-acute induce morphological alterations and disrupt the biochemical constituents of the selected organ.

Acknowledgement

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1.10 Anuran Inventory of The Montane Forest in Cameron Highlands, Pahang, Malaysia

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Introduction

Cameron Highlands is located on Banjaran Titiwangsa range in central of Peninsular Malaysia. This hill district encompasses an area of 712.18 km^2, covers the boundaries of state of Kelantan on the north and state of Perak on the west respectively. Cameron Highlands is the smallest district in the state of Pahang is known for cold weather, tea plantations, and its highest peak of Mt. Brinchang (2031 metres) (Gasim et al., 2009; Kumaran & Ainuddin, 2006).

Herpetofaunal surveys in Cameron Highlands begin in 19th century by Boulenger (1912), followed by Smith (1930), Smedley (1931), Berry (1975), Vogel and David (1999), Leong et al. (2003), Sanders et al. (2004), revised taxonomy of geckos by Kumaran and Ainuddin (2006) and Grismer (2008), lastly, herpetofaunal diversity by Norhayati et al. (2011) with 15 species of amphibians and six species of reptiles were recorded.

Highlands hosts to great diversity of herpetological fauna (Matsui & Ibrahim, 2006; Norsham & Lim, 2002) and Cameron Highlands is in the list. Although studies have been made for number of years, however, the gap between years were too long and continuous inventory and surveys are still required to monitor species composition and diversity (Norhayati et al., 2011). Therefore, this study aimed an updated of the species richness and anuran inventory within the forest area.

Materials and method

Study area

The sampling was conducted in a secondary forest of Terla A-1 and Terla B-1 in Cameron Highlands. This forest reserve is located at 2°59'34 N and 101°42'45 E, 4°34'42 N and 101°22'30 E with elevation of 1500 and 1300 m above sea level. The study area is a lower montane forest, also known as an oak-laurel forest which is dominated by Fagaceae and Lauraceae flora families. Average temperature recorded is 18°C with mean minimum and maximum temperature of 15°C and 22°C respectively. Total annual rainfall in Cameron Highlands is 2000 mm to more than 3000 mm (Kumaran & Ainuddin, 2006; Chan, Suritati & Norizan, 2006; Leong, 2006).

Frog Sampling

The sampling was carried out nocturnally, 19:30 to 22:30 from June 2018 until February 2019. Anuran collections were conducted by Visual Encounter Survey (VES) (Crump & Scott, 1994) and Call Survey (Zimmerman, 1994). Each collection was caught by using bare hands or plastic container with the help of handheld or head torchlight. During the survey, five people surveyed a transect of at least 400 m long along a stream or a forest trail. The criteria of the study sites were identified by the accessibility and the presence of water bodies. The information such as microhabitat and location of each sample were recorded. The measurements of the specimens include the snout-vent

length (SVL) and tibia length (TL) which were measured by using vernier caliper. The weight of the specimens were also taken by using spring balance. Photographs were taken for each species for identification. Identification process was done using reference from Berry (1975) and Norhayati (2017). Each individual was tagged using paint marking technique and was released back to its habitat (Neitfeld, Barrett, & Silvy, 1994; Muhammad Faris et al., 2016).

Statistical Analysis

The Shannon-Wiener Index for diversity analysis was generated by using MVSP software version 3.1.

Results and discussion

A total of 71 individuals of anurans belonging to 13 species were recorded. The 13 species comprised of five families namely Dicroglossidae (five species), Megophryidae (one species), Microhylidae (one species), Ranidae (two species), and Rhacophoridae (four species). *Rhacophorus bipunctatus* from the family Rhacophoridae was found the highest in the study with 52.11%, followed by *Pulchrana banjarana* (12.68%) from the family Ranidae (Table 1). According to Norhayati et al. (2011), Ranidae and Rhacophoridae were recorded to be the most abundant during a survey in the forest of Cameron Highlands. This is because Ranidae was often documented compared to other families as the low temperature in the highland provides a conducive habitat, contributing to the higher records of Rhacophoridae family (Norhayati et al., 2011). Table 2 shows that Terla B-1 (H'= 1.67, E= 0.86) has higher species diversity and evenness compared to Terla A-1 (H'= 0.95, E= 0.49). This showed that the habitat in Terla B-1 may be preferred by some species while in Terla A-1 has lower species diversity due to human disturbance and also limitation in accessibility of sampling area.

Table 1: The anuran species recorded in Cameron Highlands, Pahang and their IUCN status.

No	Species	Common name	Total	IUCN Status
	Dicroglossidae			
1.	*Fejervarya cancrivora*	Crab-Eating Frog	1 (1.41)	Least Concern
2.	*Fejervarya limnocharis*	Asian Grass Frog	1 (1.41)	Least Concern
3.	*Limnonectes deinodon*	Corrugated Frog	1 (1.41)	Least Concern
4.	*Limnonectes malesianus*	Malesian Frog	1 (1.41)	Near Threatened
5.	*Limnonectes nitidus*	Tanah Rata Wart Frog	5 (7.04)	Endangered
	Megophryidae			
6.	*Xenophrys longipes*	Long-legged Horned Frog	5 (7.04)	Near Threatened
	Microhylidae			

7.	*Microhyla annectens*	Larut Hills Rice Frog	6 (8.45)	Vulnerable
	Ranidae			
8.	*Odorrana hosii*	Poisonous Rock Frog	1 (1.41)	Least Concern
				Near Threatened
	Rhacophoridae			
10.	*Philautus petersi*	Peter's Bush Frog	1 (1.41)	Least Concern
11.	*Polypedates leucomystax*	Common Tree Frog	1 (1.41)	Least Concern
12.	*Rhacophorus bipunctatus*	Twin-Spotted Flying Frog	37 (52.11)	Least Concern
13.	*Rhacophorus prominanus*	Malayan Flying Frog	2 (2.82)	Least Concern
Total individuals			71 (100)	

Table 2: Species diversity and evenness in Cameron Highlands, Pahang.

Location	**Terla A-1**	**Terla B-1**
Shannon-Weiner Diversity Index, H'	0.95	1.67
Evenness, E	0.49	0.86

Based on 'The International Union for Conservation of Nature (IUCN)', three species were identified under 'Near Threatened' (NT) namely *Limnonectes malesianus*, *Pulchrana banjarana*, and *Xenophrys longipes*; one species was identified under 'Endangered' (EN) which was *Limnonectes nitidus*; and one species was identified as 'Vulnerable' (VU) which was *Microhyla annectens*. All of the species except *Limnonectes malesianus* were highland species. Highland species has limited distribution depending particularly on the altitude range and vegetation cover. This is added with challenges such as climate change, lack of food and habitat (Kumaran & Ainuddin, 2006). The highland species are more vulnerable to such problems which may be contribute to the species decline.

Conclusion

From the survey, it is clear that Cameron Highlands hosts various species of anurans and the status of few anuran species recorded were very alarming. It is imperative to note that the highland ecosystem must be conserved and more research on anuran should be conducted to take note on the species diversity, endemism, and their true diversity (Grismer & Pan, 2008).

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CHAPTER 2: ANIMAL PHYSIOLOGY

2.1 The effect of lactation number and lactation stage on the lactation of crossbred Sahiwal-Friesian cows in selected dairy cattle farm of Sabah

N. Navin Kumar, Mohamad Zaihan Bin Zailan and Nur Hardy Abu Daud

Introduction

Lactation is defined as the process of the production of milk. This process normally occurs in all mammalian species (Boniface *et al.,* 2007). Milk is the nutritious food product made by the lactating animals and it is rich in carbohydrates, protein, fats, vitamins and minerals (Blowey and Edmondson, 2000; Sinha, 2000). Milk is synthesized by the secretory cells in the mammary glands of the mammalian species. For the first four days post calving, the milk that was produced by milking cow is known as colostrum and it is used to feed the newborn calves. Milk production rises until peak production for about 35-50 days after calving and the most ideal lactation period is 305 days or about 10 months. Milk production per lactation will increase until the fourth lactation or when the cows are at the age of six years old, this is when the cows were calved at the age of two. When the cows reach the age of eight years old, the milk production will decrease. Some of the studies suggested that the highest milk production is when the cows are at the age of seven (Boniface *et al.*, 2007). A report by Epaphras *et al.* (2004) stated that most milk producers understand that the milk production fluctuates up and down from one lactation to the next.

About one third of the world's cattle population are found in the tropics and they are of the *Bos indicus* types. The *Bos indicus* cattle generally have a lower performance than the European breeds of cattle, *Bos taurus* in the temperate regions. This can be due to poor management, low plan of nutrition, diseases and parasites and low genetic potential. The performance of the *Bos taurus* cattle in the tropical environment is also far from satisfaction level as they are affected by the effect of tropical diseases, poor nutrition and climatic stress. However, *Bos taurus* cattle play an important role in the genetic improvement of the local cattle by crossbreeding. The progeny produced by the crossbreeding between the *Bos taurus* and *Bos indicus* cattle has shown superior performances than their parents. The crossbreds did not only have higher milk yield, but their calves produced have heavier birth weights and animals with the potential for faster weight gain (Boniface *et al.*, 2007). According to a report by Malaysian Livestock Breeding Policy (DVS, 2013), the dairy industry development project was started in the early 1970's in Sabah with the crossbreeding program between local Zebu cattle and imported Friesian cattle using the artificial insemination technique. In 1976, the dairy breeding herds were established at the Sebrang Livestock Breed stations in Keningau and Tawau. In 1980, the initial importation of Sahiwal-Friesian heifers from New

Zealand was made. This project involved the small land holders with the purpose to form the dairy industry and hence, to increase the urban residents' income in conjunction of the New Economic Foundation (Salleh, 1989).

According to a report by Ahmad *et al.* (2011), it was stated that milk and milk products are well known to form a vital balanced diet and almost a complete food for human consumption and there isn't a single food which can replace milk (Eckles *et al.*, 1951). Although milk is an important source of diet for humans, however the production of milk is not at a satisfactory level even though Malaysia have a huge number of milking cows and cattle populations. A report by The Star (2019) has stated based on the Malaysia agricultural sector report that the local liquid milk production covers only about 5% of the domestic needs. With rise in industrialisation and education, this has led to a more health-conscious population and a rise in the demand for dairy products.

A number of factors have been reported to affect the production of milk in the tropics and they may include the genetic, climatic, diseases, feeding, year of calving and managerial factors (Payne and Wilson, 1999; Msanga *et al.*, 2000). Animal factors such as breed, age, lactation stage, parity and milking frequency have also been reported in other studies (Tekerli *et al.,* 2000; Johnson *et al.,* 2002). Milk yield and quality is significantly affected by the lactation (Ahmad *et al.,* 2011). With the advances in the lactation number, the milk yield of the cows rises gradually. The lactation stage does also significantly affect the milk yield. The milk yield decreases as the lactation stage advances. Several researches other than this was conducted all around the world to study on the effect of lactation number and lactation stages on the milk yield of dairy cattle. However, very few researches had been done in our country regarding this topic. Hence, the present study was undertaken to investigate the effect of lactation number and lactation stage on the lactation of the crossbred Sahiwal-Friesian cows at a selected dairy cattle farm in Sabah, to evaluate the relationship between milk yield with lactation number and lactation stage and to investigate the effect of lactation number on the peak yield, time to peak and peak holding period of the crossbred Sahiwal-Friesian cows at the selected dairy cattle farm of Sabah.

Methodology

The pilot study was carried out at the Yun Fook Resources farm, a dairy farm located in Keningau, Sabah. The farm is located approximately 4.40 km from Keningau town. Yun Food Resoures farm is situated at 5°19'46.2'' North of Equator and 116°11'37.9'' East of Greenwich. The climate of the study site was characterized by the hot and humid weather. The average temperature of the farm area was approximately about 26℃ and the average humidity level was about 65％. The farm was located 300 meters above sea level and it lies on a highland area of Keningau, Sabah.

Currently the farm has dairy cattle of the Sahiwal-Friesian crossbreeds (5194), dairy goats (356) and deers (224). The lactating cows were supplemented with mixture of concentrates consisting of soy bean powders, palm kernel cake, corn and minerals. The concentrate mixture was provided at 10.5 kg per head of cow. Concentrates were only provided to the cows before each milking sessions. Napier grasses were provided to the cows *ad libitum* after each milking sessions. All lactating cows were milked using the automated milking system (AMS) for two times a day, the first session at 4 a.m. and the second session at 4 p.m. Milking was done in the milking parlour.

Data was collected based on the slight modifications of the method by Bondan *et al.* (2018). Daily milk production data of the cows were recorded by the DelPro 5.2.1 software. During this study, the records of the milk yield for 100 lactating dairy cows on the farm were used for the study. Data used were for the cows of 1-220 days in milk (DIM). All animals included in this study were the Sahiwal-Friesian crossbreed cows, with a body condition score (BSC) of 2.75 to 3. Stage of lactation was classified into three categories according to days in milk (DIM): 1 to 60 (early), 61 to 120 (middle) and 121 to 220 (late). 25 cows were selected based on their DIM for each of the lactation number groups (first lactation, second lactation, third lactation and fourth lactation).

Average daily milk yield was measured in liters/cow/day and recorded into Microsoft Excel program. From the daily milk yield data, the averages of the peak yield, time to peak and peak holding period was determined. Peak yield is the point of milk yield, in which the cow reaches the highest milk production level during the entire lactation. Peak yield was measured as liters/cow. Time to peak was defined as the number of days taken for a cow to reach the peak yield and was measured as days/cow. The peak holding period was defined as the period in which the highest milk production was maintained in the entire lactation and it was measured as days/cow.

For the statistical analysis, One-way Analysis of Variance (ANOVA) was used to interpret the average daily milk yield, peak yield, time to peak and peak holding period data. Meanwhile, Two-way ANOVA was used to interpret the interaction between the effect of lactation number and the lactation stage on the average daily milk yield. This analysis was performed using SAS version 9.4 for Windows program software. Results are expressed as the mean ± standard error of the mean. Comparison between the means were evaluated using *Duncan's Multiple Range Test (DMRT)*. A probability of less than 0.05 was considered significance ($p<0.05$) for the average daily milk yield, peak yield, time to peak and peak holding period data analysis. Meanwhile, a probability of less than 0.0001 was considered significance ($p<0.0001$) for the interaction effect between the lactation number and lactation stage data analysis.

Results and discussion

Milk yield, peak yield, time to peak and peak holding period at different lactation numbers

Table 1: Mean (± standard error) of milk yield, peak yield, time to peak and peak holding period of crossbred Sahiwal-Friesian cows with different lactation numbers.

Lactation number	Milk yield for 220 days (liters/cow/day)	Peak yield (liters/cow)	Time to peak (days/cow)	Peak holding period (days/cow)
1	14.11±0.32^{ab}	30.85±0.92^{b}	52±5^{a}	60±3^{a}
2	14.57±0.29^{a}	34.01±1.93^{ab}	42±3^{ab}	45±5^{b}
3	14.88±0.27^{a}	37.25±1.77^{a}	36±4^{bc}	40±5^{b}
4	13.14±0.50^{b}	37.35±2.10^{a}	28±3^{c}	35±4^{b}

*Different letters among lactation number indicate significant differences (P<0.05).

The average daily milk yield, peak yield, time to peak and peak holding period of Sahiwal-Friesian cows at different lactation numbers was shown in Table 1. It was found that the average daily milk yield of Sahiwal-Friesian cows at the 1st, 2nd, 3rd and 4th lactation was 14.11±0.32, 14.57±0.29, 14.88±0.27 and 13.14±0.50 liters/cow/day, respectively. Statistical analysis showed that there was a significant difference ($p<0.05$), within the daily milk yield of different lactation for crossbred Sahiwal-Friesian cows. Milk yield increased gradually from first to third lactation. The highest milk yield was recorded in the third lactation cows (14.88±0.27 liters/cow/day) and lowest was in the fourth lactation cows (13.14±0.50 liters/cow/day).

A report by Epaphras *et al.* (2004) stated that the lactation number or parity was positively associated with the milk yield, supporting the results of the current study. Johnson *et al.* (2002) reported that the capacity to produce milk by the older cows were higher than the younger cows in association with their greater feed intake. However, cows in the fourth lactations or more were no longer better milk producers than the cows in the third lactation. The older age of the cows may cause the decrease in the milk produced by the turnover rate of the secretory cells, with higher number of cells dying compared to the active secretory cells that are newly produced. Fat tissue cells normally substitute the dead secretory cells. Not only that, the results reported by Vijayakumar *et al.* (2017) is in support with the findings of the current study. The increase in the milk yield with the lactation number is maybe due to the increasing development and size of the udder with an increase in the secretory cells (Sorensen *et al.,* 2006). Vijayakumar *et al.* (2017) have also stated that the lower milk yield in first lactation cows is due to the early lactation of cows are not yet in the productive stage but remain in the growing stage with their mammary gland and mammary vein are not well developed at this

stage. As the current study have shown that the highest milk yield is in the third lactation cows. Thus, cows with more than the third lactation have exhibited the tendency for total milk yield to decrease. Ray *et al.* (1992) have reported that as lactation number rises, the total milk yield increases but at a decreasing rate with lactation number. From Table 1, we can say that the cows with more than three lactations are not good candidates for maximizing milk yield with the automatic milking system.

The peak yield of the Sahiwal-Friesian cows at the 1st, 2nd, 3rd and 4th lactation was 30.85±0.92, 34.01±1.93, 37.25±1.77 and 37.35±2.10 liters/cow, respectively. This shows that with the increase in the lactation number, the peak yield of the Sahiwal-Friesian cows also increases. Thus, the highest peak yield was produced by the cows in the third and fourth lactation. This can be explained that the peak yield produced by the third and fourth lactation cows were highest due to the well-developed mammary gland of the cows. Meanwhile, the time to peak of the Sahiwal-Friesian cows at the 1st, 2nd, 3rd and 4th lactation was 52±5, 42±3, 36±4 and 28±3 days/cow, respectively.

The current study results showed that the cows of the fourth lactation have reached their peak yield much earlier than the other lactation cows. This is due to the reason that the fourth lactation cows have well developed mammary glands with secretory cells that would allow the production of more milk at the earlier days of the lactation cycle of the cows. The first lactation cows were found to reach their peak yield later than other lactation cows as this was caused by the mammary gland and mammary vein were not well developed and thus, it takes some time for more milk to be produced and hence, causing the cows to reach their peak yield at a later time.

Besides that, it was found that the cows of all four lactations have reached their peak yield at the earlier stage of lactation (1-60 days) and this can be supported by the finding of Vijayakumar *et al.* (2017), in which the highest peak of milk yield was obtained in the early stage (1-100 days). This can be explained by the effect of the changes of hormones causing the deterioration of the mammary gland, nutrient requirement of the fetus and insufficient nutrition for milk production. Next, it was found that the peak holding period of the cows decreases as the lactation number increases. The cows of the first lactation have the longest peak holding period (60±3 days/cow) compared to the fourth lactation cows that have the shortest peak holding period (35±4 days/cow). This is due to the cows of the fourth lactation have less secretory cells than the first lactation cows during the peak holding period as higher number of cells died compared to the active secretory cells that are newly produced when the age of the cows increase (Bondan *et al.,* 2018).

Effect of lactation stage on milk yield

Table 2: Means (± standard error) of milk yield at different stages of lactation of crossbred Sahiwal-Friesian cows.

Lactation stage (days)	Milk yield (litres/cow/day)
1-60	14.59±0.34[a]
61-120	14.46±0.37[a]
121-220	13.22±0.24[b]

*Different letters among stages indicate significant differences (P<0.05).

The main predictor of milk yield was the lactation stage. The average milk yield at different stages of lactation of the crossbred Sahiwal-Friesian cows are presented in Table 2. The average milk yield of crossbred Sahiwal-Friesian cows at early, middle and later stages of lactation were 14.59±0.34, 14.46±0.37 and 13.22±0.24 litres/cow/day, respectively. The results revealed that lactation stage had a significant effect (P<0.05) on the average milk yield. The results indicated that the cows in the early and middle stage had the highest milk yield. This is because the highest peak of milk yield of the cows was obtained at the early and middle lactation stage and remains high for a while and then as the lactation progresses, the milk yield gradually decreases. This result are reasoning can be supported by the findings of Vijayakumar *et al.* (2017), in which it was reported that the results are due to the hormonal changes causing the deterioration of the mammary gland, nutrient required by the fetus and the lack of nutrition for milk production.

Figure 1 below shows the average milk yield of the first, second, third and fourth lactation of the crossbred Sahiwal-Friesian dairy cows at early, middle and late stages. The results of the current study showed that the average milk yield of the first, second, third and fourth lactation of the dairy cows at early, middle and late stages were 14.43, 14.99, 13.21; 15.06, 14.52, 13.60; 15.81, 15.66, 13.87; and 13.15, 12.69, 12.21 litres/cow/day, respectively. The current study results were found in supportive with the results of the studies by Sekerden (2002), in which it was stated that the milk yield decreases at the late stage of lactation. However, the results of the current study were not supportive to Sekerden (2002) study, at which in the current study highest yield was observed in the early stage instead of the middle stage of lactation. This difference can be due to the better feeding management and the genetics of the cows used for the current study. Based on the statistical analysis conducted, it was found that there were no significant interaction effects between the lactation numbers and lactation stages on the milk yield of the crossbred Sahiwal-Friesian cows (P=0.9148, P>0.0001).

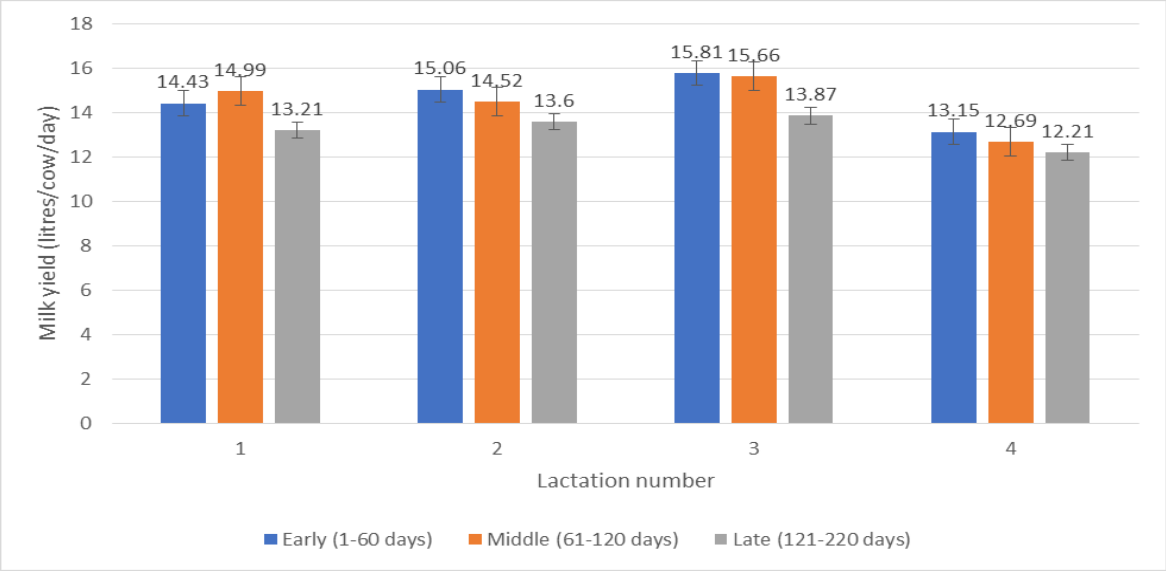

Figure 1: Average milk yield of crossbred Sahiwal-Friesian cows at different lactation numbers and lactation stages.

Conclusion

Lactation number and stage of lactation does play a role in the lactation of the crossbred Sahiwal-Friesian cows. However, it was found that the lactation number and lactation stages have no significant interaction effects on the milk yield of the cows. Meanwhile, the lactation numbers of the cows have significant effects on the milk yield, peak yield, time to peak and peak holding period of the cows. Besides, the different lactation stages of the cows significantly affect the milk production of the cows. Overall, by ranking, cows of the third lactation was the most productive. Cows in the early and middle lactation stage were the most productive. Overall, the production of cows had not reached the level of satisfaction as it is a need to improve the quality of nutrition of the individual cows. With the lactation trends obtained from the current study, farmers will be able to identify the weakness and strong point for the improvement plan and the other future undertakings of the farm. Farmers must be encouraged to keep continuous record of the daily milk yield and construct the lactation curve especially with the peak milk production based on the cows different lactation numbers (age or parity of cows) and lactation stages. I would like to recommend that further studies regarding this topic should be conducted in Malaysia such as the milk production of different crossbred cows.

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2.2 Evaluation of baseline diagnostic lethal concentration for adult A*edes albopictus* in Kuantan, Pahang

Ilma Syawati Osman, Norazsida Ramli, Hamdan Ahmad, Abu Hassan Shaari Abd Kadir, Jaiza Jaafar and Fatin Mardhiah Ramli

Introduction

Dengue is a vector borne disease transmitted from infected female mosquitoes. Principal vector for dengue virus in Malaysia is *Aedes aegypti* due to the preference human habitat and takes multiple blood biting. However, *Aedes albopictus* is a suburban mosquito also documented as sole vector where *Ae. aegypti* was not present or had a reduced (Rezza 2012; Peng et al. 2012; Kasai et al. 2011; and Kyle Jl and Harris GE, 2008). Moreover, Norafikah et al. 2019 found the existence of *Ae. albopictus* almost in all study sites located in Peninsular Malaysia compared to *aegypti.* Transition to secondary vector has greatly increased the chances of dengue transmission through close contact to human. At present, there is no definite antiviral therapeutics to treat dengue, nor an effective vaccine to prevent infection. Tetravalent dengue vaccine (Dengvaxia) developed by Sanofi Pasteur was still in evaluation as many complications arise (WHO, 2017). Currently, the authorization relied heavily on vector control method to reduce transmission of virus either in larvae or adult stage. Fogging is rapid approach to curb the transmission once dengue case notified. However, frequent insecticide exposure will develop insecticide resistance in mosquito (Regan D et al. 2016). Insecticide resistance is a major risk to the future elimination of infected mosquito (IRAC 2011). To date, the discriminating dose of insecticide for respective species of mosquito was adhered to standard WHO which applied in worldwide generally to kill the infected vector. In this study, we aimed to establish diagnostic doses of Deltamethrin as the alternative chemical control tool based on localities in Kuantan, Pahang.

Materials and methods

Mosquito collection

Aedes albopictus and *aedes aegypti* samples collection for laboratory strain were initiated in November 2018. All wild-caught immature stages of both species were brought to the insectarium at Jalan Gambut, Kuantan which was under Vector Control Unit of Jabatan Kesihatan Negeri Pahang. The samples were reared from egg to adulthood under standard insectarium conditions recommended by the World Health Organisation (WHO) (WHO, 2009). The insectarium were maintained at temperature 28 ± 2°C, room humidity 80 ± 10% and photoperiod of 12h day/night. The larvae were fed daily on mosquito larval food made of fine powdered beef liver. Upon pupation, all wild populations from each site were separated into vials and species identified after adult emergence on the basis of morphological characteristics (Rattanarithikul et al, 2010).

Adult were provided with 10% honey solution using soaked cotton wicks and were allowed to mate. Susceptible strain of *aedes* mosquito has been maintained more than F2 generation to ensure free from any insecticide resistance.

Susceptibility of mosquitoes toward deltamethrin

25 non-blood-fed female mosquitoes introduced into each of the holding tube and held for one hour for acclimatization. Each test consisted of 5 replicates with insecticide exposure and 2 for control. Then, susceptible strains of mosquitoes were transferred gently into exposure tube lining with impregnated insecticide paper. The tubes placed vertically for one-hour exposure of insecticide and the knockdown rate was recorded every 5 minutes.

At the end of exposure, the mosquitoes were blown back into holding tube and placed vertically for 24h with glucose solution on cotton wool. The mortality rate of mosquitoes was counted after 24h. Each test was valid when the mortality of control tube met below than 5% under standard condition. The serial dilution of deltamethrin was varying from 0.005% to 0.06% (5 concentrations). The methodology adhered to guideline provided by WHO (WHO, 2016) and the impregnated insecticide paper were prepared by USM Penang. Probit analysis determined the LC_{50} and LC_{99} values and twice amount of LC_{99} established the diagnostic concentration to be used.

Results and discussion

The susceptibility of the laboratory inbred strain was confirmed by 100% mortality of *Ae. albopictus* against standard dosage of insecticide. According to WHO (2016), susceptibility to deltamethrin is indicated if the mortality between 98% - 100% while resistance is suggested if the mortality is less than 98%. Table 1 showed the result of percentage mortality of *albopictus* exposed to several type of insecticide.

Based on the result obtained, almost all the *albopictus* mosquitoes were susceptible to insecticide. For this study, *albopictus* strain at F2 generation can be used as a reference for laboratory strain since the susceptibility towards different type of insecticide was higher. Deltamethrin and pemethrin are pyrethroid group of insecticide while primiphos-methryl is an organophosphate group. Variable group of insecticide act different mode of action against mosquito. Most current insecticides act on nerve and muscle targets (IRAC). Organophosphate inhibits AChE which causing hyperexcitation while pyrethroid group keeps sodium channel open. *Albopictus* strain was free from any insecticide resistance where the sample was collected in rural area far away from agriculture that have lower tendency of pesticide exposure.

Table 1: Susceptibility of laboratory strain towards variable of insecticide

Type of insecticide	Standard concentration (%)	No. of female mosquito	Replication	No. of dead	Mortality %
Deltamethrin	0.05	100	5	100	100
Pemethrin	0.75	101	5	101	100
Primiphos-methyl	0.21	115	5	114	99.13

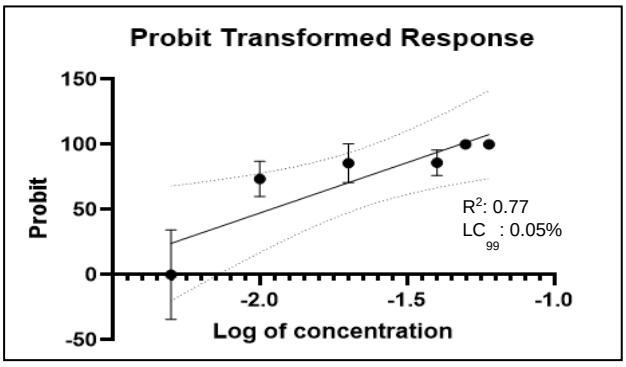

Figure 1: Probit analysis for a serial dilution of deltamethrin against *albopictus* mosquitoes in Kuantan.

Albopictus strain has been verified as susceptible strain towards insecticide and tested to a serial dilution of Deltamethrin (0.06%, 0,04%, 0.02% 0.01% and 0.005%). Probit analysis obtained 0.05% as the LC_{99} for *albopictus* towards Deltamethrin (Graph 1). Diagnostic concentration set at twice the amount of minimum concentration to kill more than 99% mosquitoes. Currently, standard discriminating concentration used for deltamethrin against *aedes* mosquito is 0.05%. However, this concentration might not be applicable or reliable for all mosquito vector species. Then, the new baseline diagnostic concentration was measured at 0.1% which was higher than standard dosage endorsed by WHO. It can be deduced that the level of resistance mosquitoes is higher based on specific location in Kuantan. Similarly, a recent study carried out in Thailand established a higher concentration of pemethrin and deltamethrin for their resistance monitoring programme of *Ae. albopictus* (Thanispong et al. 2015). The study used different diagnostic concentration than the one recommended by WHO. This happen because there are different geographical structures that enable different life cycle and behavior of vector. The weather also contributes to different traits of vector.

Conclusion

Dengue vector control strategies are depending on the use of chemical insecticides. An established laboratory strain of *albopictus* is required to be used as the reference for susceptible strain. The increased number in generation of mosquito offspring has lower tendency towards insecticide resistance. In addition, condition and human activity at the collected sample will induce the resistance in mosquito. Routine for resistance monitoring is part of an effective intervention to reduce dengue transmission. The

findings in this research can be proceed for wild strains mosquito collected from different localities in Kuantan to detect the resistance level. However, it is undeniable that survival rate of adult mosquito is quite short and requires lengthy of time to maintain many adult female msoquitoes for sample collection. A proper plan and management should be optimized to produce a stable colonization of mosquito in insectarium.

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2.3 Physichochemical properties of Khaki Campbell, Muscovy and Pekin duck feet gelatin extracted with acetic acid

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Introduction

Gelatin is a high molecular weight polypeptides with water soluble properties that was derived from collagen by thermal treatment hydrolysis (Bailey & Light., 1989; Kuan et al., 2016). The major source of gelatin production in Europe is 60% from pigs and another 40% of gelatin production are from bones and skin of cattle and other animal (Aykin-Dinçer et al., 2017). Nevertheless, the market for non-porcine and non-bovine gelatin has been increased recently by Kosher, Muslims and Vegetarian regarding the halal food issue and occurrence of diseases from bovine such as Bovine Spongiform Encephalopathy (BSE) and food and mouth diseases (FMD) (Karim & Bhat., 2009; Samsudin et al., 2018). Hence, another alternatives to gelatin is from mammalian-derived source such as avian sources for example from duck feet (Kuan et al., 2016).

Poultry intake per capita per year in Asia has raised by 30.5% which is from 6.6 kg to 9.5 kg with Malaysia reached 36 kg per capita per year (Supriatna, 2016; Wahyono & Utami, 2018). There was 0.8% increment of duck livestock population in Malaysia from 9.2 million to 10 million in 2017 and 2018 respectively (DVS, 2018). The increment in livestock production will also increase the waste production from duck. Hence, the duck feet can be turned into something beneficial such as gelatin from natural resources. This study focused on production of gelatin from acid pretreatment and comparison of physicochemical properties between three breeds of duck, Khaki Campbell, Pekin and Muscovy with commercial bovine gelatin.

Materials and methods

The Pekin duck feet was purchased from Perak Duck Food Industries Sdn Bhd, Khaki Campbell duck feet was purchased from 4R Agro Enterprise, Pasir Mas, Kelantan and Muscovy duck feet was purchased from duck farm at Kota Bharu, Kelantan. The raw materials were transported under refrigerated condition to Muscle Lab, Faculty Bioresources and Food Industry, University Sultan Zainal Abidin and stored at -18°C prior to use. Commercial bovine gelatin (CBG) was purchased from Halagel (M) Sdn Bhd, Sungai Petani, Kedah Darul Aman. Chemicals and reagents that were used were hydrochloric acid (J.T Baker, Center Valley, PA), citric acid (Bendosen, Batu Caves, Selangor), 1000 Kjeltabs Cu/3,5 (Foss Analytical A/S, Hillerod, Denmark), sodium hydroxide (Merck, Darmstadt, Germany), sulfuric acid (Merck, Darmstadt, Germany), boric acid (HmbG, Eschborn, Germany).

Gelatin extraction was done by following method by (Kuan et al., 2017). Frozen duck feet was thawed overnight in a refrigerator at 4–5 °C. The duck feet then was grounded into small pieces and soaked in acetic acid (4.0%) for 16 hours. The raw materials were rinsed with running tap water until they reached pH 5.5, and then subjected to hot extraction for 12 hours. The liquid was sieved and then filtered. The filtered liquid was oven dried at 50 °C for 12 hours. Lastly, the gelatin sheets was grounded and store at ambient temperature. The percentage of the gelatin yield was obtained by measuring the weight of gelatin powder (Nik Muhammad et al., 2018). The protein content of gelatin was determined according to AOAC (2005). The protein content was determined by Kjeldahl method AOAC (2005) with a protein factor of 5.55 to convert the nitrogen value to gelatin protein. The pH of gel is determined at room temperature (27 ± 1^{o}C) by preparing solution at concentration of 6.67% by mixing 6.67 g of gelatin powder into 100 ml distilled water (Samsudin et al., 2018). The bloom strength of gelatin are determined using texture analyser TA.XT plus (stable Micro Systems). 6.67 g of gelatin was mixed with 100 ml distilled water in the bloom jar to produce 6.67% (w/v) gelatin solution to determine the bloom strength (Mhd Sarbon et al., 2013). Gelatin sample was subjected to FTIR analysis using an ATR-FTIR spectrometer, Shimadzu IR-Prestige-21 (Ahmad & Benjakul, 2011). The data were analyzed using statistical one-way analysis of variance (ANOVA) followed by Tukey test for mean comparison of Statistical Package for Social Science version 25 (SPSS inc, Chicago, Illinois, U.S.A). Significant different was define at $p < 0.05$.

Results and discussion

Yield and protein content

The yield and protein content of commercial bovine gelatin (CBG), Khaki Campbell duck feet gelatin (KCDFG), Muscovy duck feet gelatin (MDFG) and Pekin duck feet gelatin (PDFG) were shown at Table 1. The yield of duck feet gelatin extracted from the three different breeds of duck were significantly different at ($p < 0.05$). The gelatin yield was calculated as grams of dry gelatin per 100 g of raw duck feet with PDFG has the highest yield (1.91%) followed by KCDFG (1.65%) and lastly MDFG (1.40%). Nik Aisyah et al. (2014) reported that yield of Pekin Duck Feet Gelatin that was treated with 0.1M acetic acid was 2.48%. Meanwhile, in another study, (Mokhtar et al., 2017) reported that yield of chicken feet that was treated with acetic acid and enzyme papain is 12.63%.The differences percentage obtained in gelatin were causes by age of animals, species, degree of cross linking in the raw material, collagen content, proximate composition, and different method of extraction (Widyasari & Rawdkuen, 2014; Abedinia et al., 2017). Low yield percentage might be happen due to the various way of extraction method that can affect the properties of collagen extracted and different value of yield (Silva et al., 2014; Mokhtar et al., 2017).The loss of extracted collagen in the pretreatment process through leaching that happen during washing or incomplete

hydrolysis of collagen are factors that contribute to lower yield of gelatin (Mhd Sarbon et al., 2013; Widyasari & Rawdkuen, 2014).

The gelatin sample showed high value of proteins with low moisture content value that range around 10% to 11%. Consumable gelatin is prescribed to has moisture content value that less than 15% (GME, 2005; Abedinia et al., 2017). Besides that, a low moisture content gelatin can prevent gelatin from become sticky and also prolonged the shelf life of the gelatin (Mohd Nazri & Syed Khair Azlan Jamalulail, 2012; Widyasari & Rawdkuen, 2014). Duck Feet Gelatin has a protein content value around 66.03% to 73.39% with Muscovy Duck Feet Gelatin (MDFG) has the highest amount and Khaki Campbell Feet Gelatin (KCDFG) has the lowest amount among the three breeds. There were significantly difference of protein value between Commercial Bovine Gelatin (CBG), Khaki Campbell Feet Gelatin (KCDFG), Muscovy Duck Feet Gelatin (MDFG) and Pekin Duck Feet Gelatin (PDFG). Widyasari & Rawdkuen. (2014) reported that the protein content of chicken deboner residue extracted with hydrochloric acid is 85.67%. Meanwhile, in another study (Abedinia et al., 2017) reported that the protein content of duck feet gelatin that was extracted with 0.05M acetic acid is 81.38% that is higher than this finding. Low protein content of gelatin sample is caused by termination of high hydrogen bonds and the excess opening of collagen structure which can cause amino acid extracted and separated from collagen and carried away by washing process (Samsudin et al., 2018).

Table 4: Yield and protein content of commercial bovine gelatin (CBG) and four different species of duck feet gelatin (DFG)

Sample	Yield	Protein
CBG	-	87.64 ± 0.57^{d}
KCDFG	1.65 ± 0.05^{b}	66.03 ± 0.18^{a}
MDFG	1.40 ± 0.03^{a}	73.39 ± 0.28^{c}
PDFG	1.91 ± 0.04^{c}	67.87 ± 0.13^{b}

Means with different superscript letters within the same columns are significantly different at $p < 0.05$.

pH and Bloom strength

The pH and Bloom Strength of commercial bovine gelatin (CBG), Khaki Campbell duck feet gelatin (KCDFG), Muscovy duck feet gelatin (MDFG) and Pekin duck feet gelatin (PDFG) were shown at Table 2. pH value of gelatin that using acid as pretreatment process must be within range 4.5 to 6.5 according to Gelatin Manufactures Institute of America (GMIA) standard 2013 (Samsudin et al., 2018). pH value of the extracted DFG ranged from 5.54 to 6.25 which were acidic with the value of CBG close to the value of PDFG indicating they are type B gelatin (Aykin-Dinçer et al., 2017). There was no significant difference of pH value between CBG, KCDFG and MDFG but significantly

different with PDFG. MDFG has the highest pH value, 6.25 followed by KCDFG, 6.18 and PDFG 5.54. Chemical type and strength that was used during extraction process affected the pH value of the gelatin (Songchotikunpan et al., 2008; Widyasari & Rawdkuen, 2014). Samsudin et al. (2018) reported that the effectiveness of washing chemical from raw material before doing extraction process can produce a higher pH value gelatin.

The gel strength of the gelatin can be categorized into three which are high (< 200), medium (120-200) and low (> 120) bloom (Rafieian & Keramat, 2015). MDFG has the highest bloom value compare with other DFG and CBG. The gel strength of CBG and PDFG can be classified as medium gel value meanwhile MDFG is categorized under high gel value and KCDFG fall under low gel value. The difference of gel strength between different types of gelatin possibly due to difference in amino acid content and difference in protein chain composition (Kuan et al., 2017).The gel strength of duck feet gelatin might be related with the degree of protein degradation that happened during swelling and various heating process (Park et al., 2013). Gel strength is a function of complex interaction determined by amino acid composition and the a-chain and the amount of b-complex (Lassoued et al., 2014). The lower value of gel strength are due to the lower proline (Pro) and hydroxyproline (Hyp) content (Rafieian & Keramat, 2015).

Table 5 : pH and Bloom Strength of commercial bovine gelatin (CBG) and four different species of duck feet gelatin (DFG)

Sample	pH	Bloom Strength
CBG	5.97 ± 0.02^{b}	150.71 ± 0.52^{c}
KCDFG	6.18 ± 0.00^{b}	63.78 ± 0.15^{a}
MDFG	6.25 ± 0.09^{b}	285.05 ± 0.00^{d}
PDFG	5.54 ± 0.28^{a}	139.87 ± 0.00^{b}

Means with different superscript letters within the same columns are significantly different at $p < 0.05$.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of commercial bovine gelatin (CBG), Khaki Campbell duck feet gelatin (KCDFG), Muscovy duck feet gelatin (MDFG) and Pekin duck feet gelatin (PDFG) were shown at Figure 2. The FTIR fingerprint of all gelatin for three different species of duck feet (DFG) and CBG displayed the amide region as major peaks. The peak of amide bands for DFG exhibited the wavenumber around 1600 – 1755 cm^{-1}, 1521 – 1563 cm^{-1}, 1363 – 1433 cm^{-1}, 3134 – 3601 cm^{-1} for amide I, II, III and A respectively. Meanwhile, FTIR spectra for CBG were not significantly different from all DFG with amide I, II, III, and A were 1631 cm^{-1}, 1539 cm^{-1}, 1394 cm^{-1} and 3284 cm^{-1} respectively. Amide A band location of the gelatin samples around 3400-3440 cm^{-1} was closed to the free NH stretching frequency which indicate the involvement of NH group peptides in hydrogen bonding in the primary structure of gelatin (Shurvell, 2006; Kuan et al., 2017). Besides

that, amide band A also related to the CH stretch peak when carboxylic acid group existed in stable dimeric associations (Abdelmalek et al., 2016).The carbonyl bond (C=O) stretching of C-N bond that occurs at 1684 - 1634 cm^{-1} in DFG and 1631 cm^{-1} in CBG indicate amide I in existence in gelatin sample (Chakka et al., 2017).

The amide I band that located at 1684 - 1634 cm^{-1} was associated with the secondary structure of protein and indicated the characteristics triple helix and random coil structure of gelatin (Muyonga et al., 2004; Kuan et al., 2016). The DFG sample also had peak at 1521 – 1563 cm^{-1} and 1363 – 1433 cm^{-1} which represented amide II band and amide III band. Meanwhile the position of amide II band and amide II band of CBG are 1539 cm^{-1} and 1394 cm^{-1} respectively. Amide II was an assigned of an out of phase combination of –CN stretch and in plane –NH deformation mode of the peptide group (Lavialle et al., 1982; Xu et al., 2017). The peak of amide III reflects the involvement of NH bending in the random coil or disordered structure (Kuan et al., 2016). The amide III vibration mode is the combination peaks between C-N stretching vibrations and N-H deformation from linkages as well as absorptions arising from wagging vibrations from CH_2 groups (Widyasari & Rawdkuen, 2014).

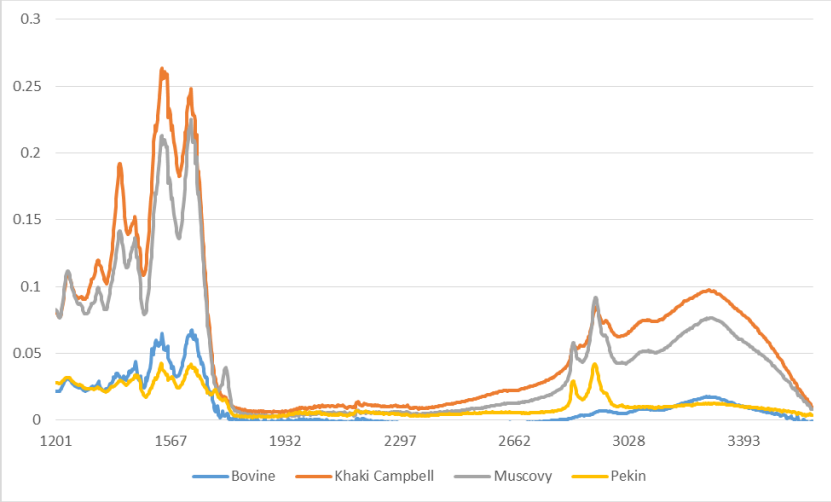

Figure 2 : FTIR spectra of commercial bovine gelatin (CBG) and four different species of duck feet gelatin (DFG)

Conclusion

Gelatin from the duck feet from three different breed of duck, Khaki Campbell, Muscovy and Pekin were successfully extracted by using acetic acid. Among all the Duck Feet Gelatin, PDFG has the highest yield followed by KCDFG and MDFG even though the yield were low compared to other study. Muscovy Duck Feet Gelatin (MDFG) has the highest amount of protein content and Khaki Campbell Feet Gelatin (KCDFG) has the lowest amount of protein content among the three breeds. pH value of the Duck Feet Gelatin are acidic indicating that they are type B gelatin with PDFG has the closest value to CBG

followed by KCDFG and MDFG. Meanwhile MDFG has the highest bloom strength value and KCDFG has the lowest bloom strength value. PDFG has the lowest melting temperature followed by MDFG and lastly KCDFG. The FTIR fingerprint of all gelatin for three different breeds of duck feet (DFG) displayed the amide region as major peaks. The peak of amide bands for DFG exhibited the wavenumber around 1600 – 1755 cm^{-1}, 1521 – 1563 cm^{-1}, 1363 – 1433 cm^{-1}, 3134 – 3601 cm^{-1} for amide I, II, III and A respectively. Research on Khaki Campbell and Muscovy duck should be continuing on the future due to limitation of data in duck feet gelatin from these breed. Duck feet gelatin can be an alternative source for the production of gelatin from natural resources.

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2.4 Physicochemical properties of duck (Khaki Campbell) skin gelatin pre-treated with acetic acid and citric acid

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Introduction

Gelatin is the main derivative of a collagen obtained by partial hydrolysis of collagenous material and has unique properties which are the ability to form thermo-reversible gels and emulsion (Rasli and Sarbon, 2015). These properties contribute to a wide range of application in many industrial fields such as food industry, pharmaceutical, cosmetic and photographic industry due to its functionality. According to Sompie et al. (2015) the higher quality of gelatin depends on its physical and chemical properties, rheological properties and manufacturing method. The awareness of the halal product is not only concerned for the muslim consumers but also non-muslim consumers. The non-muslim consumers preference about the halal issue is related to some factors such as hygiene, quality and safety of the product provided from a halal product. There are many issues related to the halal and one of them is gelatin. In addition, the halal issue is arisen when the gelatin used comes from prohibited sources such as porcine, the animal that is not slaughtered according to shariah law such as bovine (Fathin et al., 2018).

For this study, Khaki Campbell duck species is chosen as a source of gelatin as it is known as one of the best, famous and most popular eggs laying breed of domestic duck. Nowadays, poultry skin, feet and bone have increased as sources to replace mammalian resources. Most of the previous study are focusing on the extraction of gelatin from pig skin, chicken skin and duck feet by using different extraction method and condition (Kim et al., 2014; Widyasari & Rawdkuen, 2014; Sompie et al., 2015). Duck skin might be used as a substitution source of bovine and porcine gelatin as reported by Lasekan et al., (2013) since gelatin from poultry by products receives some attention due to the waste generated during processing contains varying amount of protein and rich in collagen protein. Moreover, Malaysia is one of the leading countries that produce and export duck meats (Abedinia et al., 2017) which by this can supply sufficient duck skins as raw material for gelatin production in the industry. Therefore, the aim of this study was to investigate the suitability of duck skin as raw material for gelatin powder production in term of physicochemical characteristic and the yield of the gelatin extracted with two different acids (acetic acid and citric acid) and compared with the commercial bovine gelatin (CBG).

Materials and methods

The ducks were obtained from 4R Agro Dedak at Pasir Mas, Kelantan. All the duck skins were kept in a freezer with temperature below -18°C. Acetic acid and citric acid were

used in the gelatin extraction while 1-butanol was used to remove fat in the duck skins. The commercial bovine gelatin was purchased from Halagel (M) Sdn. Bhd. All the chemicals and reagents used were analytical graded.

Sample preparation

Duck skin gelatin was extracted according to Lee et al. (2012) with a slight modification. The frozen duck skins were thawed in the chiller at 5°C for 24 hours, cut into small pieces followed by fat removal. Duck skins were homogenized with 10% butanol by using blender followed by suspending in 10% (v/v) butanol solution with a ratio 1:8 (w/v) at 5°C to 7°C for 48 hours. The 10% (v/v) butanol solution was poured and changed for every 24 hours. Defatted duck skins were treated with 0.1 M acetic acid (CH_3COOH) and 0.1 M citric acid ($C_6H_8O_7$) respectively at a ratio 1:7 (w/v) for 40 minutes with gentle stirring by using magnetic stirrer. This step was repeated three times with alternated changed of acetic acid and citric acid solution. Then, duck skins were subjected to neutralize under running tap water approximately for 3 hours until obtained pH value 5. After that, the gelatin was extracted by water at a ratio of 1:1 (w/v) with the temperature of 65°C in water bath for 2 hours. The vacuum filtration was used to filter the gelatin solution. Duck skin gelatin (DSG) was then placed in the freeze dryer at temperature of -38°C for 72 hours until the moisture content of duck skin gelatin achieved was below 10%. Duck skin gelatin powder was kept in the tight container until used for the next analysis.

Yield of gelatin

The percentage of the gelatin yield was calculated according to Bueno et al. (2011).

Protein recovery

Protein recovery value was obtained according to Boran et al. (2009).

Chemical composition of gelatin

The chemical composition of duck skin gelatin powder were determined according to the method described by AOAC (2005).

Bloom strength

Bloom value of gelatin gel was determined according to Norziah et al. (2013). Texture analyzer TA-XT PLUS was used to determine the gel strength of the gelatin (Rahman & Jamalullail, 2012).

pH determination

The pH value was determined according to See et al., 2010).

Melting temperature determination

The melting temperature of gelatin were measured using differential scanning calorimetry (DSC)(Norziah et al., 2013).

Statistical analysis

The data was subjected to the analysis of variance (ANOVA). A mean comparison was analysed by using Tukey Tests. Significance of difference was defined at $p<0.05$. The statistical package used was SPSS 20 (SPSS Inc, Chicago, IL).

Results and discussion

Based on the result in Table 1, the percentage yield of duck skin gelatin extracted using acetic acid obtained was 1.70 % and higher compared to duck skin gelatin extracted using citric acid, 1.32 %. Both acids were suitable to be used in gelatin extraction due to their smallest molecular size, low ionization constant (K) and ionic strength (Mahmood et al., 2016). However, the yield (%) of both DSGAa and DSGCa was lower than chicken skin gelatin extracted by using acid pre-treatment which was 2.16 % (Sarbon et al., 2013). The gelatin extraction yield on chicken feet skins and tendons was 7.83 % higher than chicken feet paws which was 7.37 % (Almeida & Lannes, 2013). The yield of chicken feet gelatin was higher than DSGAa and DSGCa due to the chicken feet had different amino acid composition compared to duck skin. Table 1 also showed that the protein recovery for DSGAa was 22.29% while for DSGCa was 27.98 %. According to Teng (2014), the highest protein recovery of gelatin found in silver carp skin was 78.1 %. The poultry skin had abundant of stroma protein and the main content of stroma protein was collagen (Nik Aisyah et al., 2014).

Table 1: Yield and protein recovery of duck skin gelatin acetic acid (DSGAa) and duck skin gelatin citric acid (DSGCa)

Samples	Yield (%)	Protein Recovery (%)
DSGAa	1.70	22.29
DSGCa	1.32	27.98

Based on the result in Table 2, there was significant different ($p<0.05$) in moisture content value between these three gelatins. The moisture content of DSGAa, DSGCa, and CBG were 9.39%, 6.47% and 11.21% respectively. The moisture content for DSGAa and DSGCa was lower than CBG which meant they undergone good drying process. Gelatin

absorbed or released moisture based on the humidity of the surrounding. Gelatin Manufacture Institute of America (GMIA) had prescribed the limit of moisture content for edible gelatin in the range of less than 15%. Thus, all these three gelatin had moisture content within the prescribed limit. Based on the previous study, moisture content for gelatin extracted from chicken skin gelatin was 9.81% (Sarbon et al., 2013) and chicken feet skins and tendons were 10.39% (Almeida & Lannes, 2013). When the moisture content exceeded 16% there was the increased risk of lump formation and microbiological growth.

The low moisture content increased the shelf life of the gelatin with proper storage condition (Fathin et al., 2018). The value of protein of and DSGCa was 55.86% and 70.08% respectively lower than commercial bovine gelatin (CBG) which was 86.20 %. The protein content of DSGCa had no significant different ($p>0.05$) with both CBG and DSGAa while DSGAa had significant difference ($p<0.05$) with CBG. The extraction temperature of DSGAa and DSGCa used was 65°C for 2 hours. Due to the elevated temperature during extraction, the gelatin might be degraded then caused the lower protein content. Based on the previous study by Mohd Nazri et al. (2015) showed that the protein content of chicken feet gelatin extracted at 75°C for 2 hours was 67.40%. The analysis of the protein was required due to the quality of the gelatin in which the bloom strength was related to the protein content (Fathin et al., 2018). From Table 2, the fat content showed a significant difference ($p<0.05$) between these three gelatins. The fat content in CBG was 0.18 % lower than DSGAa which was 23.76 % and DSGCa which was 16.66 %. Based on previous study conducted by Almeida and Lannes (2013), they have found 12.8 % fat in chicken feet, which was higher than the fat content of CBG in this study but lower than the fat content of DSGAa and DSGCa. This difference might be due to the greater efficiency of the filtration step, beyond the longer time the pre-treatment acid of the raw material that contributed to the separation of fat and consequently a lower content of lipids in the final product.

According to Sebastian (2014), skins poultry contained a lot of fat and industry usually used separators high performance and the fat obtained was purified. The ash content of CBG, DSGAa and DSGCa was 3.57%, 6.00% and 6.05% respectively. There was no significant difference ($p>0.05$) of ash content between DSGAa and DSGCa but there was significant difference ($p<0.05$) of ash content between these two gelatins with CBG. According to Food Act (2001) and Food Act 1983 and Food Regulations 1985, the permitted ash content of gelatin must not exceed 3% (Mohd Nazri et al., 2015). The ash content for CBG was still within an acceptable range but the ash content for DSGAa and DSGCa exceeded the prescribed limit. Previous study conducted by Huda et al. (2013) stated that the ash content for duck feet collagen was 28. 60% while Kuan et al. (2017) reported that ash content for duck feet gelatin was within the range which was 2.47%. From the previous study by Almeida and Lannes (2013) the ash content of gelatin from the whole chicken feet was 6.03% and paws was 6.38% which was higher than

recommended value. This might due to the large amount of calcium and other salts present in bones.

Table 2: Chemical composition of DSGAa, DSGCa and CBG

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
DSGAa	9.39±0.33^{b}	55.86±4.62^{a}	23.76±2.41^{c}	6.00±0.87^{b}
DSGCa	6.47±0.42^{a}	70.08±11.42^{ab}	16.66±0.11^{b}	6.05±0.42^{b}
CBG	11.21±0.16^{c}	86.21±0.04^{b}	0.18±0.02^{a}	3.57±0.02^{a}

Means with different superscript letters within the same column are significantly different at $p < 0.05$. DSGAa, duck skin gelatin treated with acetic acid; DSGCa, duck skin gelatin treated with citric acid; CBG, commercial bovine gelatin. Data expressed as means of duplicate ± standard deviation

The pH of DSGAa was 5.12 and DSGCa was 5.00 had significant different ($p<0.05$) to CBG which was 5.60 (Table 3). DSGCa had the lower pH of gelatin compared to other but it was still in the range of pH 5. The pH of gelatin could be affected by the types and strength of chemical used for pre-treatment according to Ratnasari (2016). Almeida and Lannes (2013) stated that the higher the pH value of gelatin was because of the effectiveness of washing the raw material after chemical treatments before extraction of gelatin. The low pH value for both DSGAa and DSGCa was due to the acid pre-treatment used before the extraction and differences in the type and strength of acids employed during the extraction procedures (Teng, 2014). GMIA standard (2013) stated that the pH value using the acid pre-treatment within the range of 4.5 to 6.0. Thus, the pH value for CBG, DSGAa and DSGCa were in the suitable range of pH. Perfect process of neutralizing and washing the raw materials before the extraction process resulted to have neutral pH values and could minimize the contamination (Sompie et al., 2015). DSGAa obtained the highest gel strength which was 143.86g with no significant different ($p<0.05$) with DSGCa which was 143.61g. The CBG had the lowest gel strength which was 115.47g. Bloom strength was the key to measure the quality and the amount of the gelatin needed for application in products (Almeida and Lannes, 2013). The measurement of this property was important both from the point of view of control as an indication for gelatin required for a particular application and that value was obtained according to standard methods developed by Gelatin Manufacturers Institute of America (GMIA, 2013). The higher the bloom value, the stronger was the bloom strength (Abdullah et al., 2016). According to Nik Aisyah (2015), commercial bovine gelatin had values of gel strength range from 50g to 300g. Based on the result in Table 3, the bloom strength of CBG, DSGAa and DSGCa in this present study was within the range. The gel strength values or bloom values were categorized in term of low (<120 g), medium (120-200 g) and high (>200 g) bloom (Rafiean et al. 2015). Thus, DSGAa and DSGCa gelatins were grouped as medium quality of gelatin. The temperature where the gelatin gel softens known as melting temperature and it was one of the most important

physical properties to determine the quality of gelatin gels (Mariod and Adam (2013). Based on Table 3, the melting temperature of DSGAa, DSGCa and CBG was 37.06°C, 36.15°C and 30.70°C respectively. From the previous study reported by Park et al. (2013), gelatin extracted from duck feet had the higher melting temperature which was 38.69°C. There was no significant difference ($p<0.05$) between these three samples in terms of melting properties. According to Sarbon et al. (2013), the melting temperature of gelatin was greatly affected by the bloom strength and molecular weight distribution. Normally, gelatin with high bloom strength had the higher melting temperature of the gel at the same concentration (Fathin et al., 2018). In addition, Norziah et al. (2013) reported that the low melting temperature was due the weak structural stability of gelatin. Thus, based on the result, the melting point of DSGAa and DSGCa were comparable to the CBG.

Table 3: pH value, bloom strength and melting temperature of DSGAa, DSGCa and CBG

Samples	pH	Bloom Strength (g)	Melting Temperature (^oC)
DSGAa	5.12 ± 0.07^a	143.86 ± 4.77^b	37.06 ± 0.73^a
DSGCa	5.00 ± 0.05^a	143.61 ± 5.50^b	36.15 ± 3.21^a
CBG	5.60 ± 0.10^b	115.47 ± 2.56^a	30.7 ± 0.16^a

Means with different superscript letters within the same column are significantly different at $p<0.05$. DSGAa, duck skin gelatin treated with acetic acid; DSGCa, duck skin gelatin treated with citric acid; CBG, commercial bovine gelatin. Data expressed as means of duplicate ± standard deviation.

Conclusion

Gelatin from duck skin was successfully extracted by using acetic acid and citric acid solution. The gel strength of DSGAa and DSGCa were categorize as medium bloom and has potential as source of high quality of gelatin. Method on extracting gelatin from duck skin should be studied to find the optimum condition for duck skin gelatin with the highest yield and protein recovery. A more efficient defatting duck skin should be studied as duck skin contain high fat content which will affects the purity and as well as the turbidity of gelatin extracted. Malaysia is the third largest country producing duck meat, thus the duck skin as by product can be fully utilized to produce gelatin as gelatin from duck skin show good physicochemical properties.

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2.5 Calcium and phosphorus requirements of female *Ayam Kampung* MARDI (AKM) in pullet phases

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Introduction

Poultry production has an important in economic, social and cultural benefit and as role a nutrition supply in development country (Desalew Tadesse et al., 2013). Village chickens are the most common type of livestock in many rural areas (M Chibinga, 2016) and developing countries as a food source (Muhiye, 2007). *Ayam Kampung* MARDI (AKM) introduced at the Malaysia Agriculture, Horticulture and Agro-Tourism Exhibition (MAHA) 2012. The AKM slower growth rate in growth and production, poor laying ability and smaller egg size as compared to commercial breeds. It was to produce uniform in terms of body size, physical characteristics, growth and better laying chickens compared to the original breed.

Calcium (Ca) and Phosphorus (P) is one of the key nutrients required for bone, egg quality, growth performance, layers and breeder. The Ca well over 90% found in the bone where it combines with P as second most important mineral element in bone, to from calcium phosphate crystals (Gordon & Roland, 1996) In feed nutrition other important element for body system including Na, Mg, Fe and Fi. In addition, phosphorus as essential component of almost all metabolic processes (McDonald et al., n.d.)The P content less than from Ca between 80 to 85% of the total phosphorus found in the bones function. Phosphates are used for metabolism and energy storage in formation of sugar phosphates and adenosine di- and tri- phosphates. Furthermore, P form part of a protein that contains phosphorus, nucleic acids and phospholipids (Driver, John Patrick, 1994).

The chemical composition is also quite variable although it mainly consists of levels calcium and phosphorus in feed nutrition. In body skeleton allows an animal to stand and protects its internal organs system and tissues. The bone is living tissue and its structure is largely affected by the nature of stresses placed upon it. Village chicken as poultry have been developed for improved egg production or growth and meat production, the skeleton remains a potential in the physical support of heavier carcasses at ever younger ages (Applegate & Lilburn, 2000).

The age AKM at Weeks 16 take a bone sample part of tibia right and left to compare content for quality and mineralized the bone. From the study, quality between right and left bone no significant different based on quality. In addition, the different levels of Ca and P between percentage low, medium and high have significant difference in bone

quality. The bone measurements such as weight, length, bone density and bone ash, have been used as indicators of bone status in the mineral nutrition of poultry.

To our knowledge, a considerable amount of research has been conducted on the effect of feeding various Ca and P levels during pullet stages requirements. This experiment was conducted to investigate the effects of dietary Ca and P levels on bone quality. In addition, to compare tibia bone quality and mineralized content between right and left portion there have a difference or not. In this regard, the objective of the present study was to determine effect of difference level Ca and P in physical tibia bone quality and for part right or left bone have differences or not with pullet stages with AKM.

Materials and methods

The study preparation, sampling process, lab analysis and data analysing process conducted Malaysian Agricultural Research and Development Institute (MARDI) located at Serdang, Selangor. The department at MARDI is Livestock Science and Nutrition Program Research Centre Headquarters in Feed. The experimental design for this project is Complete Randomized Design (CRD) from Week 11 to 16. The treatment has 3 level Ca and P (Medium 0.90% (Ca) and 0.58% (P)), (High 1.15% (Ca) and 0.74% (P)) and (Low 0.75% (Ca) and 0.48% (P)). The percentage in diet based on recommended nutrients concentration levels (Lohmann Tierzucht, 2014)In this experiment have 9 cage and every cage have 13 bird per replicates, 39 bird per treatment, total bird is 117. Parameters measured are live weight (LW), bone quality by measuring the weight, dry matter (%), ash (%), length and density for both right and left tibia bone. The main sources of Ca are limestone and P is Dicalcium phosphate (DCP). Every treatment has similar ingredient for energy and protein but difference in level of Ca and P.

Table1: Treatment level of Ca and P in chicken feed

Stage	Energy MJ/kg	Protein (%)	Treatment	Ca (%)	P (%)
Pullet	11	16	Low	0.75	0.48
			Medium	0.90	0.58
			High	1.15	0.24

Before start the experiment on going for Proximate Analysis to determine feed nutrition content. The method of proximate analysis was implemented to determine the percentage of Dry Matter (DM), Ash, Crude Protein (CP), Crude Fibre (CF), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Ether Extract (EE).

Samples take after slaughter with 6 bird per treatment were randomly selected and weighed (1.6kg- 1.8 kg). The sample part removes from the body and each sample were individually packed in polyethylene bags and stored at -20°C prior to bone preparation.

Bone were cleaned of the adhering tissues and dried in the oven at 105°C for 24 h standard procedure according to AOAC Official Method 930.15. Dry weights were recorded after cooling in a desiccator. The bone parameters including dry weight, length, density and ash were determined according to Zhang and Choon (1997). Dried tibiae were ashed in a muffle furnace at 600°C for 24 h or overnight, cooled in desiccator before the weight were recorded for ash content. Data were analysed using the ANOVA using the general linear model (GLM), multiple comparison of means using Duncan method procedures of IBM SPSS Statistics.

Results and discussion

The result from study for difference part between right and left is no significant difference in bone weight, dry matter, length, density and ash. Levels Ca and P no significant difference between each treatment in bone weight, dry matter, length, density and ash.

Table 2: Effect of levels dietary calcium and phosphorus on physical tibia bone quality. Values are means represent ± standard deviation

Bone sample	Ca and P levels	Bone weight (g)	Dry matter (g)	Length (mm)	Density (g/cm^3)	Ash (%)
Right	Low	12.25 ± 1.13	8.05 ± 0.71	13.08 ± 0.36	1.22 ± 0.05	38.57 ± 3.96
	Medium	12.16 ± 1.45	8.40 ± 0.70	12.93 ± 0.37	1.21 ± 0.03	41.32 ± 1.79
	High	12.77 ± 0.43	8.40 ± 0.70	13.18 ± 0.29	1.25 ± 0.07	40.45 ± 1.57
Left	Low	12.12 ± 0.73	8.11 ± 0.70	13.11 ± 0.34	1.24 ± 0.05	40.43 ± 2.94
	Medium	12.27 ± 1.45	8.17 ± 0.87	13.01 ± 0.44	1.21 ± 0.07	40.64 ± 1.77
	High	12.81 ± 0.90	8.33 ± 0.82	13.13 ± 0.23	1.25 ± 0.06	40.93 ± 2.20

Bone weight measurements between part of right and left tibia are presented in table 2. For the part between right or left no significant. In addition, for the level Ca and P no significant between level low, medium and high. The mean each level between 12.12 g to 12.27 g for low and medium but level high achieve until 12.81 g.

In general terms, the weight of a specific ingredient come from either the moisture in the material from the dry matter (DM) portion. Dry matter refers to material remaining after removed of water, and moisture content reflects the amount of water present. For the right part have a high of value dry matter at level medium and high between 8.40 g. The left part no significant between level low 8.11 g, medium 8.17 g and high 12.81g. Dry matter physical bone quality for difference part right and left no significant difference. In bone quality for bone density is one of the most important factors to measure. In addition, bone density values are affected by many factors, such as age, sex, type of production, diet and management. From the result table 1 with difference part right or left no significant difference. For levels Ca and P no significant difference between low medium and high. High level Ca and P is the higher value for density. For

low and medium density value between 1.21 to 1.23 g/cm$^{3.}$ Bone density also be measured using bone mineral composition, bone breaking and seedor index (Address, 2006). Skinner et al. (1991) reportrd on the effects of dietary protein, calcium and phosporus on tibia length and ash. However, effects from feed restriction and environment on long bone development (Bruno et al, 2000). The correlated can be found between increased bone ash, higher amount of availeble calcium and phytate phousphorus in the diet and incresed body weight gain (Hall et al., 2003). The result from table 1 in level bone ash no significant diffrence between levals Ca and P and part of bone right or left. There are physiologically distinct regions of a growing bone, each with its own unique developmental characteristics. The tibia particularly the epiphyseal end, has been extensively studied due to cellular sensitivity to numorus dietary deficiencies. The significant differences between long bones in terms of mineralization, there have been few reports since that have specifically addressed age and body weight relationships with comprehensive long bone development (Dilworth & Day, 1965)

Conclusion

As a conclusion in study effect of level Ca and P in dietary with physical bone quality no significant difference between each level low, medium and high. For the part between right or left no significant difference during study in pullet stage with AKM. The calcium and phosphorus are important mineral in body system for growth performance, egg quality, bone mineralization, and nutrient utilization. For this study are important to determine the exact Ca and P requirements in feeds for AKM to avoid overfed or malnutrition in growth

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2.6 An assessment of reproductive and stress hormone and their relation with reproductive behaviour of captive female *Rusa unicolor* (Sambar deer)

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Introduction

Peninsular Malaysia is undergoing a tremendous encroachment and land development which results in the rainforest environment being changed drastically. Therefore, there is urgent need to constantly manage the existing wildlife and habitats, to sustain, protect, preserve and propagate wildlife species. (Zaaba *et al*. 1991). Apart from habitat encroachment, illegal hunting also caused the decreasing population of local species including the Malayan Tiger (*Panthera tigris jacksoni*). Currently, the Malayan tiger subspecies which was first formally recognized in 2004 after genetic tests is listed as Endangered by the IUCN Red List. The Department of Wildlife and National Parks of Peninsular Malaysia (DWNP) believe the status may have to be changed to Critically Endangered given the recent estimates. According to the Ministry of Water, Land and Natural Resources, to date, the Malayan tiger is less than 200 in the wild population. The main reasons are illegal trade and poaching due to high demand in the black market. In between year 2013 to 2018 has reported number of poachers saw significantly increased with 98 locals and 64 non-locals. 3,500 snares were cleared.

Another threat facing the Malayan tiger is a decline in tiger prey, pointing especially to a loss of Sambar deer to hunting and snares. In Malaysia, tiger prey populations appear to be declining. Species of Sambar deer among the favourite by local for hunting and being poached at all time. (Zaaba *et al*. 1991). Sambar deer has lost more than 50% of its habitat range, currently only a quarter is protected. Recommended stated in IUCN Red List as Endangered (Kawanishi *et al*., 2014). Ex situ conservation efforts i.e, captive breeding are being taken by government to boost Sambar deer numbers in captivity and then reintroduce in the wild to support a higher number of tigers, consistent with the goal of our National Tiger Conservation Action Plan to increase tiger numbers. The reproductive success of Sambar deers and their welfare management practices in captivity are important components for effective captive breeding programmes. DWNP has set up three breeding centres for Sambar deer at Sungkai in Perak, Jenderak in Pahang and Gua Musang in Kelantan (Habsah, 1985; Pan & Sabri, 1989). Since DWNP has implemented a captive breeding programme, efforts must be focused on the reintroduction programme. It is crucial to assess the effectiveness of this programme (Mohammad, Shukor, Mohd & Mohd, 2001).

Environment factors have significant impact on natural behaviour of animals (Kchan, 2014) especially the reproductive state, which can be affected by both daily activity and space use of the animal (Mahdive, 1984). Environmental surroundings that changes

rapidly may also triggered an increment of cortisol (an example of glucocorticoids) secretion rates which lead to phsycological stress responses in animal (Wingfield et al. 1958, Ficinero, 2002). Changes in the social environment especially through social conflicts even within their own species will intensity affect their physiological stress (Goymann *et al*. 1999. Franceschini *et al*. 2007, Kuo Jong & Lai, 2011) could lead to significant impact on their natural reproductive and stress behaviour in both captivity and wild habitat. A study conducted in Zoo Melaka concluded that Sambar deer spot less time interacting with conspecies (Sun and Rahman, 1989).

Unfortunately, throughout their study they were not being able to observe any mating as they are known to be shy. Many other species placed in captivity and continuously shown exhibit abnormal behaviour due to having poor health problem, repeative stereotypic behaviour and breeding difficulties. (Clubb and Massion, 2002, Mason *et. al*, 2007) due to the increasing number of visitors and restrictive enclosure space (Hosey, 2005). However, there is no study yet to determine whether these factors are affecting the conservation and welfare of Sambar deer.

The study of Sambar deer by traditional field methods is also difficult due to therapy, cryptic and nocturnal behaviour, solitary social structure and preference to inhabit deep tropical forest (Marine, 2003). Recent advances non-invasive measurements (Karsley and Dehirhard, 2014 Narbeth *et. al*, 2014), Bahringer and Deschiner 2017) of reproductive and stress hormones via faeces samples reflect the endocrine behavioural stress which can provide an alternative way to study the relationship of reproductive and physiological stress responses with their behaviour (Sheriff *et. al*, 2011: Cook, 2012). Responses to stressors are complex and context dependent and therefore a combination of different measurements of (physiological and behavioural) for assessment stress and reproductive behaviour should be considered.

Stress in mammals is a complex and multistage syndrome that is orchestrated by the sympathetic nervous system and glucocorticoids, a class of steroid hormones (Sapolsky, 2001). A common practical technique for assessing the stress response in animals is to monitor adrenocortical activity by measuring glucocorticoids and their metabolites in blood, saliva and body excretions. The measurement of glucocorticoid metabolites excreted in the faeces allows for collection and analysis without handling the animals. This is especially important in large mammals and endangered species where repeated capture and handling is not possible. Another advantage of faecal analysis is that it minimizes interference with natural behaviours. Animals can be observed through binoculars from a distance, defecation locations noted, and samples collected after they have moved away. Although there are many advantages, there are also several confounding factors that need to be considered and minimized when applying this technique in wildlife field studies and in investigating conservation issues (Millspaugh & Washburn, 2004; Palme, 2005; Touma & Palme, 2005; Keay *et al*., 2006).

Faecal endocrinology has important applications for wildlife conservation because it facilitates the non-invasive monitoring of adrenal activity in wild animal populations (Möstl and Palme, 2002; Sheriff *et al*., 2011; Wasser et al., 2000; Watson *et al*., 2013). The concentration of faecal glucocorticoid metabolites (fGCM) is a reliable indicator of biologically active ("free") glucocorticoid metabolites circulating in an animal body over a period of time and, importantly, wildlife faeces are easier to collect than alternative biological samples such as blood, saliva, or urine (Möstl and Palme, 2002; Touma and Palme, 2005).

Non-invasive endocrinology testing using faecal samples or another method (e.g. hair, feathers and scales) is a fast-growing research field (Brown, 2000; Ganswindt *et al.,* 2012; Hodges, 2005). The ability to carry out continuous health monitoring on free ranging wildlife without the need for capture is an attractive prospect for wildlife conservation and management.

Discussion

This study will be conducted by using non-invasive faecal sampling to obtain hormonal profiles without the animal being sedated or restrained and it has not been tested in captive Sambar deer. This will be the advantage of this research. A non-invasive method will be measured by using a faecal sample to determine the assessment of the stress hormone to relate with the reproductive behaviour. Faecal samples offer the advantage that they can be collected easily. In faecal samples, circulating hormone levels are intergrated over a certain period and represent the cumulative secretion of hormones. (Palmae, 2012). However, diminutive information reported at present, regarding reproductive traits of this subspecies in Peninsular Malaysia. Therefore, the aims of this study are to access and manuscript the reproductive and stress hormones in captive female Sambar deer by using faeces and to correlate with their behavioural patterns for a better understanding and significance of captive breeding management programmes. Furthermore, it is expected the sexual activity and the circumstance being under stress in captivity can be forecast by associating hormonal and behavioural data. In addition, this will help for enhancement welfare captive management plan as well as wildlife ex-situ programmes.

Conclusion

Conservation of Sambar deer is extremely important especially now that the species has been listed as Vulnerable by the IUCN. The study on the reproductive and stress hormone patterns with the animal's physiology and reproductive behaviour are essential for the sustainability management of this species both in the natural habitat as well as in captive environment. It is expected, the reproductive and stress hormonal practise can be predicted via behaviour pattern for future breeding programme and welfare under captivity. This assessment will be useful to the understanding of the

important and significant role of Sambar deer towards biodiversity and ecological value as well as to provide an approaching idea about the focal prey for our endangered national iconic species, the Malayan tiger.

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2.7 The effect of different concentration of livestock fertilizers on growth performances of *Azolla pinnata*

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Introduction

The demand for goat, cow and broiler meat especially in Malaysia keep increasing and sustainable supplies of these meats are very crucial. Thus, persistence of fodder resources availability in rearing the livestock able to sustain the production of animal`s product. Unfortunately, fodder resources are another aspect that draw greater concerns among the farmer to raise their livestock for a sustainable production due of the urbanization and high cost of commercial animal feed. Urbanization has leads to less availability of pastureland for animal (Banu & Fazal, 2016). Hence, these will cause shortage of fodder as the farmers cannot cultivate feed crop for their livestock. The alternative ways to overcome those problems is by using *Azolla pinnata* that is the most economical friendly, have a rapid production and efficient feed substitution (Tamizhkumaran & Rao, 2016). Yet, it has great potential in order to produce fodder to livestock.

Morphology of *Azolla pinnata*

Morphologically, *A. pinnata* is small free-floating fern that known as mosquito fern usually grows in stagnant water bodies thus it was suitable to be cultivated in ponds, pit, and tanks (Becking, 1979). This plant where able to produce fresh weight up to 600 g after 10 days. The body of this tiny aquatic macrophyte can be divided into three parts which is leaves, stem and roots. The leaves were measured approximately 1-2 millimeters ("Global Invasive Species Database", 2018) with an overlapping arrangement and the stem is often called the rhizome and the root a feathery appearance in the water (Jacono, 2016). In addition, its leave cavity had become a host for one species of cyanobacteria called *Anabaena azollae* (Hove & Lejeune, 2002) that capable in nitrogen-fixing from air (Punita & Soma, 2015). These relationships give great advantage to this plant in their composition of protein (Kamalasanana Pillai et al., 2005).

Nutritional compositions of *Azolla Pinnata*

The nutrition composition is very crucial to be tested first on feed crop before been introduce in animal diet, this due to the animals' nutrition requirement might be vary among different species such as poultry, cattle, goat and swine. Therefore, when the nutrition content of the feed crop is low these leads to malnutrition as they do not meet an enough requirement for their body to function well (Fernandez, 2017). *A. pinnata*

has been reported to consist high content of protein including an essential amino acid such as Lysine to promote growth of animal and their live performance. Moreover, this plant also has been identify growing with rich of vitamin such vitamin A, B12, Beta-Carotene and mineral (Kamalasanana Pillai et al., 2005). Therefore, Aziz (2006) had suggested that *Azolla* is one of the excellent fodder resources in livestock feeding diet. In other hand, the variation had been reported in several studies in the nutrition composition of *A. pinnata* which is from study that been done by Singh and Subudhi (1978), Anand Titus and Geetha Pereira (2007), Alalade and Iyayi (2006) and Balaji et al., (2009) is presented in the Table 1.

Table 1: The nutritional composition on dry matter basis of *A. pinnata*

Nutritional Composition	Singh and Subudhi (1978)	Alalade and Iyayi (2006)	Anand Titus and Geetha Pereira (2007)	Balaji et al., (2009)
		Nutritional value (%)		
Crude protein	24.3%	21.4%	20% - 25%	24.5%
Crude fiber	9.1%	12.7%	-	14.9%
Ether extract	3.6%	2.7%	3% - 3.5%	3.7%
Total ash	10.5%	16.2%	-	17%
		Mineral (%)		
Calcium	0.4% - 1.0%	1.16%	0.45% - 1.25%	2.14%
Phosphorus	0.5% - 0.9%	1.29%	0.15% - 11%	0.44%

(Source: Bolka, 2011)

Cultivation using organic fertilizer

Cultivation of the feed crop is very important to sustain fodder supply to support in livestock industry. Utilization of chemical fertilizer such as Triple Super Phosphate (TSF) able to speed up the crop growth and development (Singh & Singh, 1987). But it has cause serious pollution into water resources (Wu, 2017) and yet, it will increase production cost due to the pricy of the fertilizer. Therefore, the farmer tends to apply organic fertilizer such as cow manure, goat manure and chicken manure to their crop (Mahadi et al., 2013).

Materials and methods

This study was conducted from March 2018 until September 2018 and has been located at MARDI Serdang for cultivation and sampling. Next, data of plant performance was calculated at department of Biology, UPM while nutritional analysis were done at Feed Chemistry Laboratory of Livestock Science Research Centre, MARDI Serdang.

The study was started with cultivation of *Azolla pinnata* at approximately 152m^2 plot by using 15 pieces 200L plastic tank with arrangement according to Randomized Complete Block Design (RCBD) experimental design. There were 3 types of organic fertilizers been used in this experiment which is collected from MARDI's goats and chicken housing units and the small holder beef farm at Sg. Ramal, Kajang. All the animal's

manure was dried at 60°C for 48 hours using Force-air drying oven before it's been grinded into <1mm size using electric mash grinder. Then, all grinded animal manure was weighed into 50 g, 100 g, 150 g, 200 g, and 250 g which coincides with their treatment.

The cultivation of *Azolla* was carried out for 14 days using 1 meter (L) × 0.66 meter (W) in size plastic tank. Approximately 132 L of water was filled 2 days before diluting mash of animal manure with an initial 10 kg inoculum of fresh *Azolla*. As a treatment, the weighted animal manures were diluted into the water according to treatment T1, T2, T3, T4 and T5 which is 50 g, 100 g, 150 g, 200 g and 250 g, respectively. It was stirred until evenly distributed in the water. The temperature and pH were monitored using YST water quality meter while the growth rate of *A. pinnata* was measured using simple quadrat technique. Both parameters were daily monitored and recorded.

Harvesting was conducted on day-14 after cultivation. The sieve container was used to drain out water from the harvested plant before it been weighted for fresh yield recording. Next, the fresh *Azolla* were placed into the aluminium container and been dried into force-air drying oven at 60 °C for 72 hours (Ooi, Iqbal, & Ismail, 2012). The dried *Azolla* were cooled into the desiccator before being weighted using digital weight balanced.

Doubling time and the relative growth rate of Azolla were being calculated by using the formula that had been described by Subudhi and Watanabe (1981). Next, the dried sample of Azolla was ground using Waring Commercial Blender about <1 mm and to make it suitable for proximate and fiber component analysis (Kavya, 2014, p. 27; Ooi et al., 2012).

Proximate analysis has been conducted to determine a total dry matter, crude protein, crude fiber, ether extract and inorganic matter of all the treated *A. pinnata*. The procedure was done according to AOAC (2005) procedure. Meanwhile, fiber component such as NDF and ADF were identified using Van Soest's method (1981).

All data were analyzed by using IBM SPSS Statistics 23. Two-way ANOVA test was conducted to analyze the mean of the parameter with two independent factor which is three type of dung manure (goat, chicken, cow) and five different concentration of dung (50 g, 100 g, 150 g, 200 g, and 250 g) were compared using ANOVA and Post Hoc determination by Duncan.

Results and discussion

From the data obtained during the 14-days observation, relative growth rate of A. pinnata cultured in 200 g and 250 g of goat manure dilution were significantly high than other treatment as showed at Table 2.

This could be attributed with the study performed by Nordiah, Sidik, Muta Harah, Wan Hazma (2012) which has highlighting the correlation of nutrient composition from different type of water source with the growth of *A. pinnata*. There also had reported the growth performance of *A. pinnata* in different type of water source show a vary increase in plant biomass as this due to the nutrient content that present in the water.

This perception was supported with Setiawati et al. (2018) through her study that proving that the environment condition might lead to some variation of Azolla growth performance, therefore it might cause differences in growth rate of *A. pinnata*.

Table 2: The total fresh weight of the *Azolla pinnata* for all treatments

Treatment (s)	Chicken Manure (g) [c]	Cow Dung (g) [c]	Goat Dung (g) [d]
50 g	460±0.47[a]	589±0.03[a]	2000±0.31[a]
100 g	789±0.03[ab]	748±0.03[ab]	2400±0.20[ab]
150 g	922±0.04[ab]	790±0.03[ab]	2600±0.12[ab]
200 g	860±0.05[b]	884±0.04[b]	2800±0.12[b]
250 g	1211±0.12[b]	1020±0.01[b]	2800±0.12[b]

[a, b] Means with different superscripts within rows were significantly different ($p<0.05$),
[c ,d] Means with different superscripts within columns were significantly different.

Meanwhile, propagation was measured by time taken as the plant were doubling on the water surface. The shorten time will indicate the fastest doubling time and the higher biomass will be produced.

Table 3: The doubling time of the *Azolla pinnata* for all treatments

Treatment (s)	Chicken manure (days)[c]	Cow dung (days)[c]	Goat dung (days)[d]
50 g	2.97±0.79[a]	3.27±0.18[a]	2.37±0.26[a]
100 g	2.97±0.09[ab]	3.03±0.18[ab]	2.23±0.13[ab]
150 g	2.86±0.15[ab]	2.98±0.12[ab]	2.18±0.06[ab]
200 g	2.91±0.18[ab]	2.88±0.13[ab]	2.14±0.06[ab]
250 g	2.66±0.25[b]	2.88±0.13[b]	2.14±0.06[b]

a,b Means with different superscripts within rows were significantly different ($p<0.05$),
c,d Means with different superscripts within columns were significantly different.

This study has identified that utilization of goat manure was a best manure in producing *A. pinnata* compared with chicken and cow manures. 200 gram was an optimum dosage due to the insignificant changes despite using of 250 gram of goat manure as in the table 3. However, the significant different between type of manures can be related with report by Barker, Hodges and Walls (2002) which has scientifically analyze content of NPK element in goat manure compared to broiler and cow manure. *A. pinnata* able to survive even though in low nitrogen as it has symbiotically interacted with *Anabeana azollae* in fixing nitrogen from environment. (Mayashree, et al., 2017).

However, phosphorous which is identified as a limitation because it required to be converted into an anion phosphate for plant absorption purpose. Basically, high availability of phosphate will be produced high biomass production of *Azolla* as it related to the animal's diet (Mowa, 2018).

Conclusion

This study has been conducted to evaluate the growth performance of *A. pinnata* under different types of manure and concentrations. The aim of this study is to facilitate the farmers by suggesting the most suitable fertilizer that can be used to cultivate the *A. pinnata* over short period of time, whereby a sustainable *Azolla* crop production can be achieved especially in Malaysia. Based on our finding, *A. pinnata* shows an excellent growth performance under treatment of goat dung manure at concentration 200 g and 250 g, whereby, at this concentration the *A. pinnata* give the fastest rate to propagate and double from the initial value. Furthermore, this study could be suggested to farmers to use goat dung at concentration of 200 g or 250 g as the fertilizer in cultivation of *A. pinnata*. High production yield can be achieved with minimum cost by switch pelleted food to *A. pinnata* in livestock diet.

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2.8 Feed intake, digestibility and growth performance of female goats fed feeding Napier grass supplemented with *Gliricidia sepium*

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Introduction

In Malaysia, natural or cultivated forages plays a very important role in livestock nutrition (Najim, Amin, Karim, & Mei, 2015). Amongst the forages, Napier grass plays a significant role in ruminant feeding which is a multi-purpose crop and widely used as silage, rotational pastures and as soil-improving crop in semitropical and tropical region (Kidder, 2004). The Napier grass shows a high value in dry matter, crude fiber, detergent fiber and low in crude protein (CP). However, protein has contributing at least 13.24% of livestock industry including ruminant, poultry and agriculture (Parish & Rhinehart, 2008; Pothidee, Allen, & Hudson, 1999; Rana, Siriwardena, & Hasan, 2009). Furthermore, the reduced pasture area urged farmers to rely more on concentrates than roughage thus increasing the production cost (Rahman, Nakagawa, Abdullah, Embong, & Akashi, 2014). Thus, silage-making is one of the alternative solutions especially to keep forage available as the main source of feed with produce a stable feed with a high recovery of dry matter, energy, and highly digestible nutrients compared with the fresh crop (Dunière, Sindou, Chaucheyras-Durand, Chevallier, & Thévenot-Sergentet, 2013; Kung, Shaver, Grant, & Schmidt, 2018). In addition, Archimède, González-García, Despois, Etienne, and Alexandre (2010) concluded that gliricidia forage may bea viable alternative to replace conventional energy and protein supplements in animal diets.

Silage is fermented and high-moisture stored fodder (Wood, 2012; Nizami, Korres, & Murphy, 2009; Cowan, 2000) and has been traditionally used to ensure satisfactory preservation of the crops for dry season and winter feeding in Europe (Kung, Stokes, & Lin, 2003). Zailan, Yaakub and Juson (2018) reported a significant increase of CP content from 8.13% to 9.26% after ensiling process. A local study in goats showed that substitution of the Napier grass with corn silage improved the weight gain, feed intake and digestibility (Khaing, Loh, Ghizan, Halim, & Samsudin, 2015). In addition, the concentrate requirement can be reduced by corn silage inclusion into the ration (Keady, Gordon, & Moss, 2013). Napier grass is commonly ensiled with different types of legumes and forages to enhance the nutritive value of the diet feed to the dairy animals. *Gliricidia* leaves are cheap and readily available sources of protein (Hao & Ledin, 2001). Supplementation of *Gliricidia sepium* in silo can increase the nutritive value of the silage and have proven to show positive results according to National Forage Testing Association (1987). In addition, foliage from tree legumes such as *Leucaena leucocephala* or known as white lead tree and *Gliricidia sepium* can be used as

supplement to Napier Grass fodder by means of improvising the live weight gain of young cattle, the dry matter intake, and lactation performance of dairy cows (Abdulrazak, Muinga, Thorpe, & Ørskov,1997). It is essential to provide better diet due to poorer quality of fresh Napier grass and its silage due to high water content (Mendieta-Araica, Spörndly, Reyes-Sánchez, & Spörndly, 2011).

Nowadays, nutritionists have been searching for natural strategies with low cost and easy application in order to improve animal performance (Durmic & Blache 2012). Therefore, the study was designed to evaluate the nutritive value of different types of diet to enhance growth performance in female goats.

Material and methods

Experimental animals and feeding treatment

Nine non-pregnant female goats with range live weight (LW) approximately 16.00 kg to 37.50 kg and age ranged between 24 months to 60 months old were obtained from the flock at the Ladang 16, UPM. In a completely randomized design using three triplicates, the does were allotted into three treatments: Treatment 1 serves as control composed of 100% fresh Napier grass (N); Treatment 2 is 100% Napier grass prepared as silage (SN); Treatment 3 is a mixture of 85% Napier grass silage and 15% *Gliricidia sepium* leaves prepared as silage (MIX). Each animal was housed in single pent which sized 4ft x 4ft as recommended by UPM Code of Practice for The Care & Use of Animals for Scientific Purposes (IAUCUC, UPM.) The Napier was harvested from Ladang 16, UPM chopped by using mechanical chopper then fed freshly to the animals. *Gliricidia sepium* leaves were collected at Ladang 2, UPM. The mechanically chopped Napier grass and *Gliricidia sepium* leaves were undergone 3-weeks fermentation and fed to animals. The amount of Napier grass given to animals was 10% of animal liveweight based on dry matter (DM). The does were fed 3% of their body weights with the silage. Fresh water was supplied daily. The goats were fed to those treatments for 14 days adaption period before the commencement of 56-days feeding trial.

Analytical procedure

To determine the nutrient composition on each variety, representative feed samples (200 g each) were taken from each treatment group. Approximately 100g of faecal sample were collected directly from anus of each animal and kept in seal bag. Both feed and faecal samples were oven-dried at 60 °C for 48 hours, ground to pass a 1-mm mesh screen sieve and stored for chemical analysis. The nutritive values were determined by FOSS DS2500 Near Infrared Spectroscopy (NIRS) with additional calibration from the fodder samples analysed using standard laboratory procedure. The amount of crude protein (CP) was measured and calculated (N x 6.25) (AOAC, 1990). While the neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using FOSS

FiberCap 2023 System (ISO13906, 2008). Feed intake was determined on the following day by weighing the remnants and subtracting it from the feed served. Representative sample from the orts was obtained for chemical composition analysis. During the feeding trial, the liveweight (LW) was measured once weekly before the morning feed was offered. The average of LW gain daily was calculated by dividing the initial and final LW differences by total number of experimental days (56) days. Feed conversion ratio (FCR) is the ratio between feed intake and LW gain. Nutrient digestibility was calculated by dividing the differences between the feed and faecal composition values with the feed nutritive value, then converted into percentage values.

Statistical analysis

Data were statistically evaluated using an analysis of variance procedure using one-way ANOVA. Differences in $P<0.05$ were significant and all results in the text were stated as Mean values ± Standard error of means (S.E.M).

Results and discussion

Even though there is no statistically difference of final liveweight (LW) between treatment groups, goats fed mixture of 85% Napier silage and 15% *Gliricidia sepium* silage (MIX) had the lowest final LW. This perhaps is linked with the introduction period of the new types of feed. New feeds should have introduced gradually over period of days or weeks (Khaing et al., 2015). However, these goats showed the highest total weight gain (TWG) and daily weight gain (Table 1). The fermentation process softens the hard part of Napier grass and become easily eaten. The quality of feed was also improved with the odour of *Gliricidia sepium* silage able to attract the female goats. In addition, the feed conversion ratio (FCR) of MIX were observed insignificantly higher than those fed with 100% fresh Napier grass (N) and 100% Napier silage (SN) which indicated the mixture of 85% Napier grass and 15% *Gliricidia sepium* silage could be an efficient feeding strategy to increase palatability of goats.

Table 1: The growth performance of goats fed with the experimental diets

	Types of diet		
Item	**N**	**SN**	**MIX**
Initial LW (kg)	26.5 ± 6.08[a]	31.7 ± 4.36[a]	24.0 ± 6.00[a]
Final LW (kg)	30.6 ± 6.00[a]	35.0 ± 4.36[a]	28.7 ± 6.27[a]
Total weight gain (TWG) (kg)	4.1 ± 0.60[a]	3.3 ± 0.00[a]	4.7 ± 0.76[a]
Daily weight gain (g/day)	70.0 ± 10.60[a]	60.0 ± 0.00[a]	80.0 ± 13.75[a]
Daily dry matter intake (DMI) (g/day)	6.67 ± 0.70[a]	2.82 ± 0.21[b]	2.30 ± 0.19[c]
Feed conversion ratio (FCR)	4.20[a]	4.20[a]	5.00[a]

All analyses are Mean ± Standard error of means (S.E.M.) of N = 3. Means with different superscript letters in each parameter are significantly different ($P \leq .05$) using One-way

ANOVA. N 100% fresh Napier grass, SN 100% Napier grass silage, MIX 85% Napier grass silage + 15% *Gliricidia sepium* leaves silage.

The quality of forage is indicated by the nutrient digestibility of goats as presented in Table 2. Dry matter digestibility (DMD) was enhanced by the mixture of ensiled grasses ($p > 0.05$). The DMD ranged from the least (58.35%) in N to the highest (67.38%) in MIX ($p > 0.05$). The least DMD at 58.35% in diet of N was attributed to the high fibre content. Percentage fibre composition is a function of cutting intervals of Napier grass and may have effects on DMD. A decrease on dry matter digestibility with increasing age of cutting for the grass (Bamikole, Akinsoyinu, Ezenwa, Babayemi, Akinlade, & Adewumi, 2004). The highest ADF digestibility (41.19%) was observed for the MIX ($p < 0.05$). Hard stem parts of Napier grass had refused by the female goats and counted as feed refusal. The feed intake of female goats increased slowly because of this (Table 1). The hard part of stem became soft upon three-weeks fermentation process. It eases the chewing and increases palatability of female goats. There is a trend of higher crude protein digestibility ($p > 0.05$) of SN and MIX than N showed the increased palatability of female goats. Lower CP digestibility N might have promoted ammonia-N concentrations in rumen fluid which limited the growth of cellulolitic bacteria (Satter and Slyter, 1974). The feeding treatment of MIX showed high digestibility of NDF ($p > 0.05$) and ADF ($p < 0.05$) due to the high amount of fermentable carbohydrate in silage.

Table 2: Apparent digestibility (%) of chemical composition by goats fed different diets

	Types of diet		
Item	**N**	**SN**	**MIX**
DM	58.35 ± 7.48[a]	61.31 ± 8.94[a]	67.38 ± 10.05[a]
CP	18.32 ± 3.79[a]	19.42 ± 4.56[a]	20.19 ± 6.31[a]
OM	36.52 ± 18.49[a]	23.30 ± 12.56[a]	12.28 ± 6.71[a]
NDF	3.90 ± 3.35[a]	1.89 ± 1.47[a]	2.88 ± 1.68[a]
ADF	20.96 ± 0.00[a]	32.29 ± 5.73[b]	41.19 ± 6.79[c]
ADL	26.01 ± 5.03[a]	22.91 ± 5.68[a]	20.09 ± 7.52[a]

All analyses are Mean ± Standard error of means (S.E.M.) of N = 3. Means with different superscript letters in each parameter are significantly different ($P \leq .05$) using One-way ANOVA. N 100% fresh Napier grass, SN 100% Napier grass silage, MIX 85% Napier grass silage + 15% *Gliricidia sepium* leaves silage, DM dry matter, CP crude protein, OM organic matter, NDF neutral detergent fibre, ADF acid detergent fibre, ADL acid detergent lignin.

Chemical composition of all feeding treatments is presented in Table 3. Crude protein (CP) content in MIX was evidently higher than that of N due to a high protein content in the *Gliricidia sepium* and total N concentration (Richards, Brown, Ruegsegger, and Bates, 1994). The amount of dry matter (DM) in grazing grass is important as it act as an indicator of the total amount of nutrients that are available to the animal. Dry matter can affect the adequate for the ruminants it can affect their performance. The fresh Napier grass contains the highest DM percentage ($p < 0.05$) because of does not undergo fermented process that affect the DM percent in the sample. The feeding treatment of MIX was stated the lowest DM percentage ($p < 0.05$) due to the ensiling process that affect the DM percentage in the forage sample. However, this feeding

treatment had the highest organic matter (OM) percentage which is important because can affect body weight and products such as milk. On the other hand, the MIX was lower especially in ADF ($p < 0.05$) and ADL ($p > 0.05$) than that of N. This might due to the age of forage which always contribute a significant role in the nutrient composition (Ajayi, babayemi and Taiwo, 2007) and the supplementation of *Gliricidia sepium* during ensiling process. The highest NDF percentage of MIX may due to late maturity of Napier grass during harvesting (Bamikole et al., 2004). However, the relatively low ADL as obtained for MIX in this study may be desirable as source of energy.

Table 3: Chemical composition (%) of dietary treatments

	Types of diet		
Item	**N**	**SN**	**MIX**
DM	91.78 ± 0.47[a]	91.22 ± 1.15[b]	90.71 ± 1.23[c]
CP	7.10 ± 0.32[a]	7.27 ± 0.30[b]	7.38 ± 0.55[c]
OM	16.12 ± 1.34a	16.96 ± 1.26a	18.26 ± 1.83b
NDF	69.69 ± 3.04[a]	68.21±1.43[b]	70.57 ± 2.19[c]
ADF	41.03 ± 1.31[a]	37.99 ± 1.98[b]	36.87 ± 1.76[c]
ADL	39.04 ± 1.09[a]	38.96 ± 1.70[a]	37.35 ± 3.09[a]

All analyses are Mean ± Standard error of means (S.E.M.) of N = 56. Means with different superscript letters in each parameter are significantly different ($P \leq .05$) using One-way ANOVA. N 100% fresh Napier grass, SN 100% Napier grass silage, MIX 85% Napier grass silage + 15% *Gliricidia sepium* leaves silage, DM dry matter, CP crude protein, OM organic matter, NDF neutral detergent fibre, ADF acid detergent fibre, ADL acid detergent lignin.

Conclusion

The association of Napier grass with silage of legumes; *Gliricidia sepium* improved the texture and nutritive values in pasture fed to the goats. Thus, it able to improve the feed intake (palatability), digestibility and growth performance of goats.

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2.9 Divergent loci in natural populations of *Gadus chalcogrammus,* walleye Pollock

Nadiatul Hafiza Hassan, Yoshihisa Suyama, Yu Matsuki and Masakado Kawata

Introduction

Elucidating the process and driving factors underlying genetic differentiation is an important challenge in ecology and evolution. In marine fish species especially migratory species, population differentiation is weak due to high level of gene flow and migration of marine species may be restricted by a number of factors including gradients of salinity, temperature, oceanic currents and other factors. *Gadus chalcogrammus* has widespread geographical distributions and exploits different ecological niches from extremely low temperature to variable ocean temperature. Populations from latitudinal gradient provide natural replications to study the evolutionary response towards the environmental gradients. A microsatellite-associated DNA sequencing method, MIG-seq was applied to study the genetic structures and divergent loci in *G. chalcogrammus* from Bering Sea and Japans' ocean. A total of 160 SNP loci were obtained from the adopted method and population divergence and structuring were observed in both populations. The F_{ST} values confirmed the genetic break between the populations (F_{ST}: 0.05–0.07). The evidence of outlier SNPs enhanced the dramatic divergence between Bering Sea and Japan oceans' populations (F_{ST}: 0.183-0.625). A correlation study of allele frequencies at each SNP locus with environmental parameters showed the occurrence of two divergent SNP loci, which might respond to an ocean temperature and salinity gradients in the populations. Further investigation by BLAST were positioned the loci near to a gene that play a vital role in transcription of major histocompatibility (MHC) gene, an evolutionary conserved gene in adaptive immune system in vertebrates, which might explain adaptive selection occurred on the gene.

Materials and methods

We sampled 6 and 1 local populations of *G. chalcogrammus* in Japans' ocean and Bering Sea, respectively, during spawning and pre-spawning seasons (n=181). The muscle tissues were preserved in 95% of alcohol and total genomic DNA was extracted using DNeasy Tissue Kit according to manufacturer's protocol (Qiagen Hilden, Germany). To quantify genetic variation across the populations, genomewide SNPs using multiplexed intersimple sequence repeats (ISSRs) genotyping by sequencing (MIG-seq) (Suyama & Matsuki, 2015). The MIG-libraries were prepared from single end sequencing according to Suyama and Matsuki (2015) and was sequenced using Illumina Miseq (Illumina, San Diego, Ca, USA). Low quality of reads from raw sequencing data were removed suing FASTX toolkit. First SNP appeared on the sequence tags was used for population analysis to avoid linkage between SNP loci. The program Stacks 1.20 (Catchen *et al*.

2011) identified 160 SNPs loci for *G. chalcogrammus* populations. The loci were further used to assess the genetic heterogeneity and divergent loci that were correspond to natural selection in pollock populations. Four methods were used to scan for signature of divergent loci, which based on F_{ST} genome scan analysis and associative method of allele frequencies and environmental variables.

Results and discussion

The genetic structure of *G. chalcogrammus* was carried out using STRUCTURE as depicted in Figure 1 and the clustering was consistent with the F_{ST} between populations. The significant genetic heterogeneity was showed in 160 SNP loci of *G. Chalcogrammus* populations. Eventhough, pairwise estimates of F_{ST} were in higher magnitude than those reported in other marine species, but the results corroborated previous findings of significant population differentiation in the Japans' ocean that those in Bering sea (Suda et al., 2017; Liu et al., 2016; Grant et al. 2010).

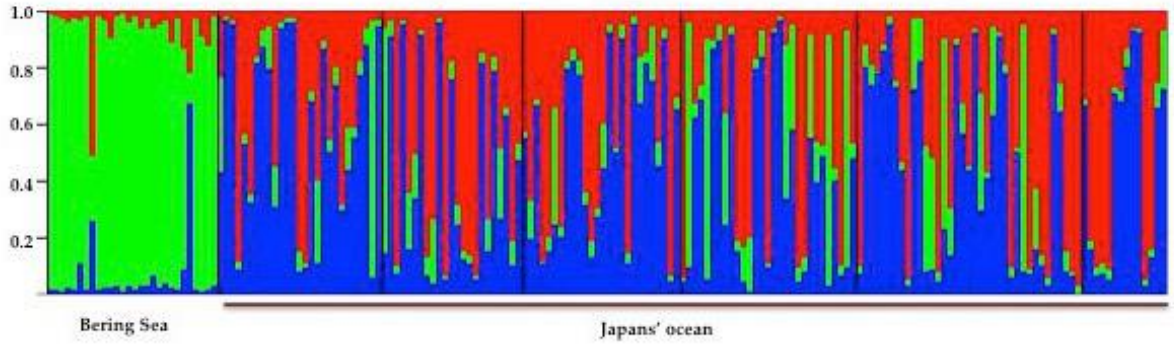

Figure 1: Genetic structure of *G. chalcogrammus* using STRUCTURE (Pritchard et al., 2000) based on 160 SNP loci. Red, blue and green represent the proporations of inferred ancestry from 2 populations; Bering Sea and Japans' Ocean.

Genome scans of 160 SNP loci using multiple platforms have revealed three divergent SNP loci associate with temperature and salinity gradient in localities of *G. chalcogrammus* (Figure 2). BLAST-searched of divergent SNP loci against Atlantic cod have located two loci near to the ZXDC gene, a known gene which encoded for protein that is important in assisting transcription of histocompatibility complex (MHC I and II) gene. The MHC is conserved adaptive gene existed in vertebrate immune system and cod was reported the first species to lost MHC II gene (Malmstrøm et al., 2016; Djikstra et al., 2013; Star et al. 2011). The two divergent SNP loci showed the significant allele frequency clines in Bering Sea population owing its properties as a distinct population from others (result is not showed).

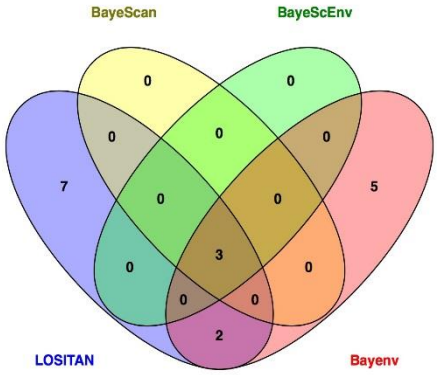

Figure 2: Venn diagram illustrating the overlap of divergent SNP loci identified by multiples genome scan platforms.

Conclusion

Despite the limited number of SNP loci, this study was able to identified adaptive genetic structure and divergent loc in natural populations of *G. chalcogrammus*. The identified divergent SNP loci were hypothesized to strongly correlate with temperature and salinity of the oceans, with two loci may have possible association with transcription of MHC gene in *T. chalcogrammus.*

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2.10 Behavioural and hormonal study of captive Sambar deer (*Rusa unicolor*) at Ladang 16, Universiti Putra Malaysia

Noorazemah Mohd Fuzi, Kushaal Selvarajah, Mohd Noor Hisham Mohd Nadzir and Geetha Annavi

Introduction

Sambar deer (*Rusa unicolor*) are classified under Cervidae family, which distributed throughout Southeast Asia region (Leslie D. M., 2011). Their habitats ranging from tropical dry forests, seasonal moist evergreen forests, subtropical mixed forests to tropical rainforests. Sambar deer are mostly nocturnal and living alone or in a very small group as they are more solitary in their way compare to the other species of deer. It is listed as vulnerable by International Union for Conservation of Nature (IUCN) in 2015 (Timmins et al., 2015). In Peninsula Malaysia, the number of Sambar deer is decreasing drastically as they are known to be hunted illegally in their natural habitat for food and trading (Kawanishi et al., 2014). Sambar deer is the key prey of *Panthera tigris* or Malayan Tiger, thus it becomes the population determinant of Malayan Tiger (Sunquist, 2010). Information regarding reproductive activity and associated behaviour are important for the progress of management strategies of this species in captivity. However, such information is still lacking. Thus, this study was conducted to determine the solitary and social behaviours of Sambar deer, to measure the level of reproductive hormone (progesterone and estradiol) in faeces of females Sambar deer and to find the correlation between behaviour with reproductive hormone of female Sambar deer. This study on both behavioural and hormonal activities of Sambar deer is essential to help to increase in the number of this species in captive breeding centres to continue the survival of both the Malayan Tiger and Sambar deer for a balance ecosystem.

Materials and methods

Study area

This study was conducted at Ladang 16, Universiti Putra Malaysia (GPS coordinates [lat, long] 2.987, 101.731). The Sambar deer were housed in an approximately 10 m x 8 m paddock surrounded by 2 m high of enclosure (Figure 1).

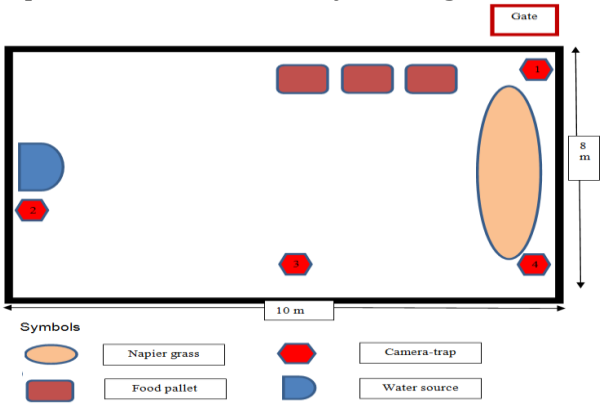

Figure 1: Paddock of Sambar deer

Behaviour recording

Two adult males and two adult females were selected for this study and they were identified by tags on their ear with different colours. Both females have their own fawn, however, the behavioural activity of the fawns was not observed. Daily observations commenced at 0900 to 1700 hours from Monday to Thursday. Behavioural study was divided into two sessions, where first observation was carried out between 0900 and 1200 and the second session was at 1400-1700. In this study, direct observation was conducted for a total of 120 hours where each subject was observed for 6 hours per week for 5 weeks between June and July 2018. Behavioural study was conducted using focal sampling method. All the subjects were observed for the same sampling period and interval. The focal sampling method was used by observing one deer per day throughout each session for a sampling period of 10 minutes with 5 minutes interval following Altmann (1974). Different types of behaviour exhibited by subjects as per described in ethogram (Table 1) was recorded on recording sheet. The deer were observed from outside of the paddock in order to avoid any disruption in their behaviour due to the presence of the observer.

As for night observations, four camera traps were deployed surrounding Sambar deer paddock (Figure 1). All the cameras were set to record between 1800 and 0900 from Monday to Thursday. Both female and male can be differentiated as they have a different physical characteristic. For night observations, the frequency of the behaviour that was captured was note down per hour.

Hormonal study

Animals and management

The two selected females which were subjected for behavioural observation in this study were healthy, non-pregnant Sambar deer between four and six years old with an average body weight 190 ± 2.90 kg. Both females were raised at the University's deer breeding unit. The females were given fresh grass of *Pennisetum purpureum* (Napier grass) on Monday and Tuesday and food pallet on Wednesday and Thursday.

Faeces analysis

The faeces were collected from Monday to Thursday. Fresh faeces of the females were collected the soonest as possible after defecation during the 5 minutes time interval of behavioural observation and the samples kept in micro-centrifuge tube. The two females can be easily identified as they have different colour of ear tag and defecated at different places each time. The faeces than were kept in ice box before transported to Genetic Lab, Department of Biology, Faculty Science, UPM and stored at -20^{o}C until further procedures. The process of faeces extractions and the analysis of faeces steroid

metabolites followed the method in Palme (2013). Plasma estradiol and progesterone concentrations of each sample were measured using ELISA kit. The estradiol and progesterone assay specificity was 100% and their sensitivity were 6.2 pg/ml and 0.045 ng/ml respectively.

Table 1: Ethogram for sambar deer

Types of behaviour	Behaviour	Code	Description of behaviour
Solitary	Resting	STD (S)	Standing motionlessly
		STD (F)	Standing while flipping ears
		STD (C)	Standing while mouth is moving (chewing)
		STD (W)	Standing while wiggling tails
		SIT (S)	Body is lying down motionlessly
		SIT (F)	Body is lying down while flipping ears
		SIT (C)	Body is lying down while mouth is moving (chewing)
		SIT (W)	Body is lying down while wiggling tails
	Locomotion	L	Trotting, running, walking, jumping
	Feeding and Drinking	F&D	Using tongue and incisors, licking and drinking
	Grooming	G	Licking and nibbling, scratching, rubbing
	Elimination	E	Defecating, urinating
Social	Sexual	S	Sniffed and lick female's vulva, male followed female, male lick female's urine
	Recognition	RG	Sniffing, groomer position to lick mouth, back of head or antler
	Adornment	A	wallow in mud, lay down body flat and move right or left on the ground, adorn its antlers
	Alarm	AL	Tail erected, head raises, ears are alert
	Vocalization	V	Bleating, mother-young relationship

Data analysis

The data were analysed in R Statistical Package Version 3.0.3 and IBM SPSS Statistic 23. For R Statistical Package Version 3.0.3, the data were analysed in lme4 package using the glmer function and set generalised linear mixed-effects model (Bates et al., 2016)

and in MuMIn package using model averaging based on information criteria, AICc (Akaike's Information Criterion) (Barto´n, 2016). The Y-axis represents the frequency of behaviours (Table 1) averaged per day for each individual. The random effects were individuals' identification number and days. Temperature, humidity, sex and session were included as fixed effects. The temperature and humidity were standardised to a mean of zero and a standard deviation of two. By their AICc value, the models were ranked, and the top model had the lowest AICc value and only plausible model if the model ranked (ΔAICc) was ≤ 7) (Burnham et al., 2011). For IBM SPSS Statistic 23, the test that was used is T-test for day versus night observation. The data obtained from the ELISA plate reader was the raw data for both progesterone and estradiol hormones and was calculated by the formula by Palme (2005).

Results and discussion

Daytime behaviour observation

Among the tested solitary and social behaviours (Table 1), only adornment was differed significantly with gender where the females had a higher a frequency than males (Figure 2). Adornment is where the deer just wallowing the body in the mud, lay down body flat and move right or left on the ground and adorn its antlers. Adornment behaviour can serve as thermoregulation process for Sambar deer (Aun and Rahman, 1989). Female usually have a higher fat content; therefore, it will tend to do the thermoregulation process more comparing to male. Female have a larger ratio of body surface to body mass thus a greater fat content has differences in resting body temperature and thermal responses (Kaciuba and Grucza, 2001). Therefore, thermoregulation process was higher in female than male and increased the adornment behaviour for females. The high temperature and the low humidity may probably be the reason as the thermoregulation of deer were served by wallowing and lay down body flat on the ground. Adornment behaviour was also related in deer to inhibit the external parasites growth on their body (Aun and Rahman, 1989).

During this study, none of males had recorded to vocalise. Only females vocalised, and this can be related to mother-young relationship (as described in Table 1). The mother bleated to find the young and to assure whether the young is within a hearing range while the young bleated to solicit attention from the mother. From the observation, the vocalisation by females was mostly common in the morning. The mother tends to roam freely without their young surrounded the paddock in the morning. While the mother is roamed freely, the young are still asleep (Aun and Rahman, 1989). As the young wake up, they might want to assure where their mother is, and this might be the reason why the frequency of this behaviour was higher in the morning session compare to the afternoon session.

As for other behaviours, there were no significant differences with both sessions and gender. However, as the duration of this research was only limited for 5 weeks due to time constraint, the accurate results might not be obtained, and longer period of study needs to be done.

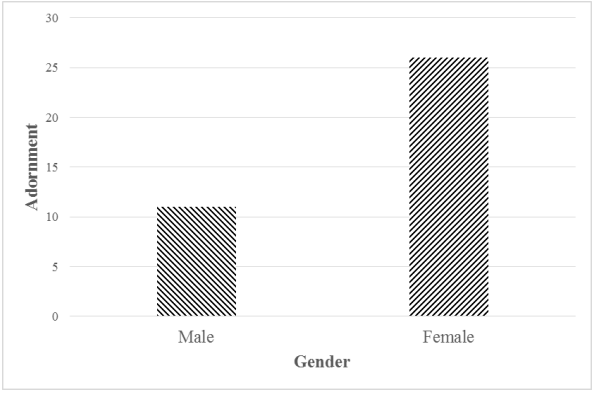

Figure 2: Total number of adornment behaviour between male and female during daytime

Day versus night behaviour observation

For night observation, only solitary behaviour was caught on the camera traps that were set up surrounded the paddock. Table 2 shows the mean values for behaviour of Sambar deer during the day and night ± Standard error of mean (SEM). All of the behaviour on both day and night showed a significant difference ($p<0.05$). Sambar deer exhibited resting, locomotion and grooming behaviour at a higher frequency during the night time compared to the day time. As stated by Caboń Raczyńska et al. (1987), where the general activity pattern of deer during the day and night were active and several peaks in activity during the 24-h period showed that the behaviour of the Sambar deer were not constant all the time. Compared to locomotion and grooming, resting was the behaviour that mostly showed by the Sambar deer during the night compared to day. However, this was contradicted to the facts that Sambar deer was a nocturnal species. A previous study by Rice (1986) stated that in order to avoid predators, selection of behaviour may become the reason for the nocturnality for Sambar deer. Another reason might due to the changes of the environment thus changed the normal behaviour as supported with the statement of individuals in captivity no longer demonstrated appropriate reactions (McPhee and Carlstead, 2010). Growing up in a captive environment that is more restrictive than the wild can alter how an animal learns and change how it responds (McPhee and Carlstead, 2010). Feeding and drinking had a higher frequency during the day. Sambar deer ate and drank more during the day was because due to the fact that the food was provided by the keeper during the day and no other sources were available in the paddock.

Table 2: Behaviour of Sambar deer during the day and night Mean ± Standard error of mean

Time	Resting	Locomotion	Feeding & drinking	Grooming
Day	112.4±14.45	27.45±3.38	167.9±14.12	2.95±0.86
Night	349±14.42	98.7±2.59	75±2.48	12.25±1.40

Hormone study

The mean concentrations level of progesterone was below 1 ng/g and estradiol was more than 1 ng/g. When the progesterone level was low, the estradiol level was high and vice versa. Progesterone level that falls below 1 ng/g shows that the females were in estrous state. From the hormone analysis females A and B were detected likely to be on oestrous for 15 and 22 continuous days respectively (Figure 3 and 4). The normal cycle of oestrous of a deer is 17 days and the concentration level of progesterone during oestrous for deer will be below 1 ng/g (Hafez et al. 2000). The results also were supported by previous study by Pereira et al. (2005), where it stated that steroid analysis of faeces is a good non-invasive method and practical to know the endocrine status of a deer.

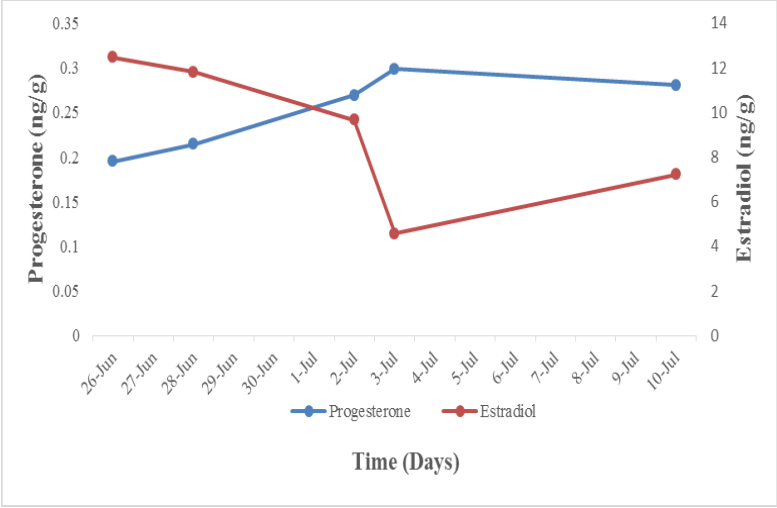

Figure 3: Concentration level of Progesterone and Estradiol for Female A

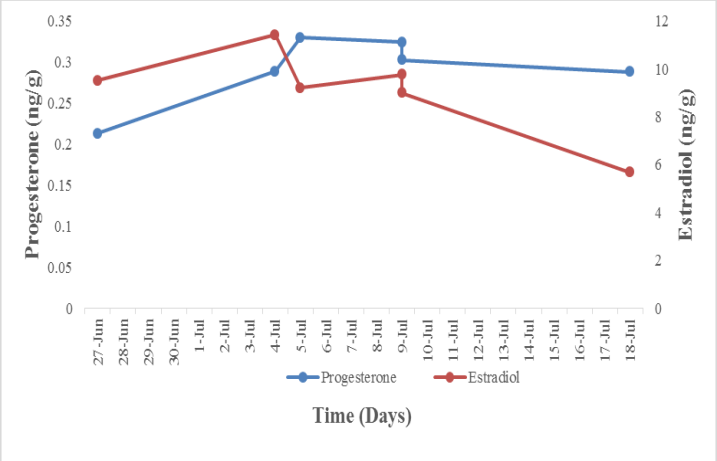

Figure 4: Concentration level of Progesterone and Estradiol for Female B

Correlation between behaviour and reproductive hormone of female Sambar deer

There was a negative correlation between social behaviour and progesterone level ($r = 0.74$, $p<0.05$). Low progesterone level shows that the deer was in estrous state and ready for mating. During mating, the females tend to show increase activities of social behaviour with males. This was supported by Adkins (2005), when females are in estrous, they do not fight males but will form social affiliations for mating. Pereira et al. (2005) also stated that there were significant correlations between fecal steroid hormones and reproductive behaviour of the deer.

Conclusion

Information regarding behavioural and reproductive activity is important for the progress of management strategies of species in captivity. For day observation, only adornment was differed significantly with gender where the females had a higher a frequency than males. The high temperature and the low humidity may probably be the reason as the thermoregulation of deer served by wallowing and lay down body flat on the ground. Besides, during this study, none of males had recorded to vocalise. Only females vocalised, and this can be related to mother-young relationship. As for other behaviours, there were no significant differences with both sessions and gender. In addition, the behaviour of feeding and drinking had a higher frequency during the day than night time. The possible reason for this because the food for the Sambar deer was given by the keeper during the day. For night observation, only solitary behaviour was caught on the camera traps that were set up surrounded the paddock and for night observation, they had a higher frequency of resting, locomotion and grooming compared to day observation. For hormone study, the reading of concentration level of progesterone was below 1 ng/g and concentration level of estradiol was more than 1 ng/g. There was a negative correlation between social behaviour and progesterone level. Low progesterone level shows that the deer was in estrous state and ready for mating. For future studies and recommendation, the behavioural study and hormonal

study need to be done in a longer period of time for better and accurate results. Addition of camera traps for both day and night observation also resulting in a better observation and data collection. It is also important to determine the accuracy of the hormone concentration to know exactly whether the female Sambar deer was on oestrous throughout the study.

Acknowledgement

We are grateful to the management officers of Ladang 16 for permitting us to study the behavior of Sambar deer. We thank all the keepers who help during field study. This study was approved by the Institutional Animal Care and Use Committee (IACUC) under reference number: UPM/IACUC/AUP-R042/2018, Universiti Putra Malaysia.

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CHAPTER 3: PLANT PHYSIOLOGY

3.1 Taxonomic value of stomatal complexes in *Shorea* Roxb. (Dipterocarpaceae)

Noraini Talip., Amirul-Aiman Ahmad Juhari, Ruzi Abdul Rahman and Hamidun Bunawan

Introduction

Surface distribution patterns of stomata based on their orientation and dispersion are found to be stable and hence could be taxonomically useful (Noraini & Cutler 2009). The developmental pattern of the stomatal complex shows absolute constancy in the majority of families (Van Cotthem 1970, 1973). The relationships discovered between some types of stomata are significant in the resolution of many taxonomic problems, such as establishing the position and size of taxa of various ranks and clarifying the relationships between different types of stomatal apparatus (Noraini & Cutler 2009). Metcalfe & Chalk (1950) reported that the stomata in the Dipterocarpaceae are confined to the lower surface, surrounded by more or less distinct subsidiary cells, the latter being particularly well-defined and parallel to the pore (rubiaceous or paracytic) in some *Balanocarpus, Shorea* and *Vateria.* The taxonomic problems in *Shorea* mostly involve identification and classification, exhibit continuous morphological variation at specific levels, the infrequent flowering and fruiting season makes the process of identification more difficult and complicated (Noraini 2006). The overall objective of this study was to determine whether stomatal anatomical characters in *Shorea* could be of taxonomic value in systematic and diagnostic investigations.

Materials and methods

Specimens were obtained from the Herbarium, Royal Botanic Gardens, Kew, Richmond, United Kingdom and the Herbarium, Forest Research Institute of Malaysia, Kepong, Malaysia and fresh specimens were from the arboretum and various forest reserves in Peninsular Malaysia. 23 species with 92 samples were collected, the leaves part were fixed, embedded and peeled followed the method described by Johansen (1940) and Sass (1958) with suitable modifications. All slides were photographed and observed using Leitz Diaphlan polarizing microscope and Reichert Polyvar 2 Microscope attach with a digital camera. Images were processed using Image Analysis and Adobe Photoshop software. Observation and measurement under scanning electron microscope follows Noraini (2006).

Results and discussion

The anatomical characters of stomata of *Shorea* species studied are presented in Table 1. The pattern of stomatal distribution in this present study was classified as scattered,

crowded or arranged in a more or less circular or semi-circular pattern. All the species examined are hypostomatic. *Shorea* found to have seven types of stomata, paracytic (Fig.1b, Fig.1d, Fig.1h, Fig.1e), staurocytic (Fig. 1d, Fig.1f, Fig.1i, Fig.1j), hexacytic (Fig.1d, Fig.1e), stauro-cyclocytic (Fig.1a. Fig.1c, Fig.1g), tetra-cyclocytic (Fig.1a, Fig.1g) and cyclocytic.

There are some species of *Shorea* that have more than one type of stoma Table 1). Baas *et al.* (1982) called the presence of several types of stomata in a single taxon 'heterogenous as to type of stomata', some authors described them as stomatal polymorphism. Baranova (1992) suggested the use of the term of 'heterostomatic' for the occurrence of several types of stomata in a single leaf and the term 'homostomatic' for taxa with a single type of stoma. Some *Shorea* species studied found to have homostomatic stomata (Table 1). Baranova (1992) and Carpenter (2005, 2006) noted that paracytic stomata, known as 'rubiaceous' in earlier stomatal classifications, is regarded as probably primitive in both dicotyledones and monocotyledones. If this is so, *S. atrinervosa, S. blumutensis, S. elliptica, S. hopeifolia, S. isoptera, S. maxima, S. maxwelliana* and *S. seminis* have a primitive type of stoma. Stauro- and tetra-cyclocytic stomata are called intermediate stomata and may occur together in some species.

Noraini & Cutler (2009) noted that the occurrence of intermediate and different types of stoma in one species or plant could have taxonomic and diagnostic value. All *Shorea* species investigated have elliptical guard cell pairs outline (Fig.2a-Fig.2o), stomatal sizes were measured directly under the scanning electron microscope and the results show that the stomata in many species are similar but some have larger or smaller stomata, some have very long and some have very short stomata. Rajagopal (1979) concluded that the stomata in monocotyledons often are 'very similar in size', whereas in dicotyledons the stomata are of 'multiple sizes'. Ten to thirteen stomata of each species were measured and analyzed and the results are summarized in Table 2. For convenience, the size of stomata, described as small, medium or large, are based on the length of the stomata. Relatively small stomata are stomata with a length of between 6 – 12 μm, medium with length of between 13 – 20 μm and large with stomata with a length above 20 μm (Table 3).

Stomata in *Shoreas* are relatively small to medium and most f species have medium size stomata. However the stomata in *S. beccariana* are the largest compared with other stomata in *Shorea,* The smallest stomata are in *S. seminis* with a width of 10 – 12 μm and a length of 6 – 8 μm. Micrographs of stomata also show an interesting feature that could have diagnostic value. For example, in *S. macrophylla* (Fig.2j), the lateral lobes have striae at right angles to the long axis of the stomata, whereas in in *S. guiso* (Fig.2h) these are tooth-like inner flanges. Another good example of a diagnostic character is in *S. atrinervosa* (Fig.2b), which has a circular, protruding stomatal rim. Resulting from these from these study, stomatal features as seen under scanning electron microscopy

together with stomatal length could be useful for authentification and identification purposes especially at species level.

Table 1: Dimensions of stomata of Dipterocarpaceae.

Species	Length (μm)	Width (μm)	Type	Guard pair cells outline	Cuticular rim
S. agamii	18(20)20	10(12)12	Tetra-cyclocytic, stauro cyclocytic	Elliptic	Narrow
S. atrinervosa	13(15)15	8(8)10	Paracytic	Elliptic	Slightly domed
S. beccariana	28(30)33	14(14)14	Cyclocytic	Elliptic	Domed
S. blumutensis	13(20)20	12(15)15	Paracytic	Elliptic	Domed
S. bracteolata	10(13)13	6(7)7	Tetra-cyclocytic and hexacytic	Elliptic	Narrow
S. elliptica	13(13)15	8(10)10	Paracytic	Elliptic	Narrow
S. gratissima	14(15)16	10(11)11	Stauro-cyclocytic	Elliptic	Narrow
S. guiso	13(15)15	9(10)10	Staurocytic, Paracytic, Hexacytic	Elliptic	Domed
S. hopeifolia	10(13)15	7(7)8	Paracytic	Elliptic	Domed
S. isoptera	15(15)18	8(8)10	Paracytic	Elliptic	Domed
S. kunstleri	20(20)20	6(6)8	Staurocytic	Elliptic	Domed
S. laevis	18(20)20	15(18)18	Staurocytic	Elliptic	Domed
S. lepidota	10(10)13	8(8)8	Staurocytic	Elliptic	Domed
S. macroptera	15(15)16	8(8)10	Tetra-cyclocytic, Stauro-cyclocytic	Elliptic	Domed
S. macrophylla	20(20)20	12(12)12	Cyclocytic	Elliptic	Domed
S. maxima	14(15)16	8(10)10	Paracytic	Circular	Slightly domed
S. maxwelliana	17(18)20	12(12)15	Paracytic	Elliptic	Domed
S. ovalis	13(14)15	10(10)10	Staurocytic	Elliptic	Domed
S. pauciflora	13(13)15	8(8)8	Staurocytic	Elliptic	Narrow
S. parvifolia	15(17)17	8(8)10	Staurocytic	Elliptic	Domed
S. platyclados	12(13)15	10(10)12	Staurocytic, Stauro-cyclocytic	Elliptic	Domed
S. rubella	15(18)18	8(10)10	Staurocytic	Elliptic	Slightly domed
S. seminis	10(10)12	10(10)10	Paracytic and staurocytic	Elliptic	Slightly domed
S. siamensis	14(14)7	6(6)8	Staurocytic	Elliptic	Slightly domed
S. singkawang	15(15)17	6(6)8	Staurocytic, tetracytic and hexacytic	Elliptic	Narrow
S. smithiana	12(15)15	8(8)8	Staurocytic, Stauro-cyclocytic	Elliptic	Domed

Note: Stomatal length and width was measured by considering the minima, maximum and common value in the majority of stomata, which is written as follows: minimum value of width or length (in brackets: the most common value of width or length of the majority of stomata) maximum value of width or length. For example; 13(14)17: 13 μm is the lowest value, 14 μm is the most common value in the majority of stomata and 17 μm is the highest value.

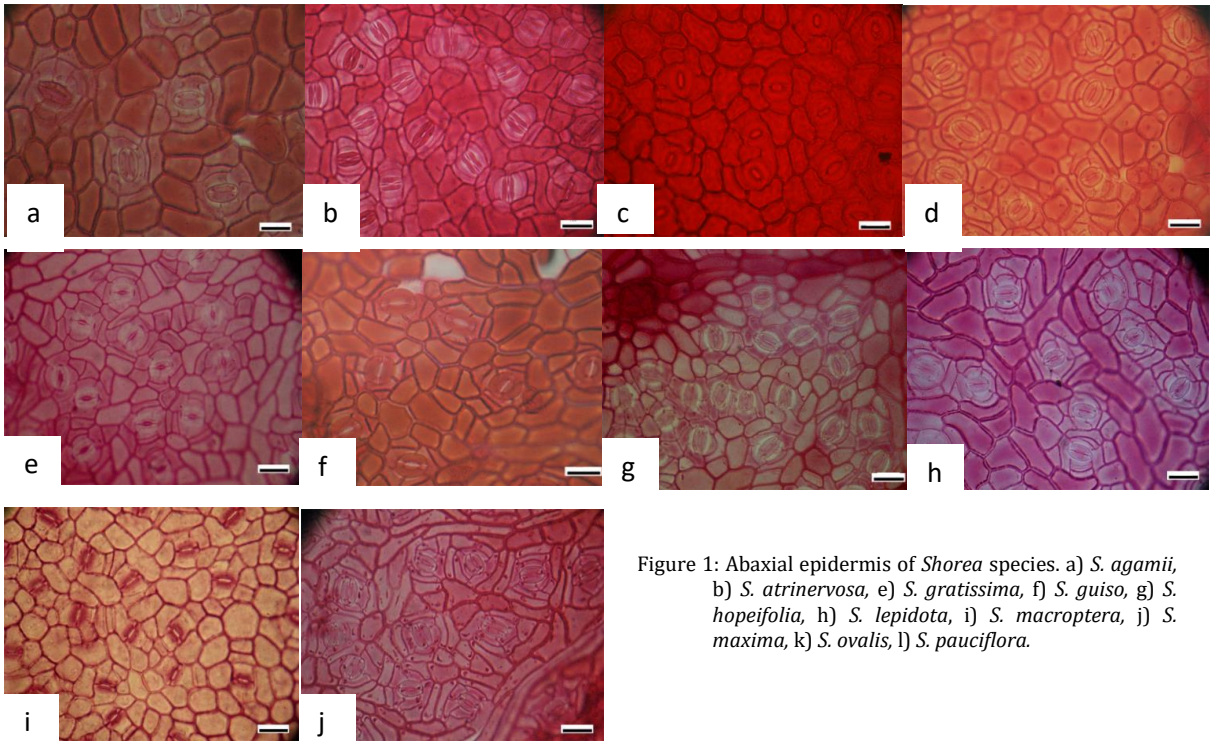

Figure 1: Abaxial epidermis of *Shorea* species. a) *S. agamii,* b) *S. atrinervosa,* e) *S. gratissima,* f) *S. guiso,* g) *S. hopeifolia,* h) *S. lepidota*, i) *S. macroptera,* j) *S. maxima,* k) *S. ovalis,* l) *S. pauciflora.*

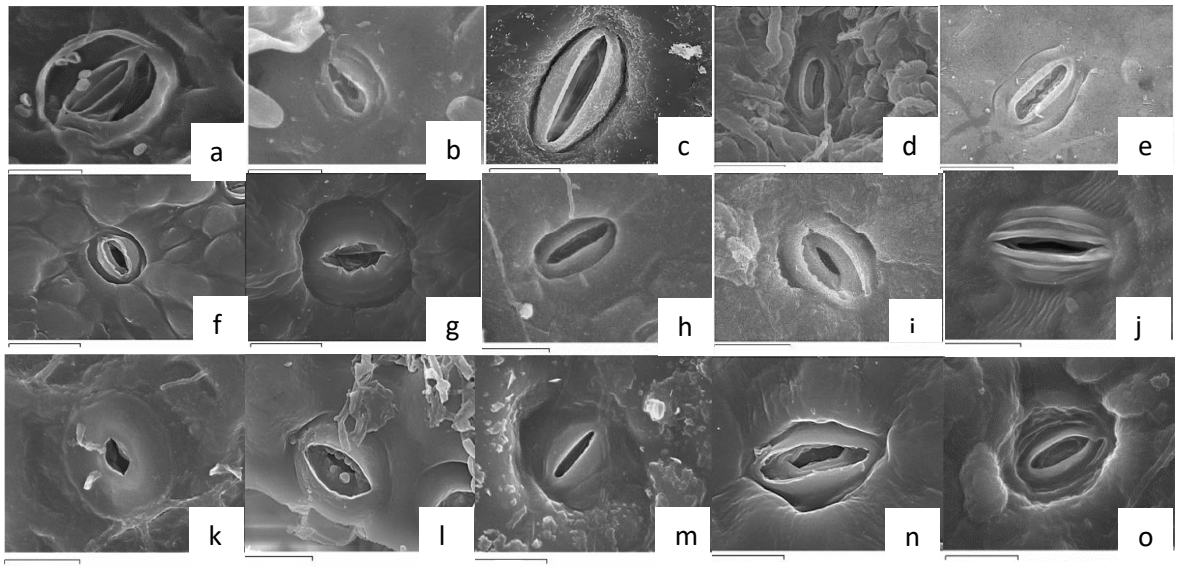

Figure 2: Abaxial leaf surfaces showing stomata in *Shorea* species. a) *S. agamii,* b) *S. atrinervosa,* c) *S. beccariana,* d) *S. bracteolata, e*) *S. guiso,* f) *S. isoptera,* g) *S. laevis,* h) *S. lepidota*, i) *S. macroptera*, j) *S. macrophylla*. k) *S. maxima*, l) *S. maxwelliana*, m) *S. pauciflora*, n) *S. platyclados*, o) *S. smithiana*. Scale bar 10 μm.

Conclusion

Shorea has seven types of stoma, with some species are homostomatic or heterostomatic. The stomata size in relatively small to medium in *Shorea*. All *Shorea* species investigated have elliptical guard cell pairs outline except circular in *S. maxima* thus diagnostic to this species. Other diagnostic characters found in this study are; lateral lobes with striae at right angles to the long axis of stomata in *S. macrophylla*, the tooth-like inner flanges in *S. guiso*, protruding stomatal rim in *S. atrinervosa*, narrow cuticular rim in *S. singkawang* and cyclocytic stomata in *S. macrophylla*. Findings in this study have shown taxonomic value of stomatal features in *Shorea*.

Acknowledgement

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3.2 Inhibitory effect of lemongrass (*Cymbopogon citratus*) leaves and stem extracts against *Fusarium proliferatum* pathogen isolated from banana fruit

Nur Baiti Abd Murad, Nur Ain Izzati Mohd Zainudin, Muskhazli Mustafa and Khozirah Shaari

Introduction

The National Geographic recognized bananas as the world's most popular fruit in 2017 and certainly, it is common in a diet of many Malaysian (Tumin and Ahmad Shaharudin, 2019). Besides being eaten raw, bananas are also the main ingredient in many of the nation's favorite tradition and modern foods. Malaysia had become a net exporter of bananas in 2017, where more than 20,000 MT of bananas have been exported which approximately 8% of estimated production with the price at RM 40.1 million. There could be a potential income prospect for the farmers and agropreneurs in Malaysia as the export has been increasing over the years.

However, exporting bananas will depend on many factors such as the ability to produce constant supply and high quality of the fruits. The average price of banana in the farms, the wholesale and retail have almost tripled from 2000 to 2018. The rising prices could be the consequence of various potential demand and supply related reasons, such as declining banana supply due to the incidence of diseases or pest and high production costs. Even some of the banana varieties have been exclusively planted for the exportation, the fruit quality still needs to be more improved in the future (Darvari *et al.,* 2010) since bananas are always at high risk for fungal infection in the field and post-harvest.

In the previous study, the most significant disease severity in banana fruits was caused by *Fusarium proliferatum* (Abd Murad *et al.,* 2017). The fungus has started the infection at harvest stage and the first symptoms appear after packaging and shipping from the production center to the marketplaces. The infection might occur on the exposed tissue such as the crown part of the fruits with the presence of developed mycelia, followed by internal development in the softest tissue of the fruit such as peduncle area, hence, resulting in softening and blackening the whole fruits.

There are many synthetic chemical treatments have been applied to control fruit rot disease in banana. However, utilization of these synthetic fungicides continuously may cause accumulation of toxic residuals in the fruit tissues leading to the development of resistance stage by the pathogenic fungi and eventually affecting human health and environment. Therefore, finding an effective alternative to naturally control this disease is increasing urgency. On the other side, as one of the tropical country, Malaysia has been

blessed with a diversity of plants with antimicrobial properties. *Cymbopogon citratus,* commonly known as lemongrass is one of them that can be found easily and abundantly throughout the country. Previously, it was reported that different type of *C. citratus* extracts showed several important therapeutic potentials such as antibacterial, antifungal, antitumoral, anticancer and insecticide activities (Zulfa *et al.,* 2016). Lemongrass is well known to comprise numerous bioactive compounds reside in its essential oil and aqueous extract found to be useful in some health issues (Olorunnisola *et al.,* 2014). Thus, this study was conducted to examine the antifungal activity of lemongrass extracts using a different type of extraction solvents against *F. proliferatum*.

Materials and methods

Plant material

Cymbopogon citratus (lemongrass) was collected in a vegetable farm in Tanjong Karang, Selangor. The plants were collected using a hoe and cutter. The leaves and the stem part of the lemongrass were cleaned and kept separately until the next use.

Fresh lemongrass leaves and stems extracts

One hundred gram of lemongrass leaves and stem were blended in 1000 mL of sterile distilled water. The mixture was homogenized using a blender (Waring Commercial Blender, Waring Products) under high mode for 2 minutes. The extract was then filtered through four folds of sterile cheesecloths followed by a layer of sterile Whatman filter paper (No. 1). This extract was considered as 10% w/v concentration and was evaporated to dryness using freeze dryer to obtain the powdered extract. The powdered extract was kept at 4°C until consequent use (Nur Fatimma *et al.,* 2018).

Dried lemongrass leaves and stems extracts

The freeze-dried lemongrass leaves and stem were ground and sieved. The powdery leaves and stem were then stored in airtight container respectively at 4°C. Thirty gram of leaves and stem powder were weighed and separately added into 300 mL of cold water, hot water, ethanol and hexane in Erlenmeyer flasks. These mixtures were considered as 10% w/v concentration and agitated in a rotary shaker at 150 rpm and 30°C for 24 hours. The extracts were filtered through four folds of sterile cheesecloths followed by a layer of sterile Whatman filter paper (No. 1). The solvents were evaporated to dryness using a rotary evaporator. The dried extracts were kept at 4°C until further analysis (Samsudin *et al.,* 2018).

Source of fungal culture

Fungal pathogen used in this study is *F. proliferatum* (B2433B) which is pathogenic to banana fruits. This culture was obtained from Mycology Laboratory of Department of Biology, Faculty of Science, Universiti Putra Malaysia. The fungus was cultured on potato dextrose agar (PDA).

Screening of antifungal activity of lemongrass extracts against *F. proliferatum*

Plant extracts were prepared in 10% dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL and sterilized by filtering through syringe filter with 0.22 μm of pore size. The antifungal screening test was conducted by poisoned food method. The plant extracts were added to the PDA in Petri plate (90 mm) aseptically and allowed to solidify. A mycelial disc of 5 mm diameter of the pure culture from 7 days old culture was inoculated on the center of the medium by using (5 mm) cork borer and incubated at room temperature 25 ºC ± 2 ºC. The plates with 10% DMSO alone and 0.1% carbendazim were included as treatments, while plates with no plant extracts and other treatments served as controls. Five replicates were used and the whole experiment was repeated once in a completely randomized design (CRD). The mycelial growth was determined at day 7 after inoculation (Sundis & Baharuddin, 2012).

Data collection

The diameter of the inhibition zone was measured in each case of successive seven days. Percentage inhibition was calculated from data obtained by using the formula (Mondali *et al.,* 2009): % FG = [(DC – DR) / DC] x 100; where % FG = percentage of inhibition of fungi growth; DC = diameter of control; DR = diameter of test.

Statistical analysis

The experimental data were analysed using IBM SPSS Statistics 22 for Windows System. *In vitro* study of mycelial growth was measured in diameter (mm) with a data type being converted to percentage inhibition. The data were analysed using a one-way analysis of variance (ANOVA). Tukey's HSD test was used to find the significant difference among means at the probability level of $p < 0.05$ (Nur Fatimma *et al.,* 2018).

Results and discussion

The extracted plant using water, ethanol and hexane have been reported to have antifungal activity at various levels and show potential for the control of phytopathogenic fungi. The diversity of bio-constituents of the plant extracts may produce different responses in inhibiting the tested microorganisms, besides the other related factors including solubility, pH, volatility, diffusion characteristics in the culture medium and the type of tested microorganisms. The use of different extraction solvents suggested a biochemically different range of bioactive compounds from polar to non-polar (Cerqueira Sales *et al.,* 2016; Samsuddin *et al.,* 2018).

The *in vitro* tests showed that the leaves and stem of lemongrass (*Cymbopogon citratus*) displayed antifungal activity against *F. proliferatum*. The results showed that different extract forms using different extraction solvents showed varied in their effectiveness in inhibiting fungi growth. Generally, the water and ethanol extracts showed good diffusion in the culture media due to their hydrophilic character, thus, making more bioactive chemical constituents available in the media compared to hexane extracts.

Hot water dried stem extract (LSH) was significantly inhibited the mycelial growth of *F. proliferatum* pathogen as shown in Figure 1 when compared to both control and other treatments. Even though it did not show a highly significant inhibition effect compared to carbendazim, yet it was showing high potential inhibition effect to combat the growth of the pathogen amongst other extract forms. Culture plate treated with DMSO did not show any inhibition zone against the tested pathogen, thus, confirmed its application as a diluent for dried crude extracts has nothing to do with the extracts' antifungal property. In addition, hexane extracts of both leaves and stem did not show any inhibition against the pathogen (Table 1). This perhaps, show the resistance of pathogens might rely on various virulence factors, and that different lemongrass parts extracted with different extraction solvents will produce different bioactive constituents which in turn can attack different aspects of pathogenicity. The data of this present study will provide an idea about the phytochemical nature of the extracts.

The mycelial growth of *F. proliferatum* was inhibited in culture plates treated with hot water extract of lemongrass stem under *in vitro* condition. It showed the presence of some of the chemical constituents that possess antifungal properties. This finding is in agreement with the results of a previous study by Dwivedi and Sangeeta (2015), where the aqueous extract of *C. citratus* possess a high degree of antifungal activities against the *Saprolegnia parasitica*, *Sclerotium rolfsii* Sacc and inhibit the growth of *Colletotrichum graminicola* by 100% concentration.

Cymbopogon citratus was reported to consist of many organic compounds such as terpenoids but the major component is citral. According to Villalobos (2015), citral is a volatile compound and many plant volatiles exhibit anti-microbial and anti-herbivore activity, which aid as indirect plant defenses. It was able to damage the cell wall and membrane of *Aspergillus flavus* spores by entering the cell and causing further damage by interfering with DNA and mitochondrial processes as well as the aggregation of protein-like molecules leading to metabolic disorder and eventually, lose the capacity of the spores to germinate. Citral also showed antifungal activity against several fungal species which cause severe postharvest diseases in fruits such as *Colletotrichum musae, Colletotricum gloeosporioides, Penicillium digitatum* and *Fusarium subglutinans* f. sp. *ananas* by altering the hyphae morphology of the fungi (Garcia *et al.,* 2008). *Cymbopogon citratus* also contains active components such as alkaloids, tannins, flavonoids, terpenes, and phenolic compounds. Phenols and flavonoids are widely being reported can cause membrane disruption while alkaloids are thought to inhibit the growth of microorganisms by affecting their genetic materials (Zulfa *et al.,* 2016).

Table 1: Antifungal inhibitory effect of lemongrass extracts using poisoned food method against *F. proliferatum* pathogen isolate

Treatments	**Inhibition of mycelial growth of (%)**	
	F. proliferatum	**Control**
LLF (cold water fresh leaves)	32.31^{e}	0.00^{a}
LLC (cold water dried leaves)	13.08^{c}	0.00^{a}
LLH (hot water dried leaves)	13.08^{c}	0.00^{a}
LLEtOH (ethanol dried leaves)	11.28^{bc}	0.00^{a}
LLHex (hexane dried leaves)	0.00^{a}	0.00^{a}
LSF (cold water fresh stem)	18.21^{d}	0.00^{a}
LSC (cold water dried stem)	11.03^{bc}	0.00^{a}
LSH (hot water dried stem)	45.90^{f}	0.00^{a}
LSEtOH (ethanol dried stem)	10.26^{b}	0.00^{a}
LSHex (hexane dried stem)	0.00^{a}	0.00^{a}
Carbendazim	56.15^{g}	0.00^{a}
DMSO	0.00^{a}	0.00^{a}

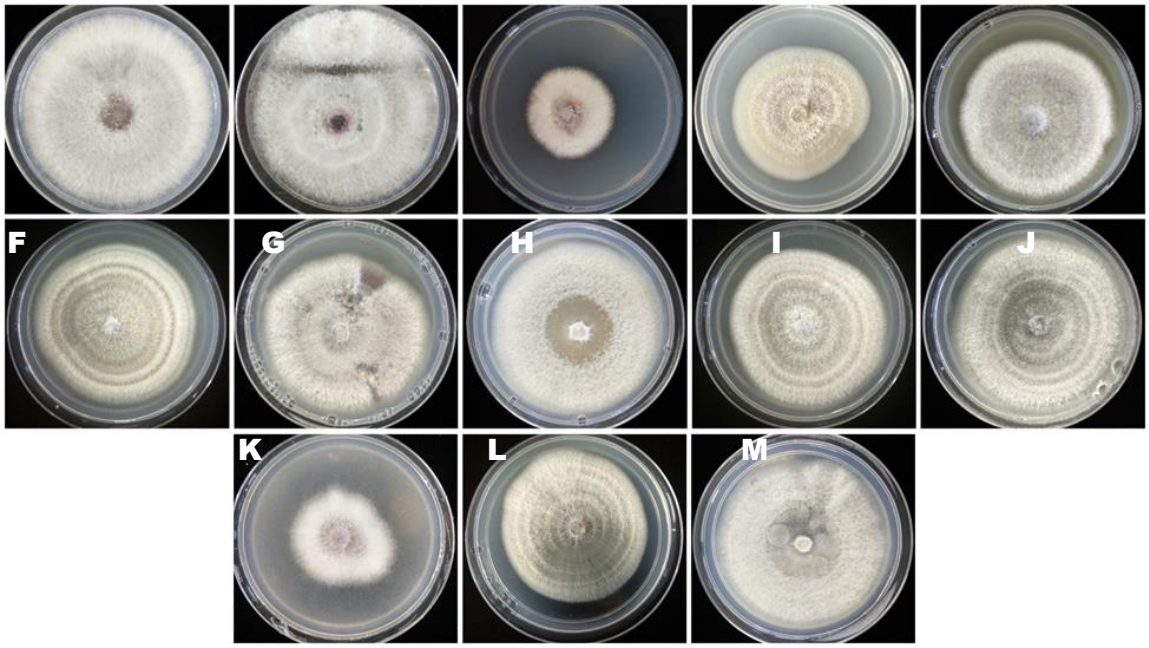

Figure 1: Effect of different type of lemongrass extracts on *F. proliferatum*. A) Control culture plate; B) Culture plate treated with 10% DMSO alone; C) Culture plate treated with 0.1% carbendazim; D) Culture plate treated with cold water fresh leaves extract (LLF); E) Culture plate treated with cold water dried leaves extract (LLC); F) Culture plate treated with hot water dried leaves extract (LLH); G) Culture plate treated with ethanol dried leaves extract (LLEtOH); H) Culture plate treated with hexane dried leaves extract (LLHex); I) Culture plate treated with cold water fresh stem extract (LSF); J) Culture plate treated with cold water dried stem extract (LSC); K) Culture plate treated with hot water dried stem extract (LSH); L) Culture plate treated with ethanol dried stem extract (LSEtOH) and M) Culture plate treated with hexane dried stem extract (LSHex).

Conclusion

In conclusion, the potential of *C. citratus* stem extract to be used as a natural antifungal agent is recommendable as antifungal activity against *F. proliferatum* were demonstrated as well as its antifungal against several phytopathogenic fungi being reported previously. Further analyses on its minimum inhibitory concentration (MIC) are necessary; besides, the active constituents and possible inhibitory mechanisms of *C. citratus* extract would be interesting. The development of natural plant extracts and active compounds would be a great alternative to the synthetic chemical fungicide.

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3.3 Nutritional values of fresh and roasted Sacha inchi kernel and its cake

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Introduction

Sacha inchi (*Plukenetia volubilis L.*), also named "Inca peanut, "wild peanut", "Inca inchi" or "mountain peanut", is a plant of the Euphorbiaceae family, which grows in the Amazonian forest (Hamaker et al., 1992). In Malaysia, Rubber Industry Smallholders Development Authority (RISDA) introduced Sacha inchi for smallholders and farmers as an effort to increase their income as they will benefit from this plant which has a lifespan of 20 years. Perak, Pulau Pinang, Kedah and Perlis will be the pioneer of Sacha inchi plantation (MyMetro, 2017). The protein content of Sacha inchi (~27%) is similar to other oil seeds such as soybean, cottonseed and sunflower (Hamaker et al., 1992). Besides that, Gutiérrez et al. (2011) also reported that Sacha inchi seeds contain many valuable minerals mainly potassium (5563.5 mg/kg), magnesium (3210 mg/kg) and calcium (2406 mg/kg) making this seed suitable to be used in human diet for supplying these elements. Apart from this nutrient-rich seed, the oilseed cake was a by-product of oil extraction process. The pressed-cake still contains various amounts of bioactive compounds such as free fatty acids, glycerides, phosphatides, sterols, tocopherols and protein fragments (Chirinos et al., 2013). As Sacha inchi kernel and its cake have many nutrients include protein, minerals and bioactive compounds, it might be concluded that this plant are nutritious as well as forming excellent sources of vegetable fats and protein.

Common process applied to Sacha inchi kernel before consumption as a snack was roasting in order to eliminate astringent off-flavors and possible antinutritional factors (Cisneros et al., 2014). The by-product after oil extraction which is the pressed-cake will be disposed. However, the by-product might also contain some valuable nutrient and minerals that would be useful as functional food ingredient. Besides that, this plant has rarely been studied and the report regarding the nutritional composition of Sacha inchi kernel as well as its cake was limited. The significance of this study was the characterization of nutritional value of Sacha inchi kernel and its cake. Other than that, the reports regarding this newly-introduced plant will increase as well as give impact towards society since it will generate income and creating new industry especially for farmers in Malaysia. Thus, the main objective in this study was to determine the proximate values and minerals content of Sacha inchi kernel and its cake.

Materials and methods

Sacha inchi kernel and its cake were acquired from local producer in Bukit Payong, Terengganu, Malaysia. Hard shell was removed to obtain the fresh kernel. For roasted kernels, the fresh kernel was roasted at temperature 120 ºC for 10 minutes (Yossaporn et al., 2017). Meanwhile, Sacha inchi pressed-cake was obtained after oil extraction using mechanical cold press machine. The kernel and its cake were ground into powder and then proceed for proximate and minerals analysis.

Proximate analysis

The moisture, ash, protein, fibre and fat contents of Sacha inchi kernel and its cake were determined according to the Association of Official Analytical Chemists (AOAC) method (AOAC, 2000). The nitrogen content was converted to protein by multiplying by a conversion factor of 5.3. Available carbohydrates were calculated by difference. The analysis of each sample was performed in triplicates. The proximate result was expressed in wet basis.

Mineral analysis

Mineral compositions of fresh and roasted Sacha inchi kernel and its cake was determined according to the method of Naozuka et al. (2011) with some modifications by using Thermo Scientific Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Thermo Fisher Scientific, USA).

Statistical analysis

The experiments were performed in triplicates and the results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was conducted using SPSS software to determine the mean values among the samples. The statistical differences were determined at $p < 0.05$.

Results and discussion

Table 1 showed the nutrients that were present in fresh and roasted Sacha inchi kernels in varied levels. The dominant nutrient content in Sacha inchi kernels were fibre, protein and fat. Both kernels have high content of fibre in the range of 14.39-17.10% so it has enough fibre for dietary nutrition which will help to maintain intestinal distention, reduce constipation, colon diseases and cancer as reported by Njoku et al. (2007). Besides, the kernels also have high protein content ranges from 26.62% to 27.33% which was

approximately like that reported by Hamaker et al. (1992) whereby the protein content is 27%. The protein content was higher than reported by Gutiérrez et al. (2011) with 24.7%. These percentages can be related to other oil seeds such as sesame (~25%), peanut (23% w/w) and sunflower (24% w/w) (Bodwell and Hopkins, 1985). Their high percentage (%) of protein will make them serve as a proper source of amino acids and protein for both man and animal and this value exceeded FAO recommended value (19.8%) (FAO, 2004). Moreover, proteins play important role in human body such as being used for the production of hormones, enzymes and blood plasma, acts as immune boosters as well as help in cell division and growth (Okeke & Elekwa, 2006). In addition, the percentage (%) of ash in the kernels (2.38 – 2.45%) indicates high inorganic matter that could be retained in the body and a reflection of mineral content preserved in the plant. Besides, Sacha inchi kernels contained high amount of fat in the range of 52.45% - 52.71%.

The high content of crude fat showed that the kernel is suitable to be used as functional food ingredient to produce more palatable food products because fats function to increase food palatability by absorbing and retaining the flavors (Okonkwo & Okafor, 2016). Table 1 also shows the calculated fatty acid for Sacha inchi kernel in the ranges of 41.97 – 42.17%. This is relatable with the finding by Maurer et al. (2012) that reported Sacha inchi oil is rich in alpha-linolenic and linoleic acids, approximately 50% and 34% respectively. The high concentration of alpha-linolenic acid making Sacha Inchi a good source of this essential fatty acid and also could be used as food supplement (Gutiérrez et al., 2011). Sacha inchi kernel contains a relatively low value of total carbohydrate in the range of 13.29 -14.61% due to the high levels of fat and protein.

The metabollizable energy (ME) of Sacha inchi kernel, calculated from the contents of fat, protein and carbohydrate, ranges from 2619.54 – 2663.37 kJ/100 g. ME has been defined as “the amount of energy available for total (whole body) heat production at nitrogen and energy balance” (Livesey, 2001). Energy-dense foods are generally described as those that are high in fat, sugar or starch (Kant, 2000; WHO, 2003). As the kernels has high content of fat, so it can be classified as an energy-dense food which will supply enough energy required by human body. As described in detail in the report of the most recent Expert Consultation on Energy in Human Nutrition (FAO, 2004), humans need food energy to cover the basal metabolic rate, the metabolic response to food, the energy cost of physical activities and accretion of new tissue during growth and pregnancy, as well as the production of milk during lactation.

Based on results stated in Table 1, the moisture content of fresh and roasted Sacha inchi kernel were 5.24% and 2.90% respectively which is a bit low when compared with legumes which range between 7.0-11.0% (Arkroyed & Doughty, 1984). This shows that the kernels are very high in dry matter content which is an advantage because it reduces

microbial activities, prevent oxidation-reduction reaction, algae and fungi growth and increase their shelf life when properly stored (Okonkwo & Okafor, 2016).

Table 1: Proximate analysis of Sacha inchi kernel

Analysis	Fresh kernel	Roasted kernel
Moisture (%)	5.24 ± 0.67[a]	2.90 ± 0.60[b]
Ash (%)	2.38 ± 0.05[a]	2.45 ± 0.02[a]
Protein (%)	26.62 ± 0.14[a]	27.33 ± 0.16[b]
Fibre (%)	17.10 ± 3.22[a]	14.39 ± 1.00[a]
Fat (%)	52.45 ± 3.87[a]	52.71 ± 9.02[a]
Carbohydrate (%)	13.29 ± 3.34[a]	14.61 ± 9.60[a]
Fatty acid (%)	41.97 ± 3.09[a]	42.17± 7.22[a]
Energy (kJ/100 g)	2619.54 ± 86.66[a]	2663.37 ± 171.51[a]

Values are mean ± standard deviation in triplicates determinations
Values followed by different letter in same row are significantly different at $p<0.05$
*a calculated fatty acid (0.8 × crude fat), *b calculated metabolizable energy (kJ/100 g) [(protein×17) + (fat× 37) + (carbohydrate × 17)] (Ekeanyanwu & Ononogbu 2010)

Proximate composition of fresh and roasted Sacha inchi cake were presented in Table 2. According to the results, protein was the largest amount in the cakes range from 52.09 to 54.08%. The results were approximately similar with Rawdkuen et al. (2016) that reported the protein content of cakes from *Plukenetia volubilis* is 56.61%. The results also shown that there was a significant difference ($p < 0.05$) between fresh and roasted Sacha inchi cake in term of moisture, ash, protein, fat, carbohydrate, fatty acid and energy value. This result was in agreement with the findings of Wollgast & Anklam (2000) which stated that the difference in proximate composition might due to roasting process that alters the structure of some molecules including proteins. Moreover, based on Table 2, roasted cake has lower moisture content than fresh cake. This might due to the thermal treatment applied to foods which is roasting process that cause moisture evaporates to surrounding (Xu & Chang, 2008).

The results of the mineral analysis in fresh and roasted Sacha inchi kernels were given in Table 3. It was observed that the major minerals found in fresh and roasted Sacha inchi kernel were potassium (2460.21-2328.81 mg/kg), magnesium (1023.32 - 1065.82 mg/kg) and calcium (650.45 – 815.91 mg/kg). Minor amounts of sodium (35.89 - 68.28 mg/kg), zinc (36.41 – 43.41 mg/kg), chromium (96.74 - 98.29 mg/kg), aluminium (41.25 – 60.09

mg/kg), cadmium (3.41 – 3.61 mg/kg), plumbum (11.52 – 14.11 mg/kg) and nickel (4.76 – 5.19 mg/kg) were also present in Sacha inchi kernels.

Table 2: Proximate analysis of Sacha inchi kernel cake

Analysis	Fresh cake	Roasted cake
Moisture (%)	9.23 ± 0.02^{a}	8.11 ± 0.01^{b}
Ash (%)	4.31 ± 0.01^{a}	4.87 ± 0.07^{b}
Protein (%)	52.09 ± 0.36^{a}	54.08 ± 0.01^{b}
Fibre (%)	2.62 ± 0.11^{a}	2.93 ± 1.06^{a}
Fat (%)	12.34 ± 0.01^{a}	9.48 ± 0.07^{b}
Carbohydrate (%)	22.03 ± 0.34^{a}	23.46 ± 0.01^{b}
Fatty acid (%)	9.87 ± 0.01^{a}	7.58 ± 0.06^{b}
Energy (kJ/100 g)	1716.54 ± 0.16^{a}	1668.86 ± 2.74^{b}

Values are mean ± standard deviation in triplicates determinations
Values followed by different letter in same row are significantly different at $p<0.05$
*a calculated fatty acid (0.8 × crude fat), *b calculated metabolizable energy (kJ/100 g) [(protein×17) + (fat× 37) + (carbohydrate × 17)] (Ekeanyanwu & Ononogbu 2010)

The values for mineral found in Sacha inchi kernels were quite different compared to study by Gutiérrez et al. (2011) whereby the values were potassium (5563.5 mg/kg), magnesium (3210 mg/kg), calcium (2406 mg/kg), sodium (15.4 mg/kg) and zinc (49 mg/kg). The difference in the mineral composition were might due to soil composition as reported by Blackwood (2007). The results show that there was no significant difference ($p > 0.05$) in mineral composition of fresh and roasted Sacha inchi kernels indicates that the roasting process at 120 ºC does not affect the mineral content in the kernels. Minerals like potassium, magnesium, calcium, chromium and nickel provide many health benefits. Potassium is an important component of cell and body fluids and also aids in controlling heart rate and blood pressure while magnesium is required for ATP production and bone stability.

Calcium plays essential role in blood clotting, muscle contraction, nerve transmission as well as bone and tooth formation (Freitas et al., 2010). Chromium were essential for metabolism of fats, carbohydrates and the synthesis of proteins while nickel play important roles in hormonal activity, lipid metabolism, activation of some enzymes as well as stabilisation of DNA and RNA (Nabrzyski, 2007; Grembecka & Szefer, 2011).

Table 3: Mineral analysis of Sacha inchi kernel

Minerals (mg/kg)	Fresh kernel	Roasted kernel
Sodium (Na)	68.28 ± 54.29[a]	35.89 ± 42.00[a]
Potassium (K)	2460.21 ± 500.80[a]	2328.81 ± 316.32[a]
Zinc (Zn)	36.41 ± 5.88[a]	43.41 ± 8.99[a]
Magnesium (Mg)	1065.82 ± 195.29[a]	1023.32 ± 111.13[a]
Calcium (Ca)	650.45 ± 210.98[a]	815.91 ± 165.96[a]
Chromium (Cr)	98.29 ± 0.51[a]	96.74 ± 2.40[a]
Aluminium (Al)	60.09 ± 53.52[a]	41.25 ± 31.69[a]
Cadmium (Cd)	3.61 ± 0.30[a]	3.41 ± 0.06[a]
Plumbum (Pb)	14.11 ± 1.57[a]	11.52 ± 0.79[a]
Nickel (Ni)	4.76 ± 0.26[a]	5.19 ± 0.19[a]

Values are mean ± standard deviation in triplicates determinations
Values followed by different letter in same row are significantly different at $p<0.05$

Mineral contents of pressed-cake from fresh and roasted Sacha inchi kernels were shown in Table 4. Potassium was the highest element for both cakes (8234.79 mg/kg for fresh and 8634.34 mg/kg for roasted, respectively). Magnesium was the second abundant mineral in Sacha inchi cake ranges from 3379.08 to 3554.70 mg/kg. The next major element was calcium in the range of 972.20 – 1003.01 mg/kg. Other elements that present in Sacha inchi cakes were sodium, zinc, iron, chromium, aluminium, cadmium, plumbum and nickel. From Table 4, it shows that there was significant difference ($p < 0.05$) in sodium, iron, aluminium and cadmium value after roasting process. Meanwhile, no significant difference ($p > 0.05$) between both cakes in term of potassium, magnesium, calcium, zinc, chromium, plumbum and nickel. This may indicate that both cakes still contained high amount of minerals even after roasting process and hence can be a new functional food ingredients for the application in food industry.

Conclusion

Sacha inchi kernel contained major amounts of protein, fibre and fat which gave many beneficial effects to human body while Sacha inchi cake contained high amount of proteins. Both Sacha inchi kernel and cake contain major minerals like potassium, magnesium and calcium which had been reported to play essential role in human health. Roasting process applied to Sacha inchi kernels at 120 ºC gave effect to proximate and minerals composition

of fresh and roasted kernel as well as its cake. From the study, it is clear that Sacha inchi kernel and its by-product (cake) could be used in food industry because of an increasing tendency towards functional food products.

Table 4: Mineral analysis of Sacha inchi kernel cake

Minerals (mg/kg)	Fresh cake	Roasted cake
Sodium (Na)	124.26 ± 0.46[a]	292.65 ± 26.23[b]
Potassium (K)	8234.79 ± 287.99[a]	8634.34 ± 167.76[a]
Zinc (Zn)	88.81 ± 7.12[a]	86.62 ± 1.67[a]
Iron (Fe)	98.39 ± 6.64[a]	250.51 ± 0.37[b]
Magnesium (Mg)	3379.08 ± 109.17[a]	3554.70 ± 73.65[a]
Calcium (Ca)	972.20 ± 238.99[a]	1003.01 ± 27.99[a]
Chromium (Cr)	97.19 ± 0.04[a]	98.14 ± 0.90[a]
Aluminium (Al)	52.56 ± 0.68[a]	70.00 ± 1.92[b]
Cadmium (Cd)	3.43 ± 0.01[a]	3.96 ± 0.01[b]
Plumbum (Pb)	17.15 ± 0.76[a]	14.44 ± 0.53[a]
Nickel (Ni)	4.92 ± 0.05[a]	4.16 ± 0.29[a]

Values are mean ± standard deviation in triplicates determinations
Values followed by different letter in same row are significantly different at $p<0.05$

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3.4 Preliminary study on antioxidant activity and total flavonoid content in four Malaysian lichens

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Introduction

Lichens are mutualistic association of a fungus and algae. Lichens ability to yield various bioactive secondary metabolites has raised interest in pharmacology due to their antioxidant potential (Fernández-Moriano, Gómez-Serranillos & Crespo, 2016). These bioactives exert antioxidant effects by reacting with free radicals, chelating catalytic metals and scavenging oxygen in biological systems (Halliwell & Gutteridge, 1984). Free radicals can cause oxidative stress in humans which leads to many complications such as cardiovascular diseases, cancer and neurological disorders (Mena, Ortega, & Estrela, 2009; Valko et al., 2007).

Previously, synthetic antioxidants were used to overcome the oxidative effects in humans. However, these synthetic antioxidants have severe side effects which can be detrimental for human health. Hence, natural antioxidants from lichens can be used as alternative in preventing and treating disease related to oxidative stress. Thus, utilization of the lichen potential as natural source of antioxidants should be explored.The objectives of this study are: (i) to determine the antioxidant activity by using DPPH analyses of acetone extracts of four types of lichen species (ii) to determine the total flavonoid compound (TFC) in the tested lichens species extracted by acetone and (iii) to determine the relationship between TFC on DPPH activity of all tested lichen species.

Materials and methods

DPPH Assay

DPPH assay was conducted as described by Shimada et al. (1992). The antioxidant activity was expressed as activity of scavenging by extract compared to standard which was ascorbic acid.

For standard preparation, 1mg/mL of ascorbic acid was prepared in methanol. 250mg/mL of stock solution of each lichen species was prepared in DMSO. The sample solution was diluted six times with DMSO to known concentration (100µg/mL – 3.125µg/mL).

As for the assay preparation, 20µL of 100mg/mL extract was added into 180µL of 84µg/mL of DPPH solution in 96-well plate. The plate was incubated at room temperature in the dark

for 1 hour. As for standard, the same step was repeated by replacing sample extract with ascorbic acid solution. Blank were prepared by adding 20 µL of DMSO into 180µL of DPPH solution. The scavenging activity was calculated from the absorbance of decolorization by doing spectrophotometry analysis at 540nm. The antioxidants activity was expressed as the half maximal inhibition concentration or known as EC_{50}.

Total flavonoid content (TFC)

The estimation of total flavonoids in the lichen extracts were carried out using Zilic method (Zilic et al., 2011). Flavonoid compound standard (quercetin) was prepared at six different concentrations (200µg/ml – 6.25µg/ml).

Then, the preparation of assay to determine total flavonoids content was performed. Firstly, an amount of 20µL of 100mg/mL sample or standard flavonoid compound solution (quercetin) was mixed with distilled water. 0.3mL of 5% of sodium nitrite solution and 10% of aluminium chloride solution were added into the sample/standard after 5 minutes. Then, 2 mL of 1mol/L of sodium hydroxide solution was added and the solution was incubated for 15 minutes. After 15 minutes, the total flavonoid content of lichen extracts was estimated spectrophotometrically at 510nm. A yellow colour indicated the presence of flavonoid compound in the extracts. All experiments were performed in triplicates.

Results and discussion

Based on Table 1, *Ramalina farinacea (*3.25 ± 0.05, $p<0.05$) exhibited highest antioxidant activity against DPPH free radicals meanwhile *Cladia aggregata (*124.24 ± 1.23) showed lowest antioxidant activity. These findings were in accordance with previous study where *Ramalina* lichen extracted with acetone showed highest potent of antioxidant among all studied species (*Bulbotrix isidiza*, *Parmotrema tinctorum* and *C. aggregata*) (Stanly et al., 2011) and *Cladonia* lichen (Gunasekaran, 2016).

R. farinacea (206.66 ± 3.33) has shown to have the highest TFC and *Parmelia* sp. (35.09 ± 0.40) showed lowest TFC among all tested species. Correlation between TFC and DPPH radical scavenging activity (expressed in EC_{50}) for three lichen species suggested strong antioxidant activity of TFC. EC_{50} value of *Parmelia* sp., *C. aggregata* and *R. farinacea* lichens have shown to be highly correlated with TFC ($r=-0.998$, $r=-0.924$ and $r=-0.596$ respectively). However, *Cladonia* sp. showed low degree correlation between TFC and EC_{50} value ($r=0.158$) although showing high antioxidant activity. The high correlation of TFC and DPPH shows that the flavonoid content may contribute to the antioxidant activity. Past study found that flavonoid content exhibited antioxidant activity in lichens (Gunasekaran, 2016).

Table 1: DPPH (EC50) and TFC in each lichen species and correlation analysis between DPPH (EC_{50}) and TFC of lichens

Lichen species	DPPH, EC_{50} (µg/mL)	TFC (µg QE/mg)	Correlation coefficient (r)	p-value
Ramalina farinacea	3.25 ± 0.05	206.66 ± 3.33	-0.596	0.593
Cladonia sp.	10.87 ± 0.62	45.00 ± 0.96	0.158	0.899
Parmelia sp.	29.65 ± 0.39	35.09 ± 0.40	-0.998*	0.036
Cladia aggregata	124.24 ± 1.23	56.48 ± 1.0	-0.924	0.250

Footnote: Correlation is significant at 0.05 level (2-tailed),

Conclusion

R. farinacea showed potent antioxidant activity with EC50 value of 3.25 ± 0.05µg/mL compared to the standard (ascorbic acid) with EC50 value of 0.0766 ± 0.000038µg/mL. The study has shown that there was high antioxidant activity of Malaysian lichens contributed by flavonoid compounds. Further study could be conducted to characterize other lichen compounds as natural sources of antioxidants.

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3.5 Isolation, growth and cellulose extraction of selected green algae

N. A. S. Amdan and S. Zulkifly

Introduction

Algae are simple photosynthetic organisms that lack true roots, stems, leaves, and vascular tissues. In terms of morphology, algae are divided into two groups called microalgae and macroalgae. Algae celluloses are of higher quality than the plant cellulose, highly valued and have wide applications in various industries. Green algae contain more cellulose in comparison to red or brown algae. Four species of green algae were selected to study their growth and cellulose contents which are *Scenedesmus* sp., *Chlorella vulgaris* (microalgae), *Cladophora* sp. and *Enteromorpha* sp. (macroalgae). The microalgae were collected at Putrajaya lakes while the macroalgae were collected from Port Dickson. These algae were cultivated in the laboratory with enriched media and nutrients for growth. Each alga was cultured in 5 replicates using 250 ml conical flasks. This study had shown that cellulose content extracted from *Cladophora* sp. algae is significantly higher than other algal cellulose. Further research should be done in order to produce more cellulose from seaweeds or macroalgae.

Materials and methods

Four selected algae were collected at two sampling sites which are Putrajaya Lakes and Port Dickson. The *Cladophora* sp. and *Enteromorpha* sp. were collected at Port Dickson while the *Scenedesmus* sp. and *Chlorella* sp. were collected at Putrajaya lake. Each isolated algae species selected were cultured in 5 replicates, each. *Bold Basal medium* was used for growth of microalgae and *SD11 medium* for macroalgae. Optimum growth of algae, pH, and light intensity as well as chlorophyll contents for each alga were recorded.

Extraction of chlorophyll from algae

The extraction of chlorophyll from algae was done by using acetone. The reading was recorded by using spectrophotometer machine at three different wavelengths which were 630, 647 and 664 nm. The wavelength was used based on the formula of Jeffrey and Humprey (1975).

Extraction of cellulose from algae

After exponential growth of algae was achieved, algal cellulose was extracted from *Cladophora* sp., *Scenedesmus* sp., *Enteromorpha* sp. and *Chlorella* sp. Cellulose was extracted from the five replicates of a conical flask of each alga. The cellulose powder

was weighed, and data were recorded. The analysis was performed using SPSS (Statistics Package for Social Science) version 20.

Result and discussion

The measurements culture and growth of *Chlorella vulgaris* for 30 days

The *Chlorella vulgaris* was incubated for 30 days for biomass production. The growth curve of *Chlorella vulgaris* through 30 days is shown in Figure 2.

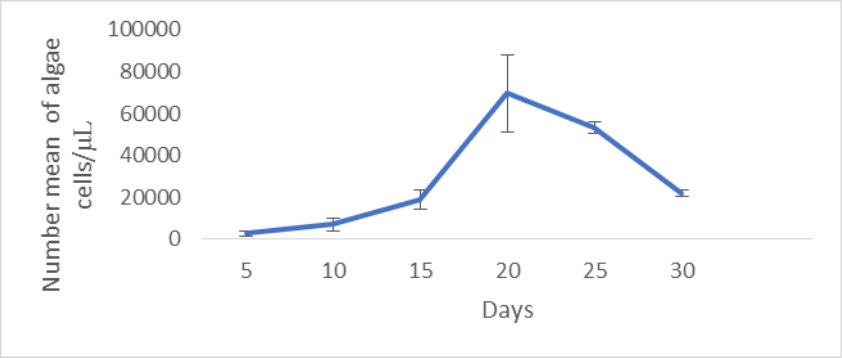

Figure 1: *Chlorella vulgaris* growth curve from day 5 until day 30.

The growth curve of *Chlorella* sp. is sigmoid. There were four growth phases shown on the graph. According to growth curve of *Chlorella* sp., at day 5 until day 10 was the lag phase. Basically, after isolation, the alga was transferred into new culture medium. The growth does not increase rapidly because the cells need time to adjust. However, after the lag phase, the algae growth will increase until they reached the exponential phase. The cultures were in exponential phase in day 10 until day 15. Stationary phase was reached after day 15 until day 20.

In the exponential phases, the light intensity, temperature, and amount of nutrients affected the growth rate. Krishnan et al. (2015) reported that the algae started to multiply continuously and have an increment in biomass because of high amount of nutrients in the medium. Therefore, the growth of algae for *Chlorella* sp. were the highest during the exponential phases until day 20. Next, in stationary phases the growth rate decreases until reached zero, however, the population still increased. Based on Krishnan et al. (2015) the cell division of algae are equal with the death cell of algae during stationary phase.

However, the algae reached the death phases on day 20 onwards. In death phases, the cell division was lower than the death rate of the algae. The population of algae starts to decrease. This is supported by Zuka, McConnel, and Farag (2012) which said that the

lysis phase is due to depleted of nutrients, limited of oxygen, pH disturbance, overheating and contamination occur.

The growth culture of *Scenedesmus* sp

The measurements culture and growth of *Scenedesmus* sp. for 30 days

The *Scenedesmus* sp. was incubated for 30 days for biomass production. Figure 3 shows the growth curve of *Scenedesmus* sp. for 30 days.

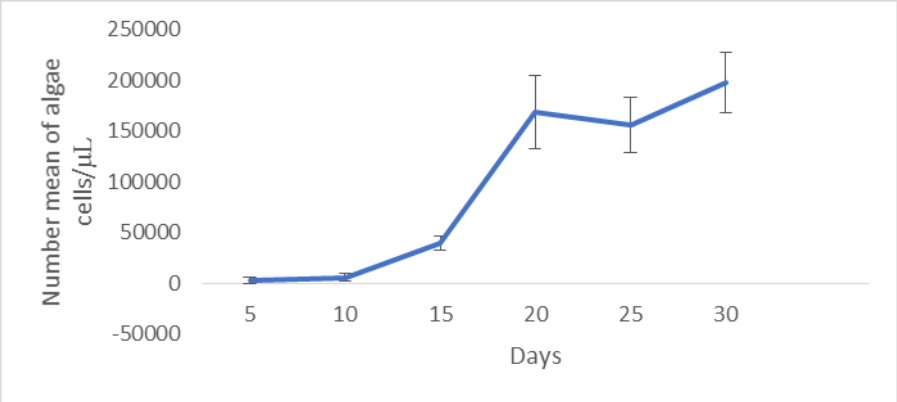

Figure 2: *Scenedesmus* sp. growth curve from day 5 until day 30.

Four growth phases were shown on the graph. The grow curve of *Scenedesmus* sp. was similar in pattern with the growth curve of *Chlorella* sp. From this research, *Scenedesmus* sp. has shown to have an increase in population growth on day 25 until 30. Possible explanation is contamination due to aeration problems. However, due to a blockage of algae at the glass tube connection, aeration was stunted, and making algae suspended at the bottom of the flask. Or that, possibly, during the transfer of flasks, some media must have been introduced to the culture.

The dry weight, cellulose biomass, and pH growth from different types of algae

Based on Figure 3, the highest mean biomass of cellulose (gram) was extracted from *Cladophora* sp. (0.0069 g), followed *by Enteromorpha* sp. (0.0041 g), *Scenedesmus* sp. (0.0027 g), and *Chlorella* sp. The lowest mean value biomass of cellulose was collected from *Chlorella* sp. (0.0025 g). However, there were 3-5 replicates collected by each different types of algae. Thus, the highest cellulose contents were recorded by *Cladophora* sp. for replicate 2 was at (0.0076 g) and the lowest was extracted by *Scenedesmus* sp. for replicate 2 at (0.0013 g).

In this experiment, cellulose contents were also extracted from four selected algae. The biomass of algae collected was extracted after exponential growth, in stationary phase to obtain the optimal cellulose in each alga. Cellulose is an essential element that can be

found in the cell wall of algae and have many applications in various industries. The algal cellulose was chosen for industrial applications because these algae cellulose production requires less energy, less cost to produce and does not pollute the environment in comparison from plant cellulose.

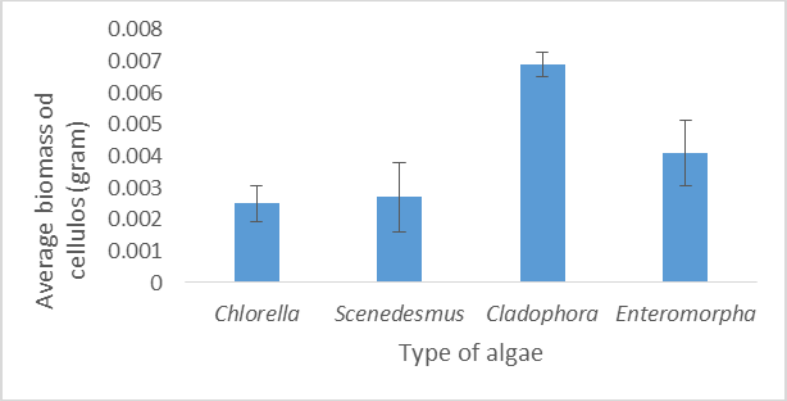

Figure 3: Type of algae (*Chlorella* sp.*, Scenedesmus* sp.*, Cladophora* sp. and *Enteromorpha* sp.) against the biomass cellulose content (gram)

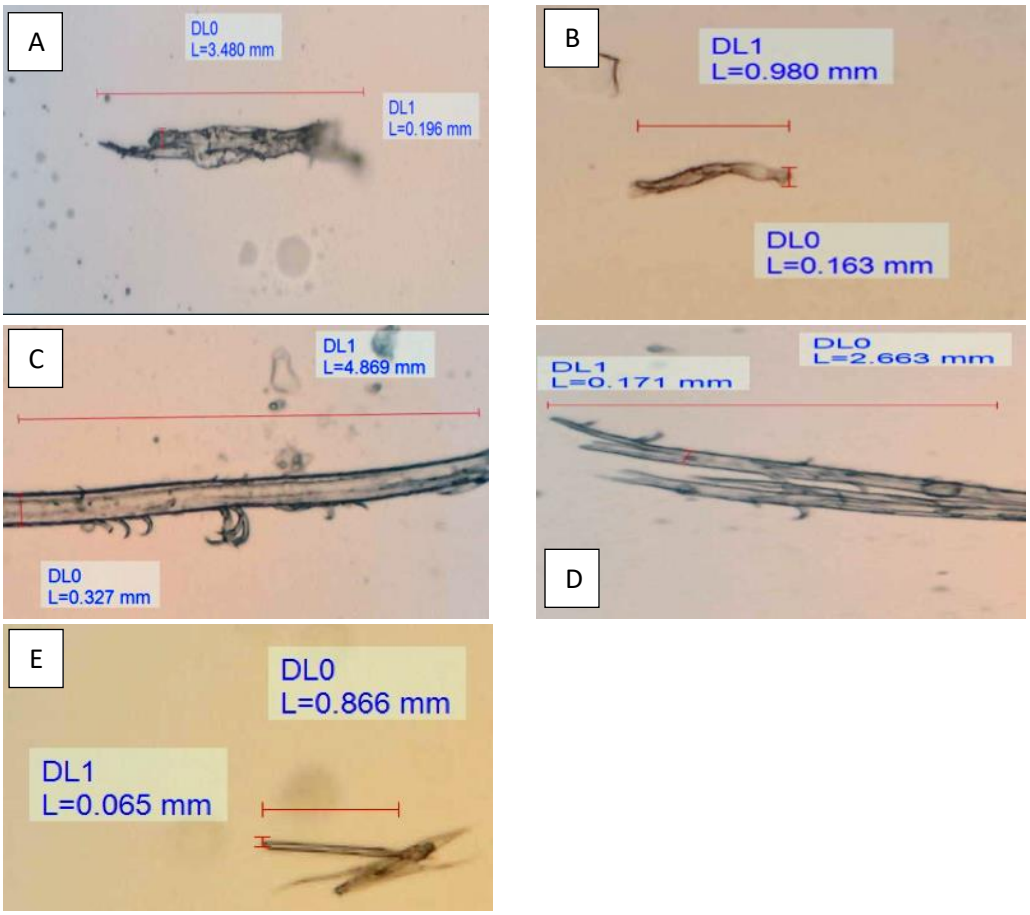

Figure 4: The observation of cellulose under 100x magnification by using Dino-Eye (Microscope Eye-Piece Camera). A) Cellulose extracted from *Enteromorpha* sp.; B)

Cellulose extracted from *Cladophora* sp.; (C-D) Cellulose extracted from *Scenedesmus* sp.; and E) Cellulose extracted from *Chlorella* sp.

Cladophora sp. have highest cellulose contents followed by *Enteromorpha* sp., *Scenedesmus* sp., and *Chlorella* sp. The result shows that the macroalgae had higher amount of cellulose contents than the microalgae. It is possible that macroalgae produced higher quality of cellulose based on the amount of cellulose produced. Based on Menon, Selvakumar, Kumar, and Ramakrishna (2017), the filamentous green algae have high amount of cellulose contents compare to others. Furthermore, the lowest cellulose contents were from *Chlorella* sp. and *Scenedesmus* sp. Algal small size may contribute to limited amounts or small size of cellulose. Improper cellulose extraction will damage the cellulose structure and decrease the cellulose contents (Baba Hamed et al., 2016). Mixing during culture will ensure all algae receive the same amount of light, carbon dioxide, air and nutrients for growth. The productivity of biomass will also be low if no aeration is provided (Muhammad Imran Khan, Shin, & Kim, 2018).

In addition, the highest amount of biomass cellulose obtained were *Cladophora* sp. at 0.0069 g (mean) while the lowest amount of biomass cellulose obtained was *Chlorella* sp. at 0.0025 g (mean) (p-value (α=0.05). The significance value is lower than 0.05 ($p = 0.018$). This means that different type of algae has different effect on production of cellulose biomass (p value <0.05). The *Cladophora* sp. have highest biomass among other green algae. In previous research, Mihhels (2017) said that the *Cladophora* sp. have high amount of cellulose contents with 95.2% of crystallinity compared to *Ulva* sp. This was supported by Mihranyan (2010) stating that Cladophora sp. contains 12-30% of cellulose.

Conclusion

This present study was conducted to investigate the relationship between the types of algae and biomass cellulose content. Four selected green algae were chosen which were *Cladophora* sp., *Enteromorpha* sp., *Chlorella* sp. and *Scenedesmus* sp. The total chlorophyll contents and growth curve of algae were also recorded. Based on the findings, the growth curve for algae is S-shaped which also known as sigmoid. There are four phases in the cycle of algae which the lag phase, exponential phase, stationary phase and death phase. As for the chlorophyll contents, in different types of algae show the roughly around the same quantity. This because the selected algae fall in same genera which Chlorophyta. Furthermore, the result for the biomass of cellulose had shown that there was significantly difference between these algae ($p=0.018$). The *Cladophora* sp. have higher biomass than *Chlorella* sp., *Scenedesmus* sp. and *Enteromorpha* sp. This is because the *Cladophora* sp. classified as filamentous green algae that have thick microfibrillar structures that produces high amount of cellulose. These marine species collected have the potential to grow well in limited resources. Due to the bigger size of the algae, high amounts of cellulose were produced. Proper aseptic

techniques need to be applied before carrying out the experiment. Any contamination that occurs will severely affect the growth of algae.

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CHAPTER 1: ECOLOGY

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