UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF ECTOPARASITES IN RED HYBRID TILAPIA (*Oreochromis* sp.) CULTURED IN EARTHEN POND

NASUHA BINTI ISMAIL

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NASUHA BINTI ISMAIL

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Identification of ectoparasites in Red Hybrid Tilapia cultured in earthen pond", by Nasuha binti Ismail and in my opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 - Final Year Project.

DR. MOHD HEZMEE B. MOHD NOOR

DVM (UPM), PhD (Queensland)

Associate Professor,

Department of Veterinary Preclinical Sciences,

Faculty of Veterinary Medicine

University Putra Malaysia

(Supervisor)

DR. HASSAN MD DAUD

DVM (UPM), MSc. (Stirling), PhD (Kingston, UK)

Associate Professor,

Department of Veterinary Clinical Studies,

Faculty of Veterinary Medicine

University Putra Malaysia

(Co-Supervisor)

DEDICATIONS

Specially dedicated to Mama, Ayah, family, friends and fish lovers :)

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

MENGENALPASTI PARASIT LUAR PADA TILAPIA MERAH HIBRID (*Oreochromis sp.*) PELIHARAAN DI DALAM KOLAM TANAH

Oleh

Nasuha Bt Ismail

2017

Penyelia: Profesor Madya Dr. Mohd Hezmee Bin Mohd Noor

Penyelia Bersama:

Profesor Madya Dr. Hassan Bin Md Daud

Tilapia merah hybrid (*Oreochromis sp*.) telah menunjukkan peningkatan dalam pengeluaran dari tasik dan empangan di Malaysia. Pada masa kini, tilapia merah hybrid telah di rekodkan sebagai ikan air tawar yang paling banyak di ternak sehingga 46% telah di perlihara di dalam kolam tanah. Malangnya, jangkitan penyakit terutamanya jangkitan parasit telah membantutkan tumbesaran dan hasil pengeluaran ternakan ikan. Kajian ini bertujuan untuk menentukan prevalens dan mengenalpasti parasit luar serta patologi di sebabkan kehadiran parasite luar dalam

tilapia merah hybrid yang di ternak di kolam tanah. Daripada 30 ekor tilapia merah hybrid yang telah dikumpulkan dari tiga ladang y di Selangor, hanya 17 (56.7%) ekor ikan di jangkiti dengan parasit luar. Morfologi parasit luar telah dikenalpasti dan diteliti dengan menggunakan mikroskop cahaya dan bilangan parasit luar dikira dan dijadualkan. Hanya satu spesis parasit luar yang dijumpai iaitu *Dactylogyrus sp*. (Monogenea) di kawasan insang. Histopatologi sampel insang menunjukkan perubahan hiperpalasia dan hakisan pada epithelium lamella. *Dactylogyrus* menggunakan opisthaptor untuk mencengkam lebih dalam pada lapisan bawah membrane insang yang telah menyebabkan reaksi hiperplastik yang merosakkan lamella kedua. Lebih banyak kajian perlu dilakukan untuk menambah baik ternakan ikan tilapia merah hibrid didalam kolam tanah untuk mengurangkan dan menhindari jangkitan parasit luar.

Kata kunci: Tilapia merah hibrid, parasit luar, *Dactylogyrus*, histopatologi.

ABSTRACT

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

IDENTIFICATION OF ECTOPARASITES OF RED HYBRID TILAPIA (*Oreochromis sp.*) CULTURED IN EARTHEN PONDS

By

Nasuha Bt. Ismail

2017

Supervisor: Associate Professor Dr Mohd Hezmee B. Mohd Noor

Co-Supervisor: Assoc. Prof. Dr. Hassan B. Mohd Daud

Red hybrid tilapia (*Oreochromis sp*.) has shown a rapid increase in production from lakes and reservoirs in Malaysia. Currently red hybrid tilapia was recorded as the most common fresh water fish being farmed, where up to 46% was cultured in earthen pond system. Unfortunately, disease infection especially parasite infestation had affected the normal growth and production yield of the farmed fish. The present study was aimed to determine the prevalence, identification and pathological lesions of the ectoparasite present in red hybrid tilapia cultured in earthen pond. In the study, 30 red hybrid tilapias were collected from three different farms within Selangor but only 17 fishes (56.7%) were infested with ectoparasites. The morphology of the

ectoparasites was determined based on the light microscopy and the number of ectoparasites observed were counted and tabulated. There was only one ectoparasite species found viz., *Dactylogyrus sp*. (Monogenea) that has infested the gills region. Histopathology of the gills samples showed hyperplastic changes of the lamellar epithelium and erosion of the lamella. Opisthaptor of *Dactylogyrus* penetrated deep into the basement of gills membrane where it induced a hyperplastic reaction which subsequently destroyed the secondary lamella. Further study is required to improve the farming of the cultured red hybrid tilapia in earthen ponds to reduce or eliminate ectoparasites infestation.

Keyword : Red hybrid, ectoparasite, *Dactylogyrus,* histopatho

1.0 INTRODUCTION

1.1 Study background

The demand for cheap and affordable animal protein has risen consistently together with the growth of human population. Besides livestock, fish is the major source of animal that subsequently protein made aquaculture to be one of the fastest growing food sectors in the world. Aquaculture sector has also been an important vector for the introduction, transfer and spread of aquatic disease and parasites (Agos, 2013). This aquaculture practices have also been significantly contributing substantially for the local food security and livelihood of fishermen and rural community (Shaharom et al., 2011). Since the last decade tilapia fish that is also known as aquatic chicken have shown a rapid increase in production in Malaysian lakes and reservoirs to meet market demand (Marcel et al., 2013). The hardy characteristic has made tilapia a major freshwater fish to be culture. Although tilapias are more resistant to unfavourable water quality compared to other freshwater fish, there were studies being reported that tilapias being infested with ectoparasite and endoparasite (Bichi, 2009). Screening of the ectoparasite infestation in tilapia fish may assist us in searching for some intervention measured in reducing the loss of fish cause by parasitic diseases. The justification of the study is that red tilapia is one of the common fresh water fish cultured in Malaysia. However, lack of information on the ectoparasites present in the fish may affect management protocol such as culture system, pond size, water source thus it will effect to the growth rate and consequently decrease in production (quality/quantity) yield. The objectives of the study were to determine the prevalance of ectoparasites in red hybrid tilapia

cultured in earthern pond, to compare the presence of the ectoparasited with the management system and to examine the pathological lesion induced by the ectoparasites in the red hybrid tilapia cultured in earthen pond. The hypothesis of this study was that there is no presence of ectoparasites in red hybrid tilapia cutltured in earthen pond. The hypothesis of this study was that there is no presence of ectoparasites in red hybrid tilapia cultured in earthen pond.

2.0 LITERATURE REVIEW

2.1 Aquaculture industry in Malaysia

The fish farming techniques were limited to freshwater fishes in the early centuries, but by the 20th century, new techniques were developed to breed saltwater species. The new successful methods for the aquaculture industry had really started blooming towards the end of 20th century and early 21st century (Dey *et al*., 2006). This industry has also quick and well developed in Malaysia and now it is an important activity. It is an important industry in Malaysia as to cope with the demand and supply of food fish that preserve as national food security, lessen pressure on capture fisheries, generate foreign exchange, provide employment, diversify or an alternative income to the fisherman and farmer and provide business and investment opportunity.

In 2008, Malaysia aims to increase fisheries production by one third - Malaysia's fisheries industry has reached the half way stage in the government's programme to increase the nation's annual fisheries production by 600,000 metric tons (mt) or one third during the current Ninth Five Year Malaysia Plan (2006-2010). The government support the efforts to develop aquaculture schemes in coastal areas and provide funding for new fish farming technology to be adopted.

As more research being carried out on the life cycles of harvestable fish and different factor, it encourages development of the industry. The fish farmer then slowly started to practice improved techniques to gain more control over the fish

health and culture. Further interventions by the scientists and farmers in fish breeding lead to adoption of the factors that were ideal for commercial farms.

There are varieties of freshwater aquaculture being culture in Malaysia. In 2012, Yusof and Aisyah reported that main cultured species in Malaysia were freshwater catfish (*Clarias* sp.), black and red tilapia (*Oreochromis* sp.), riverine catfish (*Pangasius sp.)* and freshwater giant prawn (*Macrobrachium rosenbergii*).

2.2 Tilapia

Tilapia is the generic name of a group of cichlids that is endemic to Africa. This group of fish is native to Africa, Mediterranean and Middle East. It was classified as old cultured fish as found in Egyptian tombs 2000 BC. (Brown et. al, 2010). It was introduced into many tropical, subtropical and temperate region of the world during second half of the 20th century (Pillay, 1990). Tilapia culture has been practiced in various culture system (earthen ponds, concerted tanks, super-aerated ponds, raceways and cages), management strategies (extensive, semi-intensive or intensive, monoculture, polyculture, monosex, and mixed sex) and in different environments (freshwater and saline water) (Altun et al.,2006).

Tilapia is known for their ability to sexually mature at a small size; around 8-10cm (3-4 inch) in body length and at young age when it reaches 2-3 months old (Chapman, 2015). In poorly fertilizes ponds sexually matured fish may be as small as 15g (Popma & Lovshin, 1995). Egg number is proportion to the body weight of the female. The suitable temperatures to rear tilapia at the temperature range from

31°C to 36 °C. It is an omnivorous grazer that feeds on wide range of food type such as phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus. Tilapia can live longer than 10 years and reach a weight exceeding 5 kg (FAO, 2006). According to FAO, tilapias are asynchronous breeders therefore hormones are not used to induce spawning. The spawning will continue throughout the year in tropics and during the warm season in the subtropics. It will only suppress during the cold period.

Tilapias are more resistant to viral, bacterial and parasitic diseases compared to other commonly cultured fishes. At temperatures greater than 16° to 18°C and in the absence of severe environmental stress, tilapia rarely succumbed to diseases. However, viral, bacterial and parasitic problems have all been reported, especially after stress from low temperature, handling, severe crowding or poor water quality (Popma & Lovshin, 1995).

2.3 Fish Parasite

Parasites can be found in any fish species and within any type of aquatic culture system. According to Lewis (1991), they range from Protozoans (Flagellates, Ciliates, Apicomplexans, etc.) to Metazoans (Myxozoans, Trematodes, Cestodes, Acanthocephalans, Nematodes, and Crustaceans). Parasitic diseases of fish are very common all over the world and are particularly important in the tropics (Roberts and Janovy, 2000). For cultured fish population, it was reported to be involved in serious

outbreak of disease (Kayis, 2000). The crowded culture environment, temperature and slow water flow increase the parasite multiplication and infestation (Bednarska, 2009). Mature parasites are more dangerous to fish depending on the modes of attachment, size and weight of the host (Jalali, 2010).

In Malaysia there are only a few studies on parasite in tilapia fish. Study by Bu and Leong (1997) found that monogenean species is the most common and abundance parasite on freshwater fish in tropical reservoir along the Perak River. In Borneo, Annia (2011) conducted a study at Serian Sarawak found two class of monogenea parasite and a phylum nematode from two different water quality. A recent study found six different species of monogenean gill parasite from *Oreochromis sp*.that was reared in cage culture method at Kenyir Lake by Agos *et a*l. (2013).

2.3.1 Monogenean

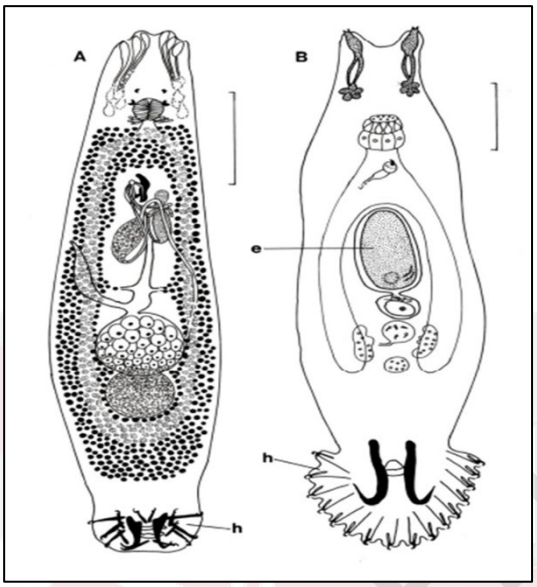

Figure 1.1: Monogenean, A: Dactylogyrus sp., an oviparous monogenean. B: Gyrodactylus sp., a viviparous monogenean. (Kearn, 201)

Monogeneans are ectoparasites and commonly known as fluke or gill fluke. This parasite usually infests the gills, skin and fin. The basis morphology character of monogenean is a posterior apparatus called an opisthaptor (Merang, 2011). Dactylogyrus and Gyrodactylus are the two most common genera of monogenean of freshwater fish (Klinger and Flyod, 1994).

2.4 Histology of Gill

The gills of fish are extremely sensitive to chemical and physical modification in the environment, mainly because of the large surface of the respiratory epithelium and high perfusion rate. Histopathological investigation had

been recognised to be a reliable biomarker in evaluation of the fish health (Hadi & Alwan, 2012). One of the advantages using histopathological as biomarker is manage to examine the specific target organ; including gills responsible for vital function as respiration in fish. (Gernhofer et al.,2001). Furthermore, the alteration found in the gills are normally easier to identify (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton & Laurén, 1990).

2.5 Water quality

El-Sayed *et al.* (1996) reported that warm water temperature (>21° C) is able to produce the best growth rate on Nile Tilapia, *Oreochromis niloticus*. Fish growth was significant reduced when exposed to water temperature below 21ºC according to their study. Fish stop feeding and developed severe stress, fungal infection and high mortality when the water temperature is below 10 degree celcius.

Dissolved oxygen (DO) refers to the microscopic bubbles of gaseous oxygen that are mixed in water and available to aquatic organism for respiration. According to Abdel *et al.* (2015), low DO level adversely affected fish growth and feed utilization. It was deduced that high growth under normal DO conditions resulted mainly from better feed consumption and nutrient digestibility. Abdel *et al.* (2015) also stated larger fish tolerated lower DO better than the small ones. There was previous report from Ross (2000) that Nile tilapia is able to tolerate DO at concentration as low as 1.0mg/L.

Nitrogen cycle is very important in fish culturing sector. The cycle begins with introducing ammonia into the water sourced from fish respiration, excessive feed, plant remnant and fish excrement and urine. The ionised form, ammonium is non-toxic to fish, whereas the unionized form, ammonia is dangerous to fish. Nitrosomonas bacteria then quickly break down the ammonia to produce nitrite. Nitrite is highly toxic to fish as well. Nitrobacter bacteria remove nitrite to produce nitrate. Nitrate is not highly toxic to fish. The live aquatic plants in ponds aid in maintaining it at low level.

Hardness is a measure of calcium and magnesium concentration in water. The desirable range of hardness is 20-150mg/L as CaCO3. It indicates the pond's ability to resist large pH changes.

3.0 MATERIALS AND METHOD

3.1 Fish sample

A total of 30 red hybrid tilapia (*Oreochromis* sp.) were collected for this study from three different farms within Selangor. 10 specimens were obtained from each farm. Two farms rear the fish in earthen pond which water supply from nearby brooklet Farm A and Farm B while Farm C rear the fish in plastic tank. Fish in Farm B were feed with processed scraps while the other farms were feed with commercial diet. The farms have different ways of culturing system to suit with the intention of the farm that are for the own consumption, local market and research respectively. The fish were caught using fish nets and fish trap . The fish were kept alive in aerated tanks until they were sacrificed and dissected (UPM/IACUC/FYP 2015/FPV.036). The water quality of the fish ponds was also determined using YSI 556 MPS and HACH Test Kit Model FF-2.

3.2 Examination of specimens

The fish were decapited and pithed prior to examination. The body weight of the fish were measured and recorded. The skin, fin and gills of each of the fish were examined for ectoparasites using wet smear technique. The pectoral fin of left and right were chopped off and placed in individual petri dish and then scrapped and smeared on glass slide. Scrapping of the body mucous for microscopic was done and placed on the glass slide with normal saline covered with a cover slip. The operculum were cut to expose the gill cavity. The gill arches were indivually

detached and placed individual labelled petri dishes filled with distilled water. It was scraped with scalpel blade and put on glass slide with cover slip then observed using compound microscope (Valladão et al., 2013). The magnification used was between 40x to 400x magnificaiton. The present or absent, number of parasites of fish sample were recorded.

3.3 Identification parasite

The parasites were identified by making sketches as observe under the light microscope and photographic record were made by using digital microscope (Moticam Pro) or digital phone camera. The identification was carried out based on pictorial guide from Kabata (1985).

3.4 Histopathological analysis.

Infested organs with ectoparasite were fixed in a buffered 10% formalin solution, processed according to usual histopathological techniques, embedded in parafin, sectioned at 5µm and stained with hematoxylin-eosin. The slides were analyzed, and photomicrographs were obtained using a digital microscope (Moticam Pro).

3.5 Data analysis

Statistical analysis was done to analyse the collected data using the GraphPad Prism 7 software. Two way ANOVA was used to study the significance between ectoparasites burden and farm location. From the analysis, (p<0.05) is considered as significant. The relation beteween the weight and the ectoparasite burden was determine by using the Pearson's correlation test.

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4.0 RESULTS

4.1 Prevalence of ectoparasite infestation on red hybrid tilapia fish

17 out of 30 red hybrid tilapia fish collected were infested with ectoparasite accounting of 56% of the total samples collected. Among the three sample locations, fish in farm B was found to have the highest parasitic infestation which was 100% of total samples collected. 70% of fish sample from fish in farm C was found to be infested and none of the fish sampled from farm A was infested with ectoparasites.

4.2 The relation of ectoparasite burden to each farm

Only one species of ectoparasite found infest the red hybrid tilapia fish. The species that found from this study was Monogenean. Eyespots seen in the flukes was confirmed as *Dactylogyru*s sp.. The flukes were found only at the gills of the fishes [Figure 2.1]. The flukes were found penetrates deeps at the tip of the gills [Figure 2.2]. The mean value for parasite burden in Farm B and Farm C on the right is 51.4, 2.1 and on the left is 43.3,1.7 respectively [Figure 2.3]. Upon statistical analysis of the comparison between the farms of the samples collected, the result was significant (p<0.05). There was no significant difference in distribution of *Dactylogyru* sp. between the left and the right gills of red hybrid tilapia.

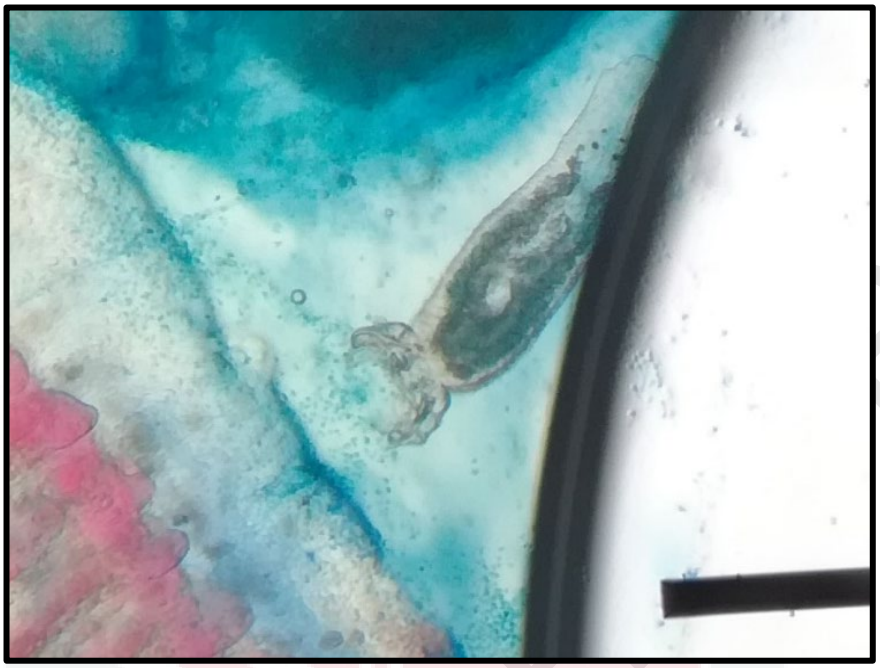

Figure 2.1: Monogenean found on the gills of fish.

Figure 2.2: Dactylogyrus found attached at the tip of the lamella.

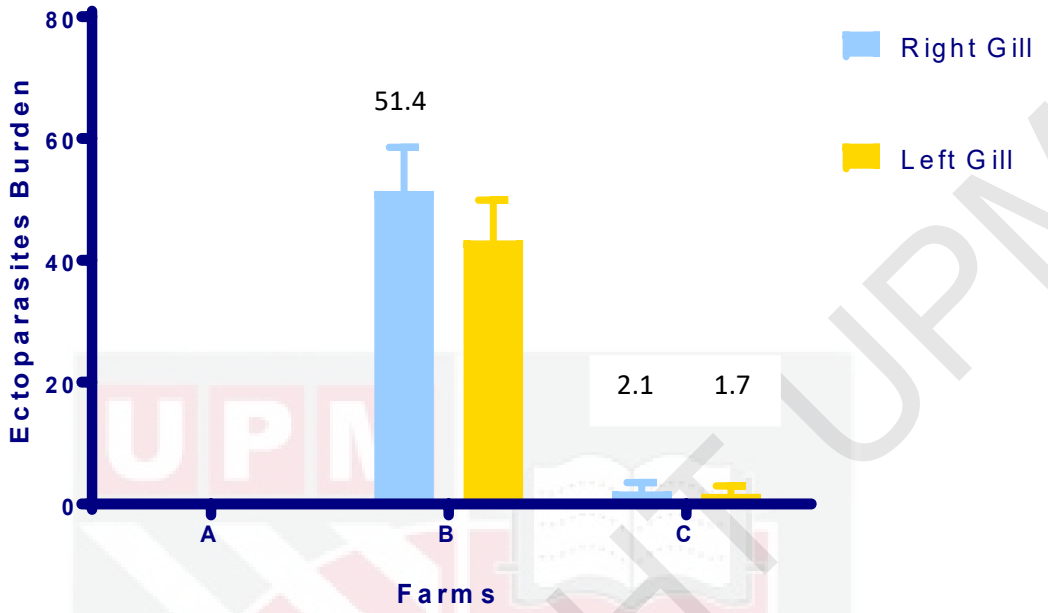

Figure 2.3: The relationship of etoparasites burden between farms

4.3 Correlation between weight to the parasite burden in each farm

From the results [Figure 3.1], it was found that there was no significant correlation (r = -0.02617) between the weight of the fish and the parasite burden in the fishes for Farm B and Farm C (r = 0.6101) [Figure 3.2].

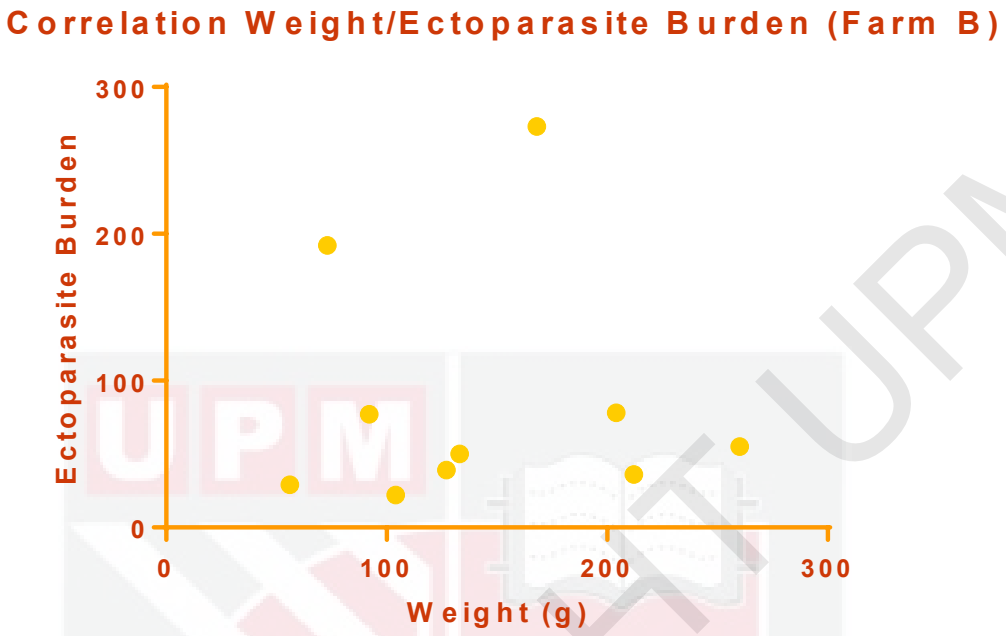

Figure 3.1: The correlation between weight and ectoparasites burden in Farm B.

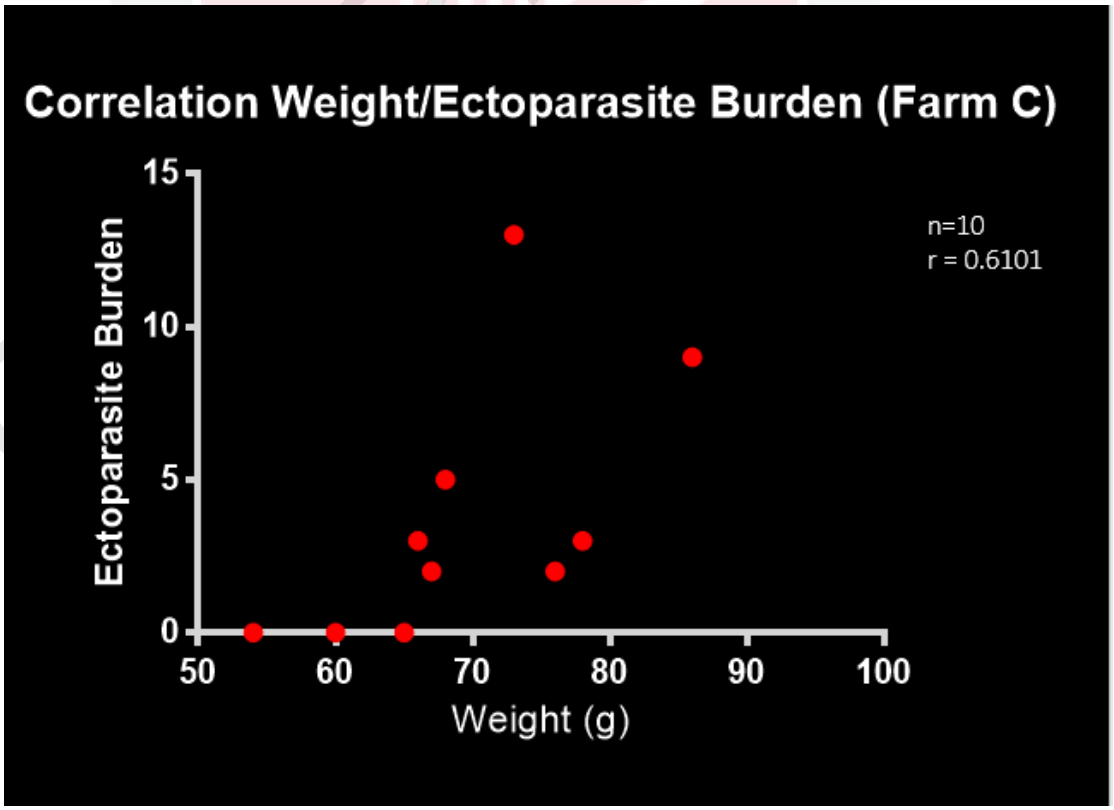

Figure 3.2: The correlation between weight and ectoparasites burden in Farm C.

4.4 Histopathological changes associates with ectoparasite infestation

The examination of histological material revealed that the opisthaptor of *Dactylogyrus* penetrates deep into the basement membrane [Figure 4.1] and the connective tissue of the primary lamella causing destruction to the secondary lamellae. The most frequent lesion observed in all analysed gills were complete and incomplete fusion of the secondary lamellae and hyperplasia of the lamellar epithelium.

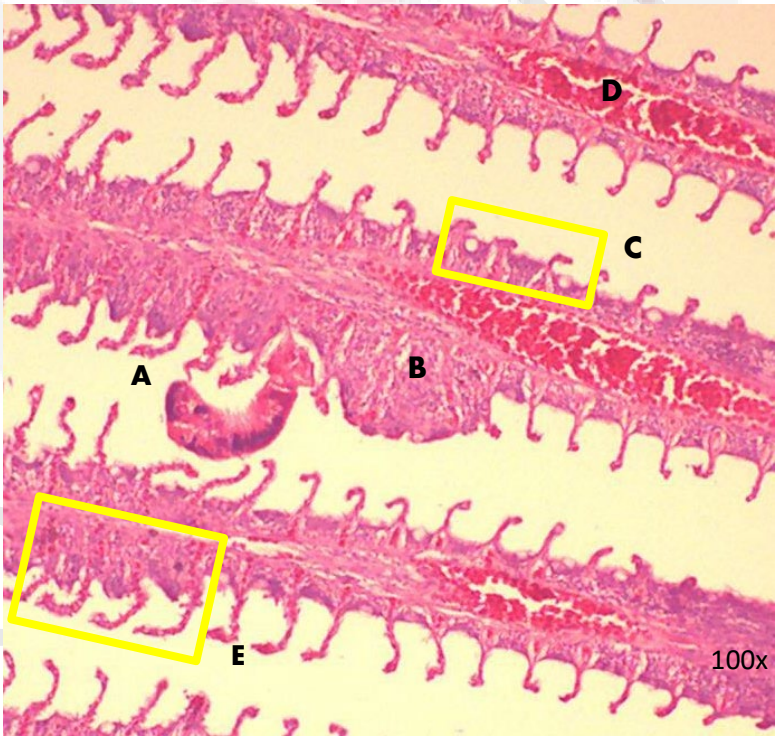

Figure 4.1: Histology of infested gill. A: Dactylogyrus penetrate deep into the epithelial membrane, B: Hyperplasia, C: Complete fusion of lamella, D: Congestion within the filament. E: Incompete fusion of secondary lamella.

4.5 Water Quality

Parameter	Farm A	Farm B	Farm C	Reference Range
Temperature (°C)	31.4 (2.30 pm)	29.4 (12.30 noon)	26.4 (12.30 noon)	
Dissolved oxygen (mg/L)	3.6	3.7	3.8	3.0-7.0 (BFAR-Philminaq, 2007)
Ammonia (mg/L)	0.006	0.008	0.001	<0.05 (Lawson T. B, 1995)
Nitrite (mg/L)	0.198	0.66	0.198	0.4 (BFAR-Philminaq, 2007)
Hardness (mg/L as CaCO3)	85	57	71	20-150
pH	5.95	4.48	6.43	6.5-9.0 (BFAR-Philminaq, 2007)

Table 1: Water quality parameter.

There were six water quality parameter determined in this study; water temperature (°C), dissolve oxygen, ammonia (NH3), Nitrite (NH2), hardness and pH. Most of the temperature were within the normal range in spite of the water temperature were taken at the highest point of temperature of the day. The water temperature at Farm C was not too low for rearing the tilapia. Farm B had the nitrite value higher than the suggested. Dissolve oxygen and hardness that measured the calcium carbonate amount in water sample all farms were within range. While ammonia and pH in all farm measured were below the reference value.

5.0 DISCUSSION

In this study, the only ectoparasite found is *Dactylogyrus* sp. from Monogenean genera. Monogenean was a common ectoparasite found in cultured fresh water fish and the ectoparasite also found in the other study being carried out in Perak and Terengganu, Malaysia (Lim et al., 2016 and Agos et al., 2016). The Monogenean found in this study was at the gills area as the adult Monogenean generally only inhibit a precise site (Kearn, 1998).

The difference in management of the farm in term of the pond preparation, types of feed and water supply can be the reasons for the significant difference in ectoparasite burden between farm. The significant difference in weight of fish in Farm C with the ectoparasite burden can be due to the fishes having low immunity. The number of ectoparasites can be low due to the ectoparasite fails to maintain proper hold on the gills (Tasawar et al., 2001). Therefore even the number of the ectoparasite burden was small in Farm C, it might lead the fish in severe condition in thesse fishes. The relation between weight and ectoparasites burden in Farm B was not significant can be due to good adaptibility of the fishes in the pond in this farm. The examination of histology material reveal that the opisthaptor penetrate deep into the basement membrane and the connective tissue of the primary lamella causing destruction to the secondary lamella.The attachment of the Monogenean to the host's gill epithelium is ensured by the dorsal and ventral spines of the opisthaptoral and by the hamuli which penetrate deep into the epithelial cell. The activity of hamuli cause the destruction of the epithelial and responsible for the detachment of gill cells cause

the area to bleed (Dezfuli B.S. et al., 2006). The blood expelled from this wound will be devoured by the parasite.

The water quality in Farm B had the nitrite value higher than the suggested. Nitrite problems are more likely to occur in closed culture systems or intensive system due to insufficient, inefficient management and malfunctioning filtration systems. High nitrite concentrations in ponds occur more frequently when temperatures are fluctuating, continuous cloudy or rainy weather conditions resulting in the breakdown of the nitrogen cycle due to decreased plankton and/or bacterial activity. A reduction in plankton activity in ponds (because of lower temperatures, nutrient depletion, cloudy or rainy weather, herbicide/insecticide treatments etc.) can result in less ammonia assimilated by the algae, thus increasing the load on the nitrifying bacteria. If nitrite levels exceed that which resident bacteria can rapidly convert to nitrate, a build-up of nitrite occurs, and brown blood disease is a risk. Although nitrite is seldom a problem in systems with high water exchange rates or good filtration, systems should be monitored year-round and managed when necessary to prevent severe economic loss from brown blood in any fish culture facility.

6.0 CONCLUSION

Dactylogyrus sp. is the most common ectoparasite infesting the red hybrid tilapia culture in earthen pond. Higher ectoparasites burden were found in low water quality. Poor management in intensive system could lead to poor water quality that directly causing stress to the fishes. The stressful condition the fishes having will cause the fishes in low immune system thus susceptible to parasite and other harmful pathogen. There were pathological changes seen in fish infested with ectoparasite in cellular even unable to be appreciated morphologically.

7.0 RECOMMENDATION

Recommendation that can be suggest for further study is to study the ectoparasites present in different life stage of fish such as fingerling, grower and finisher because there are different in immune level at different stage of life. The sampling of fish should be consider to be taken from different ponds that available for each farm. Other than that, the study can also be carried out in farm that practice polyculture and include the multiple species of fish in the study to review and relate the difference ectoparasite and the preference of the ectoparasite to different species of fish. Furthermore, larger sample size can be used to minimize statistical error. Besides that, use the quick lime for the treatment of the earthen pond can help in reducing the ectoparasites burden as the quick lime help in buffering the soil and kill all the microbes.

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