DIFFERENTIAL CELL COUNTS IN 5 COMMERCIAL ORNAMENTAL FISHES

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This project report is submitted in partial fulfilment of the requirements for the degree of Bachelor of Agriculture (Aquaculture)

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This is to certify that I have examined the final year project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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_____

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DEDICATION

To my beloved Parents

Abah Ahmad Tarmizi

and

Mama Zalehan

This humble effort is a sign of my infinity love to you!

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Thanks to Allah S.W.T. Lord of all mankind for all the countless gifts offered me and thanks to my family for their love and support for this successful research project. It is a great pleasure to acknowledge my deepest thanks and gratitude to my supervisor, Dr. Chong Chou Min for his endless support, understanding, patience, kind supervision and for pushing me farther than I thought I could go. It is a great honour to work this project under his supervision.

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ABSTRACT

The aim of this study was to determine the percentage of differential types of leukocytes in commercial ornamental fish (Green Tiger Barb - *Puntigrus tetrazona*, Rosy Tetra - *Hyphessobrycon rosaceus*, Rosy Barb - *Pethia conchonius*, Red Eye Tetra - *Moenkhausia sanctaefilomenae* and Red Zebra Danio - *Danio rerio)* and G:L (granulocyte to lymphocyte) ratio as stress index of these species. This study examined 20 samples (four specimens for each species) from the controlled conditions in good health condition. The leukocytes stained by May-Grunwald Giemsa solution and were classified to four types; lymphocytes, granulocytes, monocytes and thrombocytes. Lymphocytes were the most abundant leukocytes found in the peripheral blood of all tested species, in which the lowest percentage lymphocytes was recorded in Rosy Tetra (81.74±3.27) while the highest value was Red Eye Tetra (94.57±1.32). The G:L ratio of the tested species were lower than 0.5, where Rosy Tetra recorded the highest G:L ratio among the tested (0.120±0.021), meanwhile Red Eye Tetra was the lowest (0.007±0.003). The current result provides the reference values and background for the future experiments. Any increment from the G:L ratio of the respective species reported in this study can be an indication of stress.

Keywords: differential cell count, lymphocyte, granulocyte, monocyte, thrombocyte.

ABSTRAK

Tujuan kajian ini adalah untuk mengetahui peratusan perbezaan jenis leukosit dalam ikan hiasan komersil (Green Tiger Barb - *Puntigrus tetrazona*, Rosy Tetra - *Hyphessobrycon rosaceus*, Rosy Barb - *Pethia conchonius*, Red Eye Tetra - *Moenkhausia sanctaefilomenae* dan Red Zebra Danio - *Danio rerio*) dan indeks tekanan bagi spesies ini. Secara keseluruhannya, kajian ini mengkaji 20 sampel (empat spesimen untuk setiap spesies) dari keadaan yang terkawal dalam keadaan kesihatan yang baik. Leukosit yang diwarnai menggunakan Giemsa dan diklasifikasikan kepada jenisnya; limfosit, granulosit, monosit dan trombosit dengan nukleus bersegmen. Limfosit peratusan terendah didapati pada Rosy Tetra (81.74 ± 3.27) manakala nilai tertinggi adalah pada Red Eye Tetra (94.57 ± 1.32). Nisbah G: L yang diuji adalah lebih rendah daripada 0.5, di mana Rosy Tetra mencatatkan nisbah G: L yang paling tinggi diuji (0.120 ± 0.021), sementara Red Eye Tetra adalah yang paling rendah (0.007 ± 0.003). Hasil kajian semasa memberikan nilai rujukan dan latar belakang untuk eksperimen untuk masa akan datang. Mana-mana kenaikan daripada nisbah G: L bagi spesies masing-masing yang dilaporkan dalam kajian ini boleh menjadi petunjuk tekanan.

Kata kunci: perbezaan kiraan sel, limfosit, granulosit, monosit, trombosit.

TABLE OF CONTENT

DEDICATION i
ACKNOWLEDGEMENT ii
ABSTRACT iii
ABSTRAK iv
TABLE OF CONTENT v
LIST OF ABBREVIATION AND SYMBOLS vii
LIST OF TABLE viii
LIST OF FIGURES ix

CHAPTER 1
INTRODUCTION 1

CHAPTER 2
LITERATURE REVIEW
2.1 *Puntigrus tetrazona* 5
2.2 *Hyphessobrycon rosaceus* 6
2.3 *Moenkhausia sanctaefilomenae* 8
2.4 *Danio rerio* 9
2.5 *Pethia conchonius* 11
2.6 Differential cell count 12
2.7 Leukocyte 12
2.7.1 Lymphocyte 12
2.7.2 Granulocyte 14
2.7.3 Monocyte 14
2.7.4 Thrombocyte 15
2.8 G:L ratio 15

CHAPTER 3
MATERIAL AND METHODOLOGY
3.1 Experimental design 16
3.1.1 Blood collection 16
3.1.2 Light microscopy 16
3.1.3 Differential cell count 17

CHAPTER 4
RESULTS AND DISCUSSION
4.1 Morphology of fish 18
4.2 Granulocyte 23
4.3 Lymphocyte 24
4.4 Monocyte 25
4.5 Thrombocyte 26
4.6 Differential white blood cell count and G:L ratio 27

CHAPTER 5
CONCLUSION 29

REFERENCES 30

LIST OF ABBREVIATION AND SYMBOLS

mL	Millilitre
cm	Centimetre
%	Percent
v/v	Volume per volume percent
G:L ratio	Granulocyte to lymphocyte ratio
N:C	Neutrophil to cortisol ratio

LIST OF TABLE

Table		Page
1	Differential white blood cell count and G:L ratio of the tested ornamental fish species	28

LIST OF FIGURES

Table		Page
1	*Puntigrus tetrazona* (Source: Poissons-online)	6
2	*Hyphessobrycon rosaceus* (Source: Fish, Tanks and Ponds)	7
3	*Moenkhausia sanctaefilomenae* (Source: Fish, Tanks and Ponds)	9
4	*Danio rerio* (Source: Merifish Aquarium)	10
5	*Pethia conchonius* (Source: Aquarium Glaser GmBH)	12
6	Morphology of Green Tiger Barb. (Source: http://www.tropicalfishsite.com/green-tiger-barb-puntius-tetrazona/	18
7	Species A: Green Tiger Barb (*Puntigrus tetrazona*)	18
8	Morphology of Red Eye Tetra (Source: http://animal-world.com/encyclo/fresh/characins/redeye.php)	19
9	Species B: Red Eye Tetra (*Moenkhausia sanctaefilomenae)*	19
10	Morphology of Rosy Barb (Source: https://www.cabi.org/isc/datasheet/121017)	20
11	Species C: Rosy Barb (*Pethia conchonius*)	20
12	Morphology of Red Zebra Danio (Source: https://shop.glofish.com/products/starfire-red-glofish-danio)	21
13	Species D: Red Zebra Danio (*Danio rerio)*	21
14	Morphology of Rosy Tetra (Source: http://www.starallfish.com/Tetras-fish-export.asp)	22
15	Species E: Rosy Tetra (*Hyphessobrycon rosaceus*)	22

16	MGG staining of Granulocytes	23
17	MGG staining of Lymphocytes	24
18	MGG staining of Monocytes	25
19	MGG staining of Thrombocytes	26

CHAPTER 1

INTRODUCTION

Hematology is an approach for monitoring health status, detecting diseases, and following the diseases progress and the reaction to the therapy. Despite advances in fish medicine in recent years, the fish hematological interpretation is often hindered by the lack of successful reference value and the heterogeneity of fish species diversity (Clauss et al., 2008). Differential cell count is the determination of different cellular component of bood, it is also the stress index for fish. Based on Lewis et al. (2010), blood is an ideal tissue to determine the cellular stress response in fish. Usually, fish health research and medicine has focused on aquaculture and food fish species as food fish species are one of the main protein source to people. The need of conserving and protecting the natural sources, public and private aquarium facilities, commercial ornamental fish producers, collectors and people who likes the fishes as pets make the fish health practices are among the things that play an important roles in improving the industry of aquaculture as popular display fish (Stevens et al., 2017). Animal health, which including the fish are being consulted from the expertise in evaluating fish health because of the challenge on getting the samples and it does needs skills and techniques (Clauss et al., 2008).

Stress is an inherent component of the life of all vertebrates, including fishes. Stress measure are being applied in order gain data on how effectively a fish resists death and resets homeostatic norms when faced with noxious stimuli (Schreck and Tort, 2016). There is no precise alternative and it is hard to determine the stress index by using measurement of cortisol level due to the small size of fish (Sink et al., 2007).

Based on Department of Fisheries (2016), currently there are 259 the exporters of ornamental fish in Malaysia since this industry providing jobs and foreign exchange between countries. Ornamental fish industry is the fastest-growing industry in agriculture sector of Malaysia. Also, Malaysia is the third largest ornamental fish producer after Singapore and Indonesia with 7% share of global market in 2000. Ornamental fish industry experienced for 24% growth for exports during 1990 to 2000. The industry of ornamental fish in Malaysia is expected to increase steadily in future (MOA). These five species (*Puntigrus tetrazona*, *Hyphessobrycon rosaceus*, *Pethia conchonius*, *Moenkhausia sanctaefilomenae* and *Danio rerio)* were chosen for this study as there is no baseline data on G:L ratio reported in these species, Green Tiger Barb (*Puntigrus tetrazona*), Rosy Tetra (*Hyphessobrycon rosaceus*), Rosy Barb (*Pethia conchonius*), Red Eye Tetra (*Moenkhausia sanctaefilomenae*) and Red Zebra Danio (*Danio rerio*). Physiological and behavioural measures have been used to determine the stress level in fishes, but usually stress level is recorded by measuring cortisol which can be taken from tissues of body, whole body homogenates or by measuring the water of fish being held (Turner et al., 2003; Ramsay et al., 2006). This method is efficient to be used for small species, also for ornamental species such as *Danio rerio* (Felix et al., 2013).

Objectives

In order to explore the stress index of the fish, the current project was carried out to determine the G:L ratio (granulocyte to lymphocyte ratio) of the fish based on differential white blood cell count of five species of commercial ornamental fish, which are Green Tiger Barb (*Puntigrus tetrazona*), Rosy Tetra (*Hyphessobrycon rosaceus*), Rosy Barb (*Pethia conchonius*), Red Eye Tetra (*Moenkhausia sanctaefilomenae*) and Red Zebra Danio (*Danio rerio).*

Hypothesis

The null hypothesis (H_0) of this project is there is no difference in G:L ratio across the tested species (*Puntigrus tetrazona*, *Hyphessobrycon rosaceus*, *Pethia conchonius*, *Moenkhausia sanctaefilomenae* and *Danio rerio)* while, the alternative hypothesis (H_A) is there is difference in G:L ratio across the tested species (*Puntigrus tetrazona*, *Hyphessobrycon rosaceus*, *Pethia conchonius*, *Moenkhausia sanctaefilomenae* and *Danio rerio).*

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