ASSESSMENT OF THERAPEUTIC AND ANTIMICROBIAL EFFECTS OF LOCAL PLANTS ON BACTERIAL DISEASES IN RED HYBRID TILAPIA (Oreochromis sp.)

SHARIFAH RAINA BINTI MANAF

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By

SHARIFAH RAINA BINTI MANAF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the degree of Doctor of Philosophy

April 2016

DEDICATION

With appreciation and respect, this thesis is dedicated to:

My supportive husband, Mr Mahazan Muhammad who made this writing endurable
My beloved parents, Mr Manaf Daud & Mrs Sharifah Zainun
My beloved parents in law, Mr Muhammad Talib & Mrs Fauziah Ismail who made it possible
My son, Muslim Ramadhani Mahazan and my siblings who made it all worthwhile.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Hassan Mohd Daud, PhD
Faculty : Veterinary Medicine

Currently, aquaculture is regarded as one of the potential strategic industries for Malaysia. The percentage on its production increases year by year. The growth are obvious due to obstacle in continuous dependent on finite stocks of protein source from wild-caught fish which is shown to be over-exploited or near to be depletion. One of main problem in aquaculture is outbreaks of infectious diseases which has incurred big losses to the industry. Herbal medicine has been used in most part of the world as an alternative medicine for human being and domestic animals. The plant compounds have been shown to be beneficial and rich in a wide variety of phytonutrients, and thus used as chemotherapeutics and feed additives. Hence, the general objectives of this study were to determine the effect of herbal supplementation on the growth performance, disease resistant, disease treatment in Red hybrid tilapia (*Oreochromis* sp.). Six extracts of *Vitex trifolia, Aloe vera*, *Strobilanthes crispus*, *Clinacanthus nutans, Pereskia grandifolia* and *Peperomia pellucida* were determined for phytochemical properties and their antibacterial activities against common freshwater pathogens i.e. *Streptococcus agalactiae, Aeromonas hydrophila* and *Enterobacter cloacae.* Qualitatively screening of phytochemical properties in herbs were determined for flavonoids, tannins, saponin, alkaloids steroid and glycoside. The results showed presence of flavonoid in all plant extracts. For the antimicrobial activity, the aqueous and methanolic extracts were tested by using disk diffusion method. Antimicrobial assay of methanolic extracts (25 to 100 mg/mL) showed effectiveness against the pathogenic bacteria. Comparatively, all aqueous extracts did not show any antimicrobial activity. Moderate antibacterial activity was shown by the methanolic extracts of *V. trifolia, A. vera* and *S. crispus* while weak antimicrobial activity was shown by *C. nutans, P. grandifolia* and *P. pellucida*. From the antimicrobial result, three potential plant extracts namely *V. trifolia* (VTE), *S. crispus* (SCE) and *A. vera* (AVE) were test for toxicity and feeding experiment. The cytotoxicity test using *Artemia salina* (45.9 - 66.7 µg/mL) and BF-2 fish cell line (85.0 - 120.50 µg/mL). The median lethal dose (LD_{50}) value in *Oreochromis* sp. injected intraperitoneal were equivalent to 2.67 X 10^7

CFU/mL (*S. agalactiae*), 3.10 X 10^6 CFU/mL (*E. cloacae*) and 3.75 X 10^5 CFU/mL (*A. hydrophila*). *Oreochromis* sp. were fed with diet supplemented with VTE, SCE and AVE at 2g/kg, 5g/kg, 7g/kg and 9g/kg for 45 days, followed by challenged with 0.1 ml of 10^7 CFU/ml *S. agalactiae*. The results showed *Oreochromis* sp. supplemented with VTE-7 was the best growth promoter among treatments. It showed 57.3% in body weight gain with 100% survivability. For haematological the value of Hb after challenged with *S. agalactiae* showed significant different ($P<0.05$) compared to control except in SCE-2 and SCE-7. While, in WBC value showed significant different ($P<0.05$) except in AVE-5 and AVE-7. In MCV value showed significant different except in SCE-5, AVE-9 and in MCHC value showed significant except in VTE-2 and SCE-5. In biochemical parameters of blood, there was significant different in ALT value observed in fish fed with VTE-2, VTE-5, VTE-7, SCE-2, SCE-5, SCE-7, AVE-5 and AVE-7 compared to control (infected). In AST value showed significant different ($P<0.05$) in VTE-5, VTE-7, SCE-9, AVE-2 and AVE-7 compared to control (infected). Fish fed with SCE-5 was the best for the disease resistant showed the highest level of WBC value indicated increase immune responses and the ability of the fish to fight *S. agalactiae*. In histology examination of different selected herbal extracts showed mild degeneration in liver and kidney tissues. To determine the growth performance and disease resistant of *Oreochromis* sp. supplemented with herbal-mixed of the selected herbal extracts, the fish were fed with diet containing mixtures of AVE&VTE, VTE&SCE, SCE&AVE. Herbal mixed of AVE&VTE showed the most significant increased as compared with others. For SGR, only AVE&VTE showed promising result with significant higher ($P< 0.05$) than control. Fish survival after the disease challenge showed significant difference ($P< 0.05$) compared to control. In haematological assays, the post-challenged results showed the significant increased ($P<0.05$) of RBC, Hb and PCV values. The WBC value in pre- and post-challenged showed no significant different ($P>0.05$) of all treatments compared to control. As for MCHC value reading with the mixed-herbals, AVE&SCE was the only treatment which revealed no significant difference ($P>0.05$) in pre-challenged and showed decreased significant difference ($P<0.05$) in post-challenged in all treatments. On the other hand, MCV and plasma protein values did not show any significant difference ($P>0.05$) as compared to control. The biochemical parameters of ALT value and AST value were significantly affected by mixed-herbal supplementation. However in the post-challenged, the ALP value showed no significant difference compared to control. Histopathological changes, which included necrosis and haemorrhage in the kidney, liver and hepatopancreas tissues could be due to the cytotoxin produced by the bacteria. Also, a study was carried out to evaluate the effects of dietary supplementation of methanol extracts of *V. trifolia*, *S. crispus* and *A. vera Oreochromis* sp. as disease treatment. Results showed that RBC levels were significantly increased ($P<0.05$) in VTE-2, SCE-2, SCE-5, SCE-9, AVE-5, AVE-7 and control (OTC) as compared to control (infected). While, Hb value showed significant increased in VTE-2, SCE-5, SCE-9, in contrast to significant decreased in AVE-7 and AVE-9 as compared to control. For PCV value showed significant ($P<0.05$) increased in all treatments included control (OTC) as compared to control (infected). On the other hand, in MCHC value no significant different for SCE-5. Plasma protein value showed significant increased ($P<0.05$) after supplementation of VTE-2, VTE-5, SCE-2, SCE-5, SCE-9, AVE-2, AVE-5,

AVE-9. For ALT and AST values, they showed significant decrease in all treatments including control (uninfected) and control (OTC) ($P<0.05$). Also in ALP value, it showed significant decreased in all treatments including the control (OTC). The survivability of fish showed increase after fed with the herbal supplementation ($P< 0.05$) in VTE-5, VTE-7, AVE-2, AVE-5 and AVE-9 as compared to control (infected). All the haematological measurements in *Oreochromis* sp. that were infected with *S. agalactiae* with supplemented with VTE, SCE and AVE showed similar values compared with the control and the treatment with antibiotic. Its indicated the fish that were infected with *S. agalactiae* able to restore their haematological indices to near normal values. The results were agreeable with literatures which reported the numerous efficacies of the use of herbal in aquaculture to control various bacterial pathogens. Also from current research findings, suggested that mixed herbal of methanolic-extract given to *S. agalactiae* infected *Oreochromis* sp. had a synergistic restorative effect on the hematological variables. In conclusion, current study suggested that *V. trifolia*, *S. crispus* and *A. vera* extracts and mixed herbal were effective as growth promoters and bacterial disease treatment in *Oreochromis* sp. It also proved the potential of the selected herbal plant as aquaculture therapeutant as they were environmental friendly and it showed negligible side effect in the intended fish.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN KE ATAS SIFAT TERAPEUTIK DAN KESAN ANTIMIKROBIAL TUMBUHAN TEMPATAN BAGI PENYAKIT BAKTERIA DALAM TILAPIA HIBRID MERAH (*Oreochromis* sp.)

Oleh

SHARIFAH RAINA BINTI MANAF

April 2016

Pengerusi : Hassan Mohd Daud, PhD
Fakulti : Perubatan Veterinar

Pada masa ini, akuakultur dianggap sebagai salah satu industri yang sangat berpotensi untuk Malaysia. Peratus pada pengeluaran meningkat tahun ke tahun. Pertumbuhan akuakultur adalah jelas kerana halangan dalam pergantungan berterusan pada stok terhad sumber protein daripada ikan tangkapan liar yang menunjukkan sebagai eksploitasi terlampau atau hampir menjadi pupus. Masalah utama dalam akuakultur adalah wabak penyakit berjangkit yang telah menyebabkan kerugian besar kepada industri. Perubatan herba telah digunakan di kebanyakan bahagian dunia sebagai ubat alternatif untuk manusia dan haiwan domestik. Sebatian tumbuhan telah terbukti bermanfaat dan kaya dengan pelbagai khasiat pemakanan, dan dengan itu digunakan sebagai kemoteraputik dan bahan makanan tambahan. Oleh itu, objektif umum kajian ini adalah untuk menentukan kesan daripada diet tambahan herba terhadap prestasi pertumbuhan, resistan dan rawatan penyakit dalam tilapia hibrid merah (*Oreochromis* sp.). Enam ekstrak *Vitex trifolia*, *Aloe vera*, *Strobilanthes crispus*, *Clinacanthus nutans*, *Pereskia grandifolia* dan *Peperomia pellucida* ditentukan untuk sifat fitokimia dan aktiviti antibakteria terhadap patogen lazim air tawar iaitu *Streptococcus agalactiae*, *Aeromonas hydrophila* dan *Enterobacter cloacae*. Pemeriksaan kualitatif sifat fitokimia dalam herba telah di tentukan pada flavonoid, tanin, saponin, alkaloid, steroid dan glikosid. Hasil kajian menunjukkan kehadiran flavonoid dalam semua ekstrak tumbuhan. Untuk aktiviti antimikrobial, ekstrak akueus dan metanol telah diuji dengan menggunakan kaedah cakera peresapan asai. Antimikrobial asai ekstrak metanol (25 hingga 100 mg/mL) menunjukkan keberkesanan terhadap bakteria patogenik. Secara perbandingan, semua ekstrak akueus tidak menunjukkan apa-apa aktiviti antimikrobial. Aktiviti antibakteria sederhana telah ditunjukkan oleh ekstrak methanol *V. trifolia, A. vera* dan *S. crispus* manakala aktiviti antimikrobial lemah telah ditunjukkan oleh *C. nutans*, *P. grandifolia* dan *P. pellucida*. Daripada keputusan antimikrobial, 3 ekstrak tumbuhan berpotensi iaitu *V. trifolia* (VTE), *S. crispus* (SCE) dan *A. vera* (AVE) digunakan dalam penilaian ketoksikan dan eksperimen pemakanan. Ujian sitotoksiti menggunakan *Artemia salina* (45.9 - 66.7 µg/mL)

dan BF-2 sel turutan ikan (85.0-120.50 µg/mL). Nilai dos kematian purata (LD_{50}) dalam *Oreochromis* sp. disuntik secara intraperitoneum bersamaan dengan 2.67 X 10^7 CFU / mL (*S. agalactiae*), 3.10 X 10^6 CFU / mL (*E. cloacae*) dan 3.75 X 10^5 CFU / mL (*A. hydrophila*). *Oreochromis* sp. diberi makan ditambah dengan VTE, SCE dan AVE di 2 g/kg, 5 g/kg, 7g/kg dan 9g/kg untuk 45 hari eksperimen pemakanan, diikuti dengan dicabar dengan 0.1 ml 10^7 CFU/ml *S. agalactiae*. Hasil kajian menunjukkan *Oreochromis* sp. ditambah dengan VTE-7 adalah penggalak pertumbuhan yang terbaik di antara rawatan. Ia menunjukkan 57.3% dalam kenaikan berat badan dengan 100% kemandirian. Untuk nilai hematologi Hb selepas dicabar dengan *S. agalactiae* menunjukkan berbeza secara signifikan ($P<0.05$) berbanding kawalan kecuali dalam SCE-2 dan SCE-7. Sementara itu, dalam nilai WBC menunjukkan berbeza secara signifikan ($P<0.05$) kecuali dalam AVE-5 dan AVE-7. Dalam nilai MCV menunjukkan signifikan berbeza kecuali dalam SCE-5, AVE-9 dan nilai MCHC menunjukkan signifikan kecuali dalam VTE-2 dan SCE-5. Parameter biokimia darah, terdapat perbezaan yang signifikan dalam ALT ditunjukkan dalam ikan yang diberi makan dengan VTE-2, VTE-5, VTE-7, SCE-2, SCE-5, SCE-7, AVE-5 dan AVE-7 berbanding kawalan (dicabar). Dalam AST menunjukkan berbeza secara signifikan ($P<0.05$) dalam VTE-5, VTE-7, SCE-9, AVE-2 dan AVE-7 berbanding kawalan (dicabar). Ikan diberi makan dengan SCE-5 adalah yang terbaik untuk resistan penyakit, ini menunjukkan nilai tertinggi WBC menunjukkan peningkatan gerak balas imun dan kemampuan ikan untuk melawan *S. agalactiae*. Dalam pemeriksaan histologi daripada ekstrak tumbuhan herba terpilih yang berbeza menunjukkan pengubahsuaian kecil di dalam hepar dan tisu ginjal. Untuk menentukan prestasi pertumbuhan dan resistan penyakit *Oreochromis* sp. ditambah dengan herba-campuran tumbuhan herba terpilih ekstrak iaitu AVE & VTE, VTE & SCE, SCE & AVE. Herba campuran AVE & VTE menunjukkan peningkatan yang paling signifikan berbanding dengan herba campuran lain. Untuk SGR, hanya AVE & VTE menunjukkan hasil paling signifikan tinggi ($P<0.05$) berbanding kawalan. Kemandirian ikan selepas dicabar penyakit adalah perbezaan yang signifikan ($P<0.05$) berbanding dengan kawalan. Dalam asai hematologi, dalam pasca-dicabar menunjukkan peningkatan yang signifikan ($P<0.05$) RBC, Hb dan PCV. Nilai WBC dalam pra dan pasca dicabar menunjukkan tiada perbezaan yang signifikan ($P>0.05$) dalam semua rawatan berbanding kawalan. Bagi MCHC dengan campuran herba, AVE & SCE adalah satu-satunya rawatan yang menunjukkan tiada perbezaan yang signifikan ($P>0.05$) dalam pra-dicabar dan menunjukkan penurunan perbezaan yang signifikan ($P<0.05$) dalam pasca dicabar dalam semua rawatan. Di sisi lain, MCV dan protein plasma tidak menunjukkan sebarang perbezaan yang signifikan ($P>0.05$) berbanding dengan kawalan. Parameter biokimia ALT nilai dan AST nilai dipengaruhi dengan signifikan oleh penambahan campuran herba. Walau bagaimanapun dalam pasca dicabar, nilai ALP menunjukkan perbezaan yang signifkan berbanding dengan kawalan. Perubahan histopatologi, termasuk nekrosis dan pendarahan di dalam ginjal, hepar dan hepatopankreas mungkin disebabkan sitotoksin yang dihasilkan oleh bakteria. Selain itu, dalam kajian ini untuk menilai kesan daripada makanan tambahan ekstrak methanol *V. trifolia, S. crispus* dan *A. vera* pada *Oreochromis* sp. sebagai rawatan penyakit. Keputusan menunjukkan dalam nilai RBC peningkatan yang signifikan ($P<0.05$) dalam VTE-2, SCE-2, SCE-5, SCE-9, AVE-5, AVE-7 dan kawalan (OTC) berbanding kawalan

(dicabar). Sementara itu, dalam nilai Hb menunjukkan peningkatan signifikan dalam VTE-2, SCE-5, SCE-9 dan penurunan signifikan dalam AVE-7 dan AVE-9 berbanding kawalan. Untuk nilai PCV menunjukkan signifikan ($P<0.05$) meningkat dalam semua rawatan termasuk kawalan (OTC) berbanding kawalan (dicabar). Sebaliknya, dalam nilai MCHC tidak signifikan dalam SCE-5. Nilai protein plasma menunjukkan peningkatan yang signifikan ($P<0.05$) selepas ditambah VTE-2, VTE-5, SCE-2, SCE-5, SCE-9, AVE-2, AVE-5, AVE-9. Nilai ALT and AST menunjukkan penurunan signifikan ($P<0.05$) dalam semua rawatan termasuk kawalan (tidak dicabar) dan kawalan (OTC). Juga, dalam nilai ALP menunjukkan penurunan signifikan dalam semua rawatan termasuk kawalan (OTC). Kemandirian ikan meningkat selepas diberi makan dengan makanan tambahan herba dengan peningkatan yang signifikan ($P <0.05$) dalam VTE-5, VTE-7, AVE-2, AVE-5 dan AVE-9 berbanding kawalan (dicabar). Semua pengukuran hematologi dalam *Oreochromis* sp. yang dijangkiti *S. agalactiae* dengan ditambah dengan VTE, SCE dan AVE menunjukkan nilai yang sama berbanding dengan kawalan dan rawatan yang menerima antibiotik. Ini menunjukkan ikan dijangkiti *S. agalactiae* mampu mengembalikan indeks hematologi kepada nilai hampir normal. Keputusan adalah konsisten dengan literatur yang melaporkan pelbagai keberkesanan penggunaan herba dalam akuakultur untuk mengawal pelbagai patogen bakteria. Juga dari keputusan penemuan kajian ini, menunjukkan bahawa metanol ektrak herba campuran yang diberi pada ikan *Oreochromis sp* yang dijangkiti oleh *S. agalactiae* mempunyai kesan restoratif sinergistik dalam pemboleh ubah hematologi. Kesimpulannya, kajian ini mencadangkan bahawa *V. trifolia*, *S. crispus* dan *A. vera* ekstrak dan bercampur herba berkuatkuasa penggalak pertumbuhan dan rawatan penyakit bakteria dalam *Oreochromis* sp. Ia juga menunjukkan potensi tumbuhan herbal terpilih kerana ia merupakan terapeutan akuakultur yang mesra alam dan hanya menunjukkan kesan sampingan yang boleh abai dalam ikan yang digunakan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Hassan Mohd Daud, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Abdul Razak Alimon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Noordin Mohamed Mustapha, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name and Matric No.: Sharifah Raina Binti Manaf , GS 32480

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Signature: _____
Name of Chairman of
Supervisory Committee: Assoc. Prof. Dr. Hassan Mohd Daud

Signature: _____
Name of Member of
Supervisory Committee: Profesor Dr. Abdul Razak Alimon

Signature: _____
Name of Member of
Supervisory Committee: Profesor Dr. Noordin Mohamed Mustapha

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ALT	alanine aminotranferase
ALP	alkaline phosphatase
AST	aspartate aminotransferase
DM	dry matter
FCR	feed conversion ratio
FI	feed intake
SGR	specific growth rate
NRC	National Research Council
SE	standard error
VTE	*Vitex trifolia* extract
SCE	*Strobilanthes crispus* extract
AVE	*Aloe vera extract*
PGE	*Pereskia grandifolia* extract
PPE	*Peperomia pellucida* extract

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Aquaculture is one of a rapidly growing industry in the world. Among various kinds of cultivated organisms constitute an important industries. The percentage on its production increases year by year. In Malaysia, aquaculture is also regarded as a potential strategic industry that contribute to the economy (Ministry of Agriculture, 2015). Due to the importance of this industry, the Government has pronounced it as one of the country's transformation programme under the National Key Economic Area (NKEA). To date, the percentage of aquaculture production has been seen to be increasing year by year. In 2014, the total aquaculture production is 520,514 tonnes and the freshwater aquaculture production is 106,731 tonnes (Department of Fisheries Malaysia, 2016). Recently due to numerous intensive farming practices, infectious diseases pose a major problem in aquaculture industry, causing heavy loss to farmers. Disease occurs when the balance between hosts, environment and agent is disturbed. The pathogens such as parasites, fungi, bacteria and viruses are the known cause of many disease problems in fish. As an example, Sayuthi (1993) has estimated the cost of losses caused by one type of pathogenic bacterium was RM20 million. Thus, to handle and avoiding this problem, many chemicals and antibiotics have been used indiscriminately in large amount in fish farms, creating potential risks to ecological and human health. As alternatives, we need to replace these chemicals and antibiotics by using natural compounds.

Medicinal plants are plentiful and easy to obtained, with a broad assortment of nutrients and active compounds (Edeoga *et al*., 2010). They have been used as chemotherapeutic and feed additives in domesticated animals (Chang, 2000). Unfortunately, their information as chemotherapeutant and feed additives in fish diets is still limited in scientific researches.

Herb industry has been shown to be a definitive source of national economic growth and this initiative is one of the Entry Point Projects (EPP) under the Agricultural National Key Economic Area (NKEA) in the Economic Transformation Programme (ETP). In the effort to coordinate and monitor the effectiveness of the EPP1 High Value Herbal Products implementation, the Herbal Sub-group was established under the purview of the Ministry of Agriculture and Agro-based Industry (Ministry of Agriculture, 2014).

1.2 Background of Research

Tilapia is a popular food fish species all over the world especially in Africa and Asia. Globally the production of tilapia has shown an increased yearly. In 2015, it was forecasted that production will grow by 6% to total 5 million tonnes (FAO, 2016). The increasing global importance of cultured tilapia as a food fish has prompted significant research on improvement of the growth performance. However, a bacterial disease, Streptococcus has been reported to infect the fish culture and had caused high mortality (Austin and Austin, 2007). Commonly, the usual treatment for bacterial disease is by using antibiotic. However, antibiotic and also chemotherapeutant can contribute to various side effects especially towards humans and the development of bacterial resistance. Another method of treatment is by using vaccine, but the method is costly and only provides specific protection (Sakai, 1999). Many local plants contain antibacterial compounds which can be employed in treating health problems in fish (Citarasu, 2010). Nevertheless, there is trivial data on their role as healthcare products for fish.

As expected many local plants contain various nutrients that can promote growth and good health of animals. Thus theoretically, using local plants as whole or as extracts as additive singly or in composition, the diet may promote a positive outcome on the growth performance and productivity of fishes. Also, the usage will inevitably reduce dangerous residual accumulation in the tissues and the environment.

1.3 Problem Statement

Every year, the demand for fish and fish products from aquaculture has tremendously increased. However, the main hindrance is the cultured fish succumbing to disease infection and commonly antibiotics and chemicals are used to treat this problem. The advantages of using chemicals and drugs are that they can be applied straight using diverse applications and may give effective outcome. Unfortunately, the residual toxic chemicals or their metabolites accumulated in fish body tissues pose a health risk to consumers. Due to this reason, many drugs (antibiotics) and chemicals are now banned for aquaculture used. Most countries, including Singapore and European Union (EU) have banned imported aquaculture products which were detected to have antibiotics residues. Accordingly the EU has ratified a ban on the usage of whole sub-therapeutic antibiotics as growth-promoting agents in aquaculture practices especially for food fish. Thus, the way forward, is undertaking research focussing on alternatives to traditional antibiotics for prevention and control of diseases in aquaculture and also as growth promoters in animal feeds, such as using enzymes, herbs and probiotics. Naturally herbs and plants (phytobiotics) have been recognized to contain many compounds with properties that can promote growth in animal and antimicrobes.

1.4 Research Hypothesis

Selected local plants as feed supplement can be used to promote growth, and also for disease prophylaxis and disease treatment of red hybrid tilapia, *Oreochromis* sp.

1.5 Significance of Study

Local plants have been shown to contain certain compounds such as antioxidants, polyphenols and antibacterial properties that contribute in improving growth performance and in treating fish disease in cultivated species. Local plants can also be used as feed additives to replace antibiotics as growth promoters and treatment of disease infection. The present study will elucidate the effect of local plants for growth promoting and antibacterial properties in *Oreochromis* sp.

In relation to this study is to determine the potential of local plants as antibacterial, growth promoters, disease resistance and disease treatment in *Oreochromis* sp.

1.6 General Objectives

The general objectives of this study are:

1) To determine the effect of selected herbal supplementation on the growth performance and disease resistance in *Oreochromis* sp.

2) To determine the effect of herbal supplementation as disease prophylaxis treatment in *Oreochromis* sp.

The specific objectives of this study are :

1) To screen phytochemical properties, antibacterial activity and toxicity level of selected local plants (*Vitex trifolia, Aloe vera, Strobilanthes crispus, Peperomia pellucida, Clinacanthus nutans* and *Pereskia grandifolia*).

2) To determine the growth performance and disease resistant of *Oreochromis* sp. fed with diet supplemented with selected plant extracts at different concentrations.

3) To determine the growth performance and disease resistant of *Oreochromis* sp. supplemented with herbal-mixed of the selected plant extracts.

4) To assess the efficacy of diet supplementation of selected plant extracts for 14 days as disease treatments.

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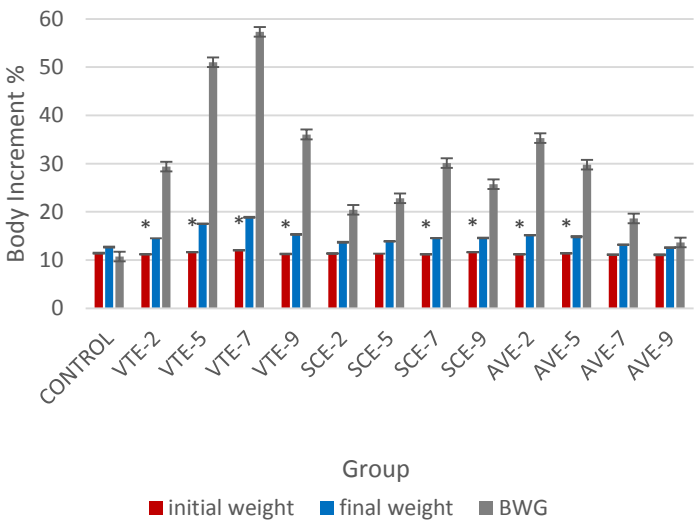
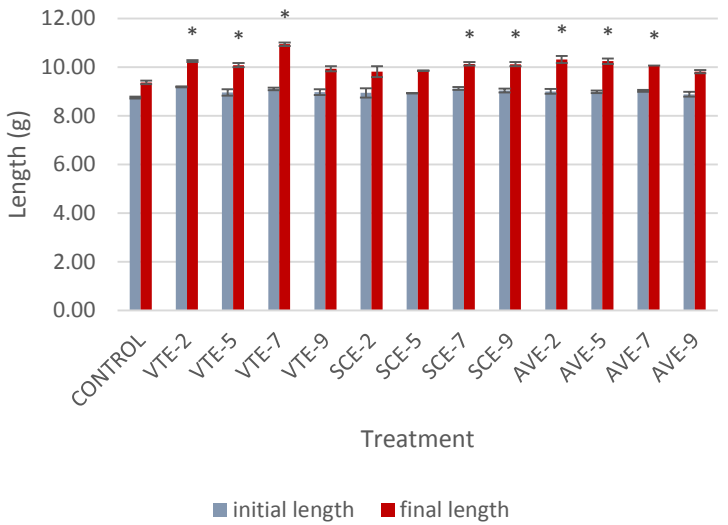
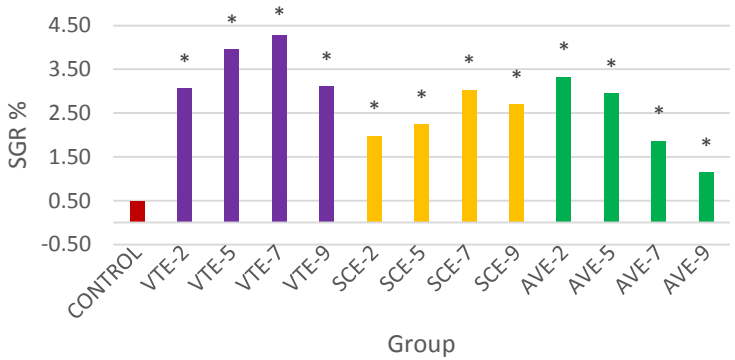
APPENDICES



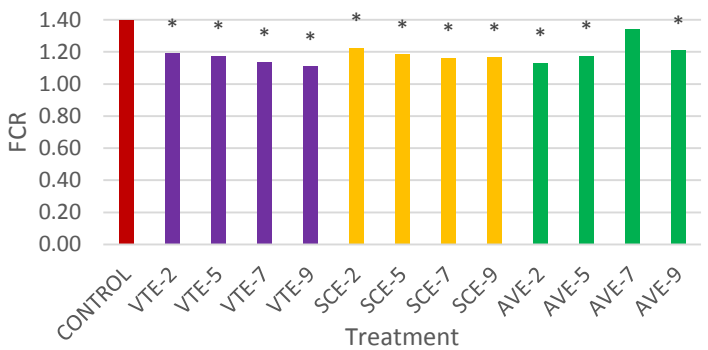
A1 Body gain weight of *Oreochromis* sp.



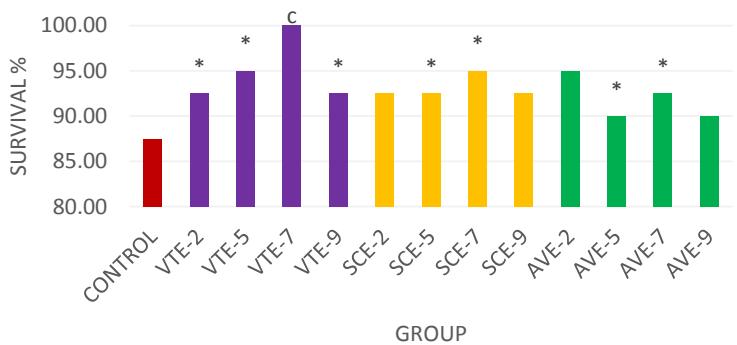
A2 Length of *Oreochromis* sp.



A3 Specific growth rate of *Oreochromis* sp.

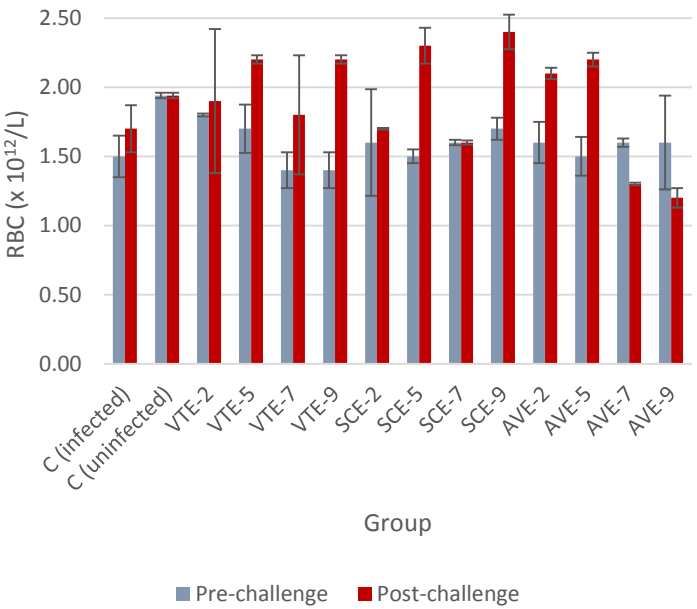


A4 Feed conversion ratio of *Oreochromis* sp.

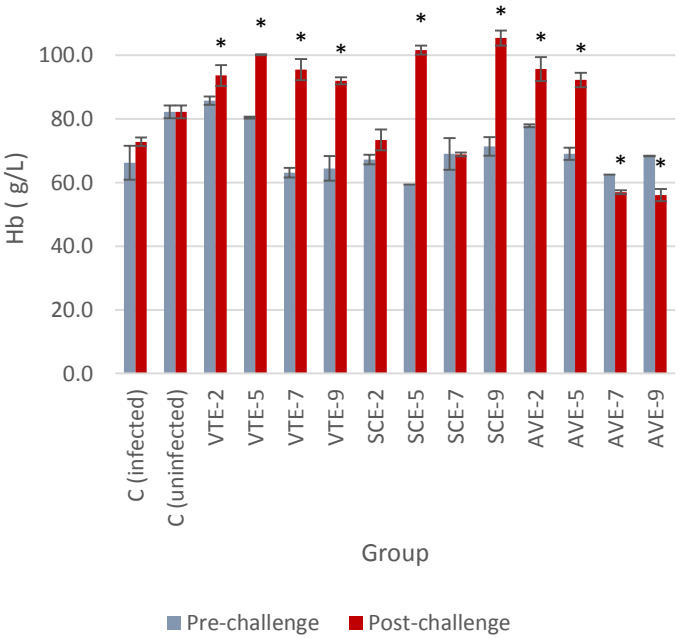


A5 Survival rate of *Oreochromis* sp.

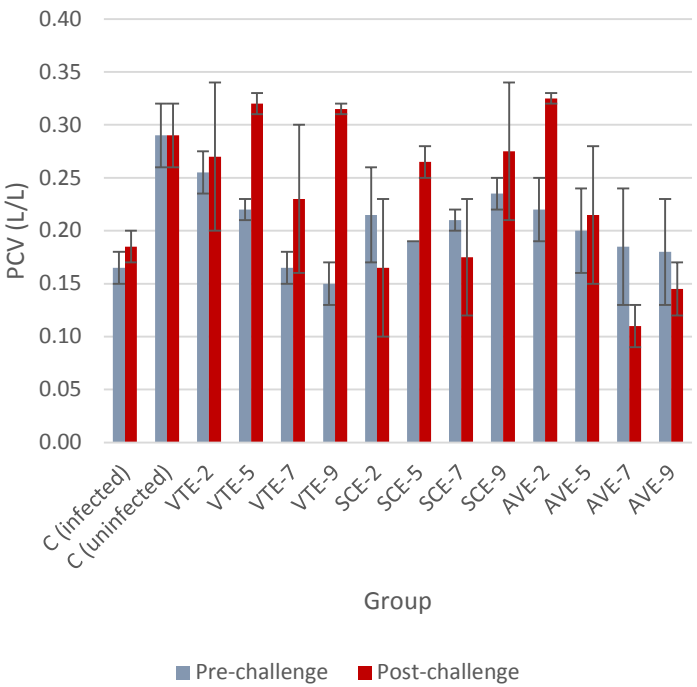
(a) RBC



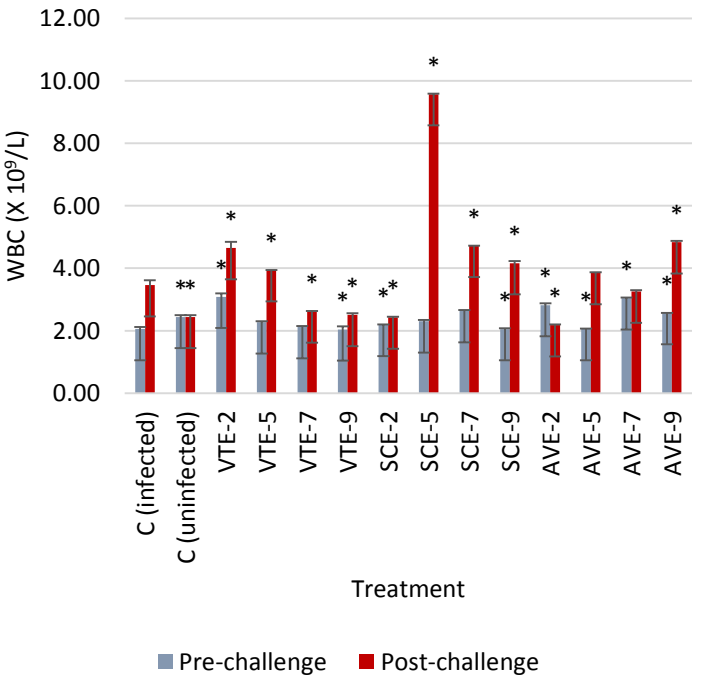
(b) Hb

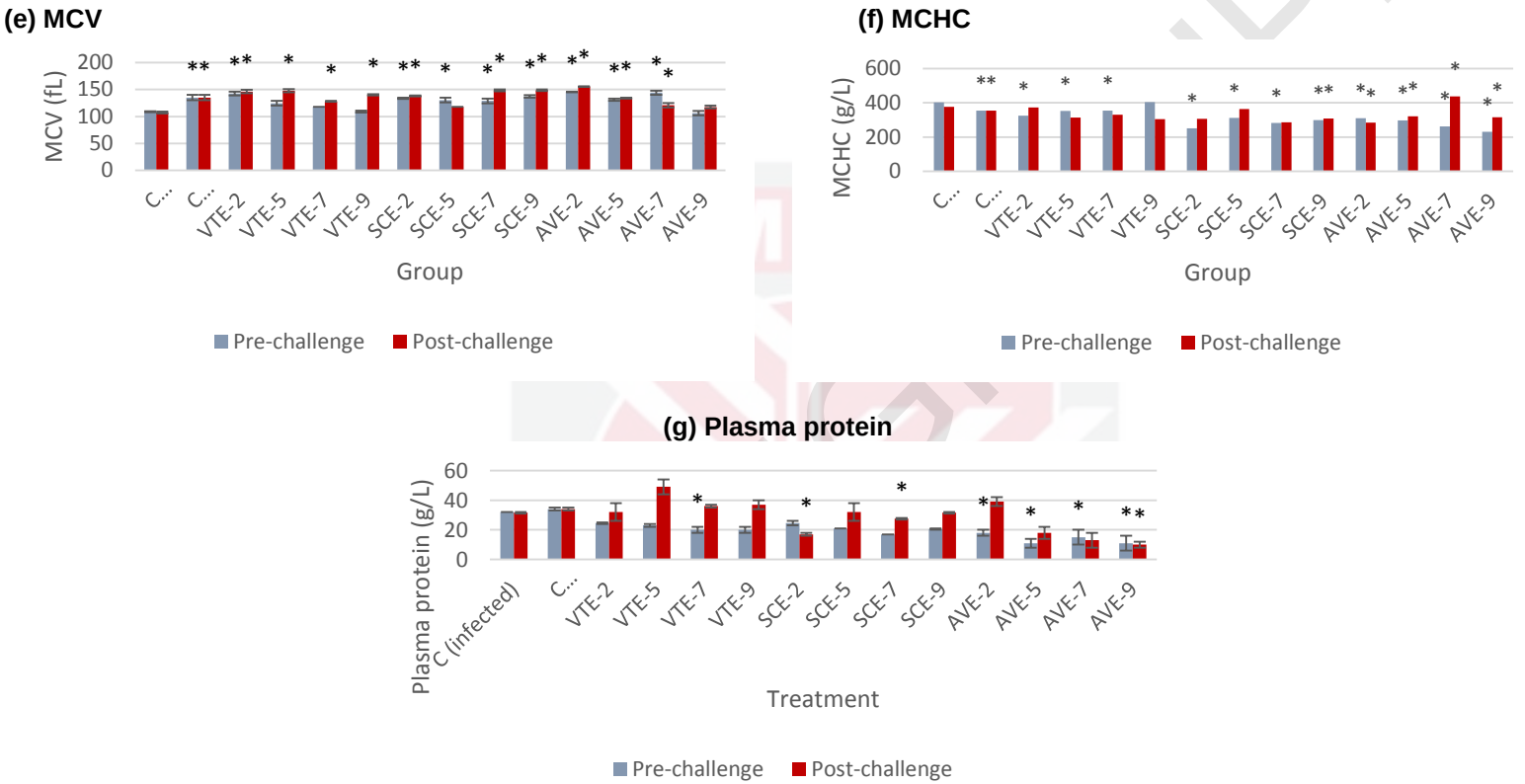


(c) PCV

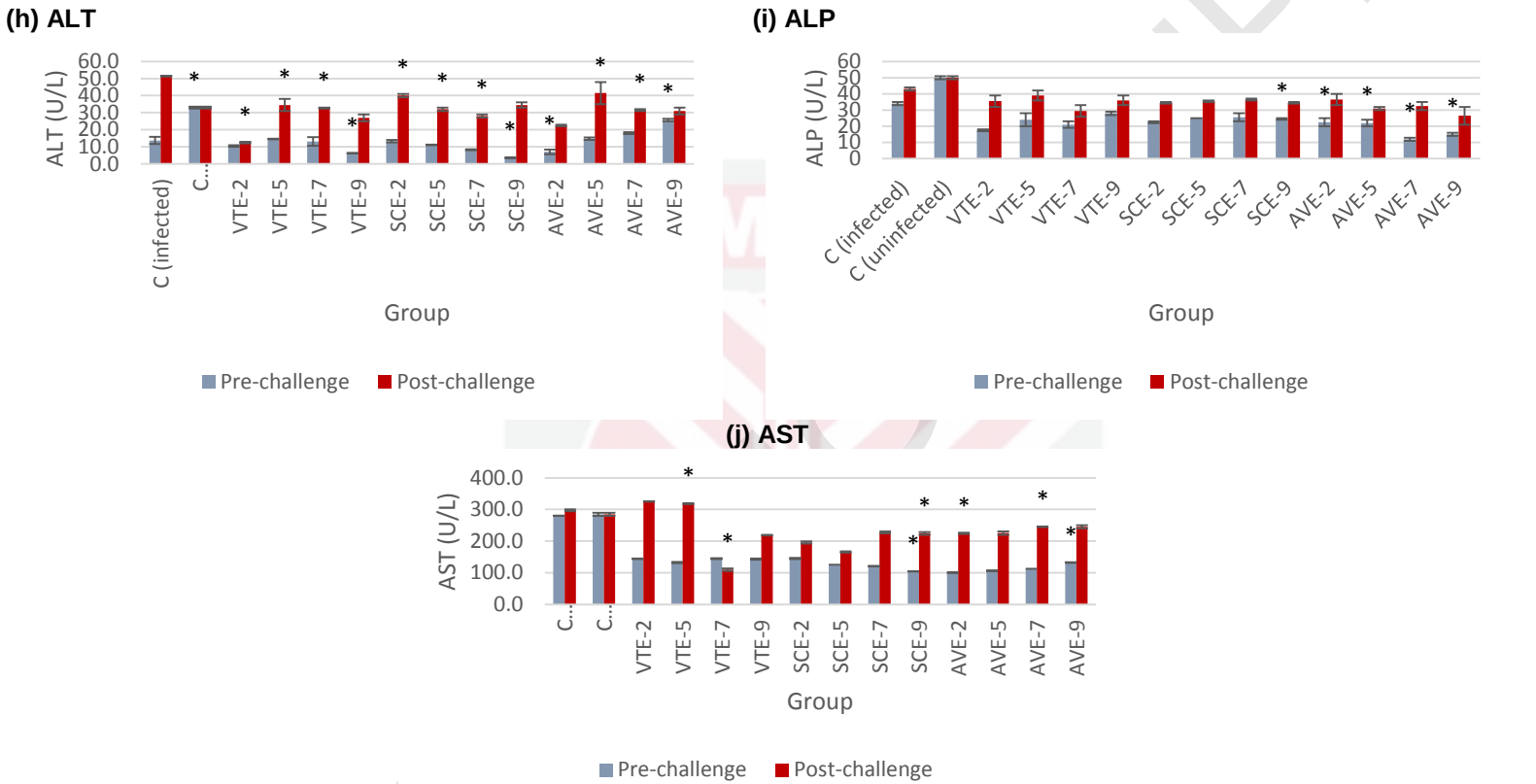


(d) WBC

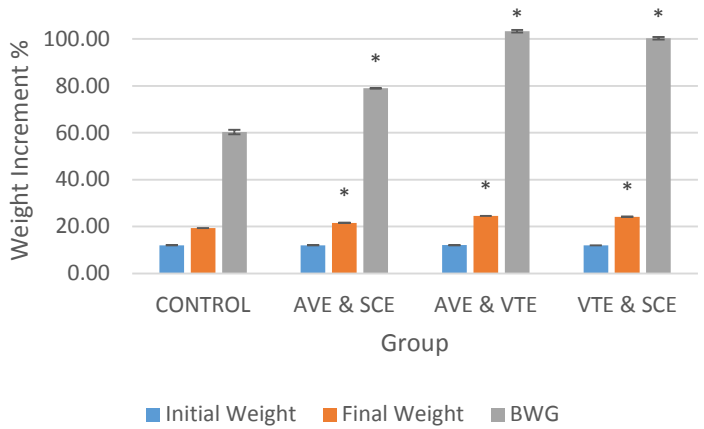




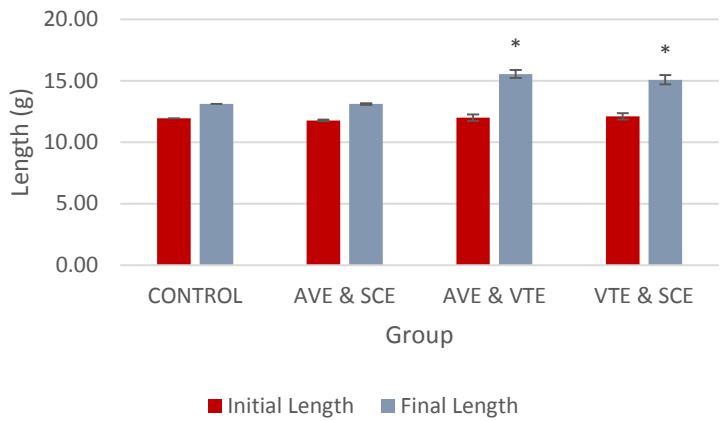
A6 Haematological parameters of *Oreochromis* sp. (pre-challenge) fed &VTE, VTE, SCE, AVE and Control diet at for six-weeks and post-challenge (two-weeks). Data are expressed as the mean ± SEM. Statistical differences (P<0.05) from the control (infected) group are indicated by asterisks. RBC = red blood cell, Hb = haemoglobin, WBC = white blood cell, PCV = packed cell volume, MCV = mean corpuscular volume, MCHC = mean cellular haemoglobin content,P.protein=plasmaprotein



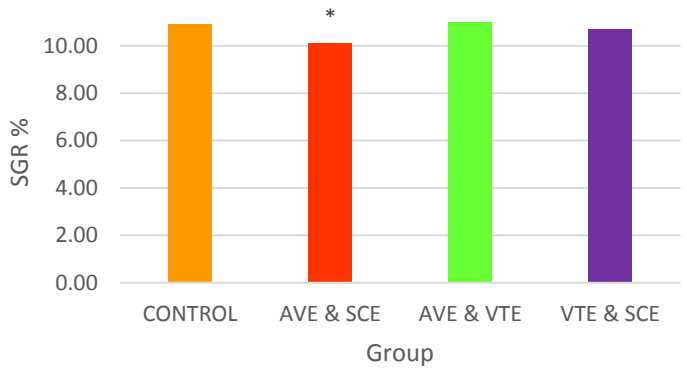
A7 Biochemical paramaters of *Oreochromis* sp. (pre-challenge) fed &VTE, VTE, SCE, AVE and Control diet at for six-weeks and post-challenge (two-weeks). Data are expressed as the mean ± SEM. Statistical differences (P<0.05) from the control (infected) group are indicated by asterisks. ALT = alkaline aminotransferase,ALP=alkalinephosphatase,AST=aspartateaminotransferase



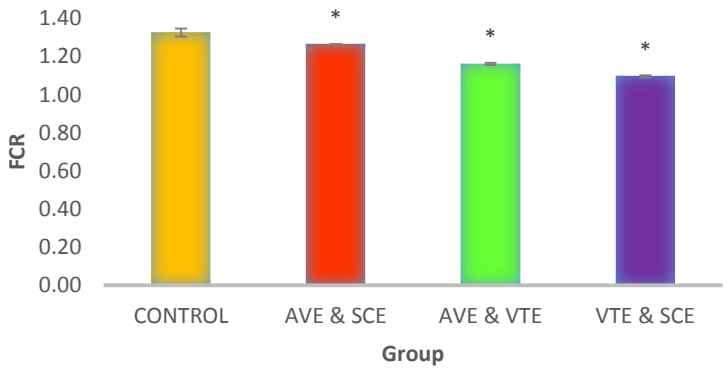
A8 Body increment of *Oreochromis* sp.



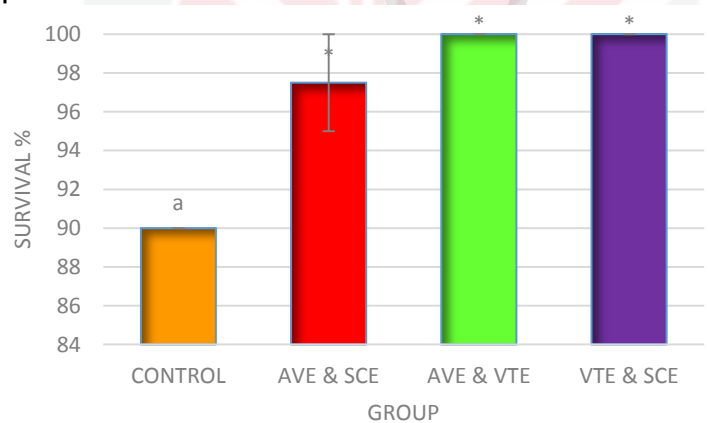
A9 Length of *Oreochromis* sp.



A10 Specific growth rate of *Oreochromis* sp.

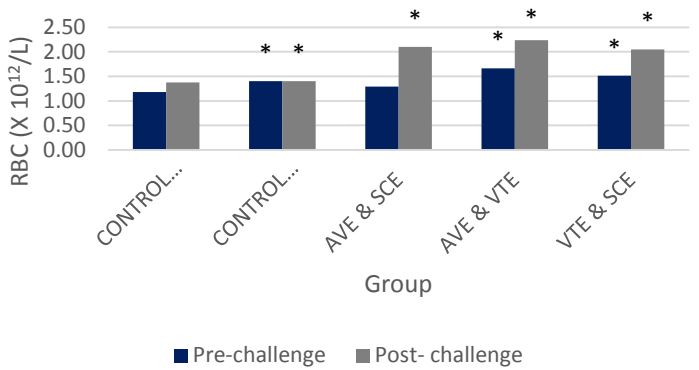


A11 Feed conversion ratio of *Oreochromis* sp.

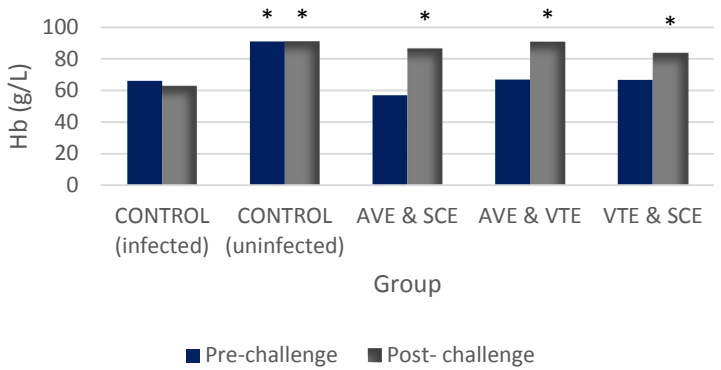


A12 Survival rate of *Oreochromis sp.*

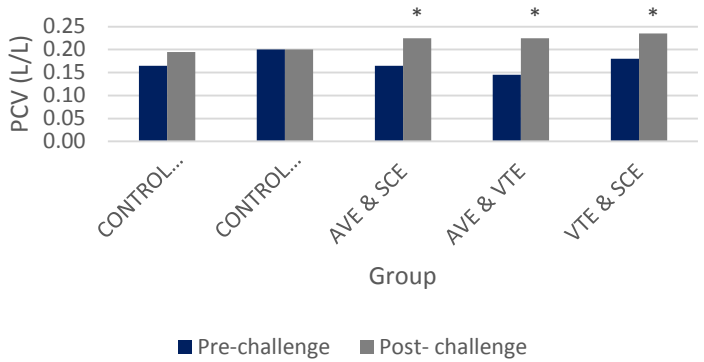
(a) RBC



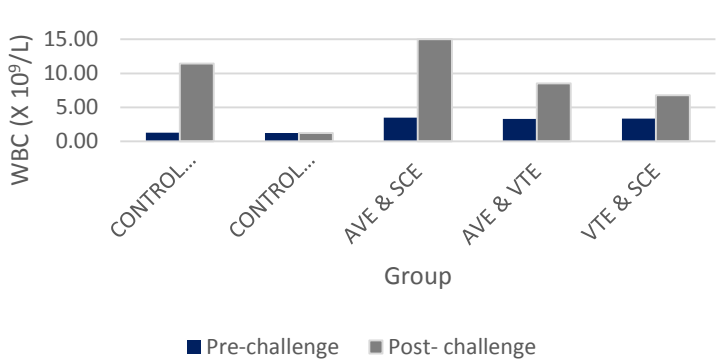
(b) Hb



(c) PCV

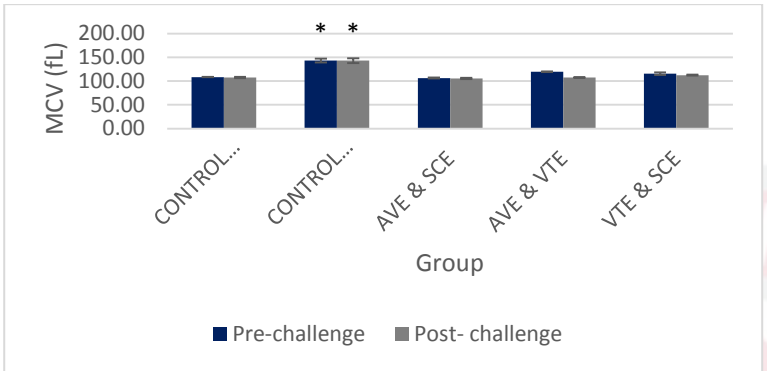


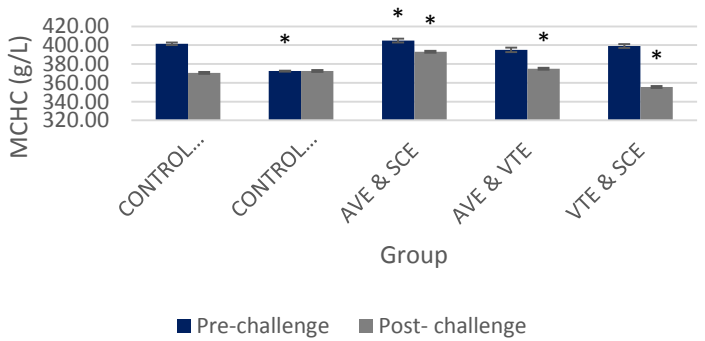
(d) WBC



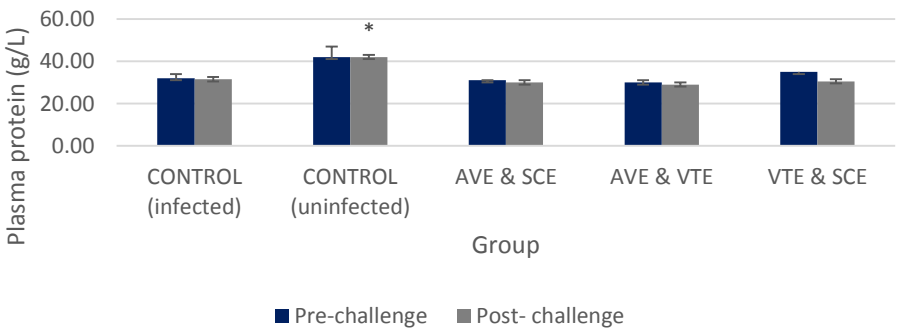
(e) MCV

(f) MCHC



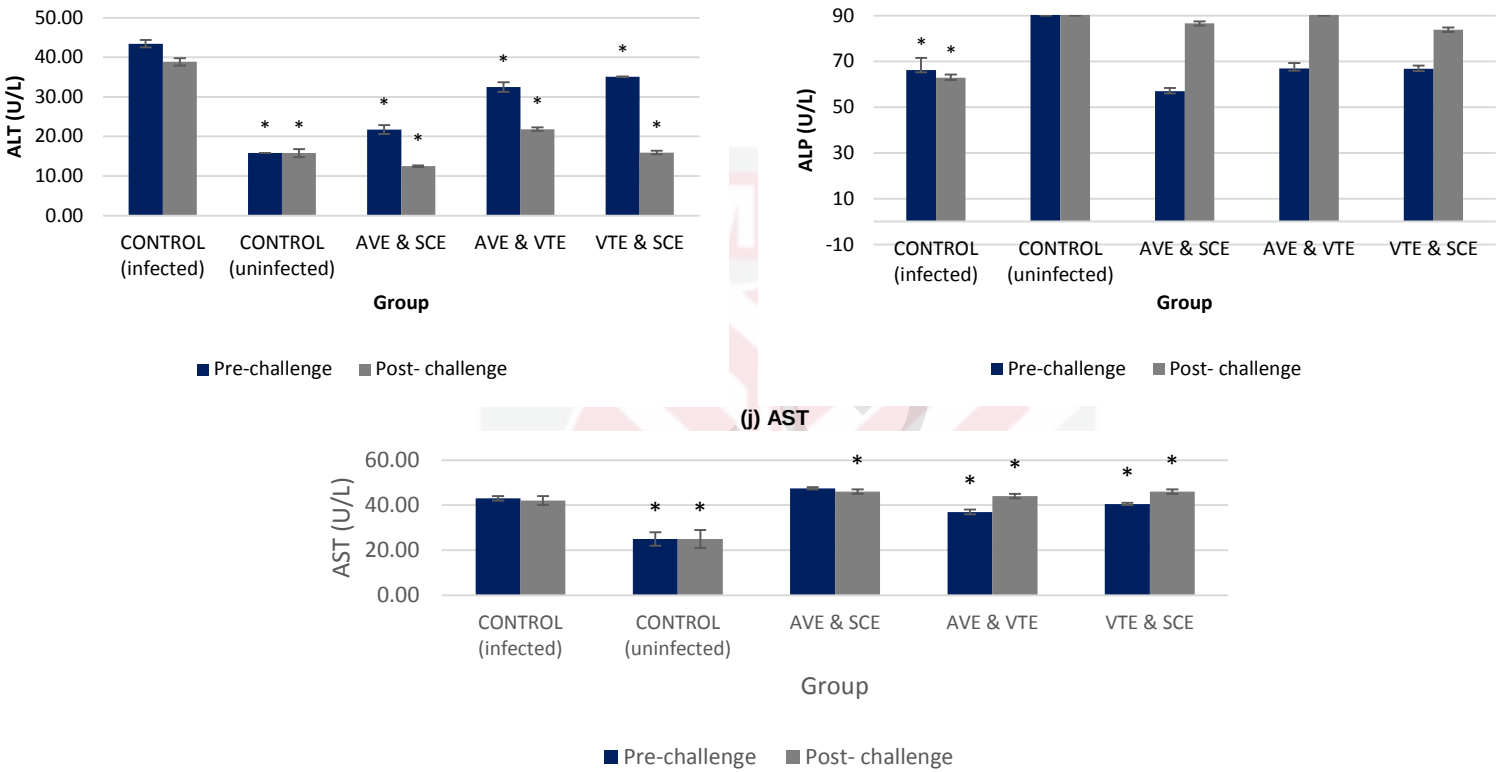


(g) Plasma protein

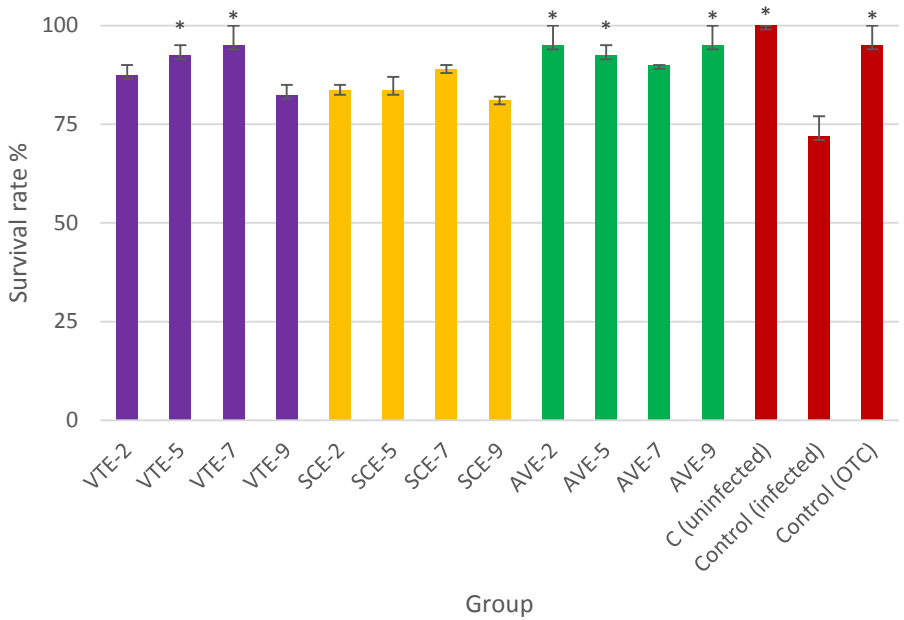


A13 Haematological parameters of *Oreochromis* sp. (pre-challenge) fed &VTE, VTE, SCE, AVE and Control diet at for six-weeks and post-challenge (two-weeks). Data are expressed as the mean ± SEM. Statistical differences (P<0.05) from the control (infected) group are indicated by asterisks. RBC = red blood cell, Hb = haemoglobin, WBC = white blood cell, PCV = packed cell volume, MCV = mean corpuscular volume, MCHC = mean cellular haemoglobin content, P. protein = plasma protein

(h) ALT **(i) ALP**

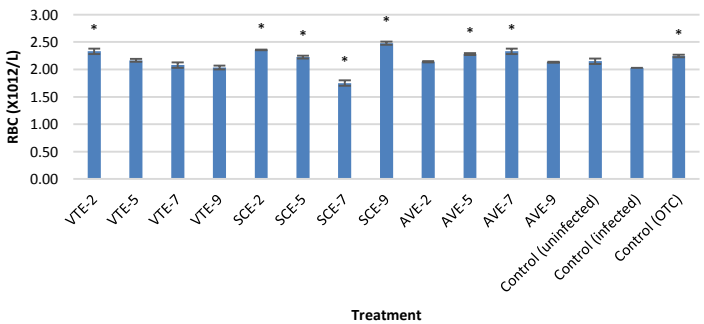


A14 Biochemical parameters of *Oreochromis* sp. (pre-challenge) fed &VTE, VTE, SCE, AVE and Control diet at for six-weeks and post-challenge (two-weeks). Data are expressed as the mean ± SEM. Statistical differences (P<0.05) from the control (infected) group are indicated by asterisks. ALT = alkaline aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase

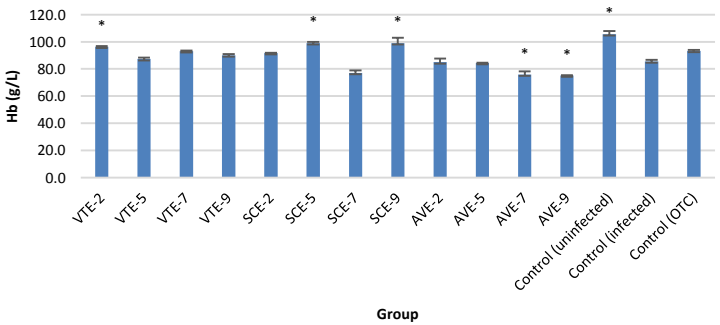


A15 Survival rate of *Oreochromis* sp.

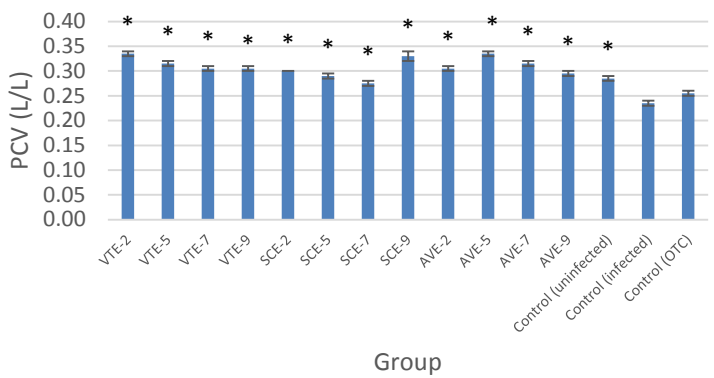
(a) RBC



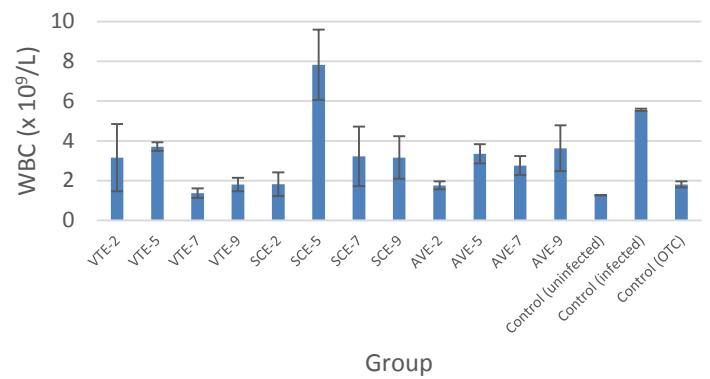
(b) Hb

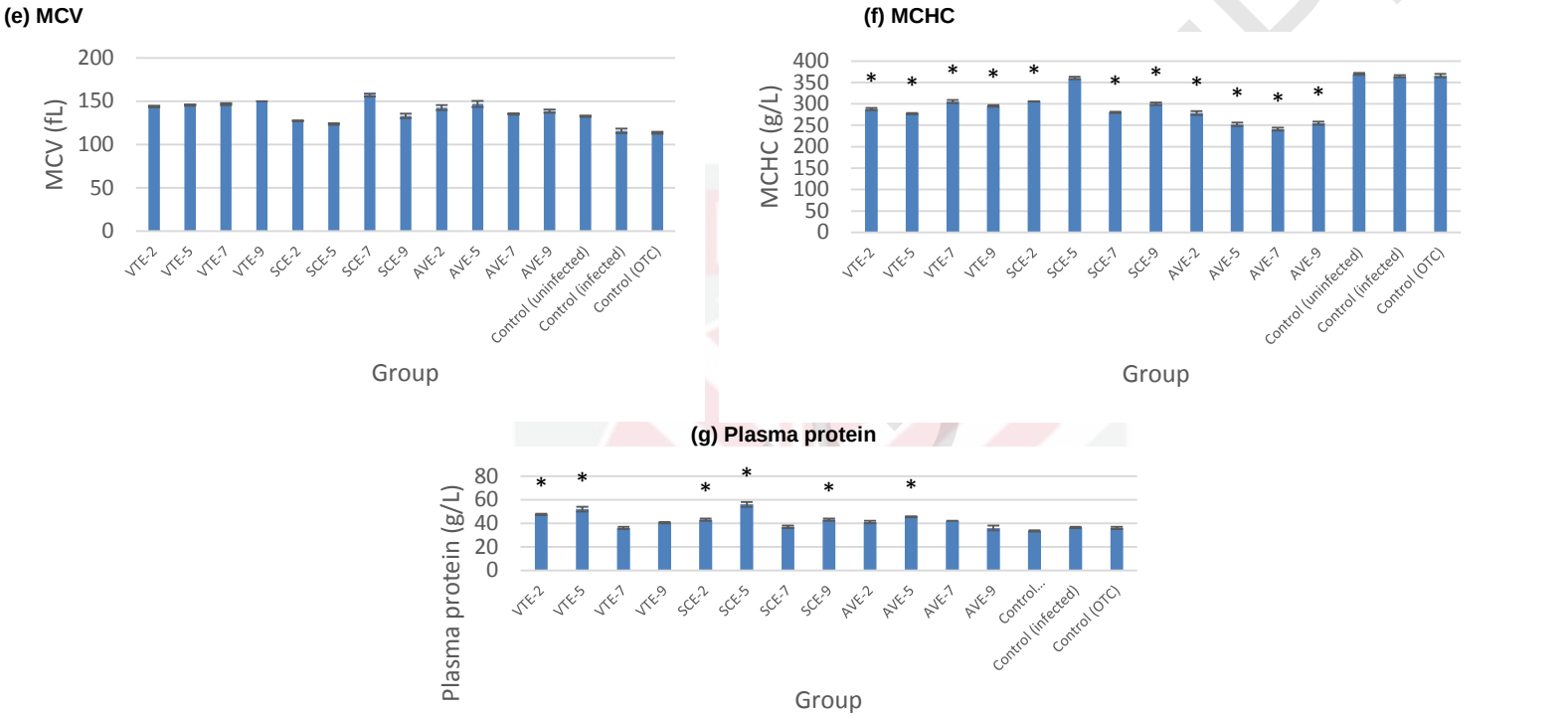


(c) PCV



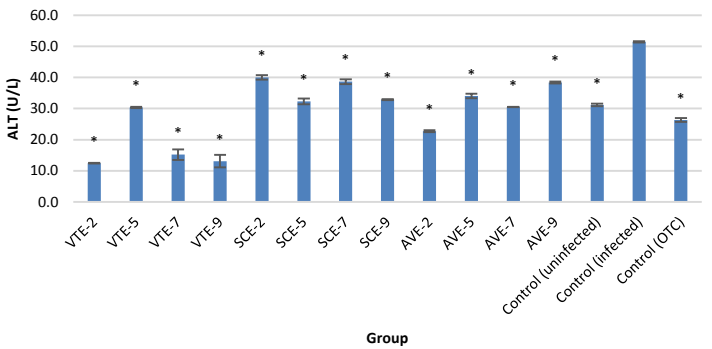
(d) WBC



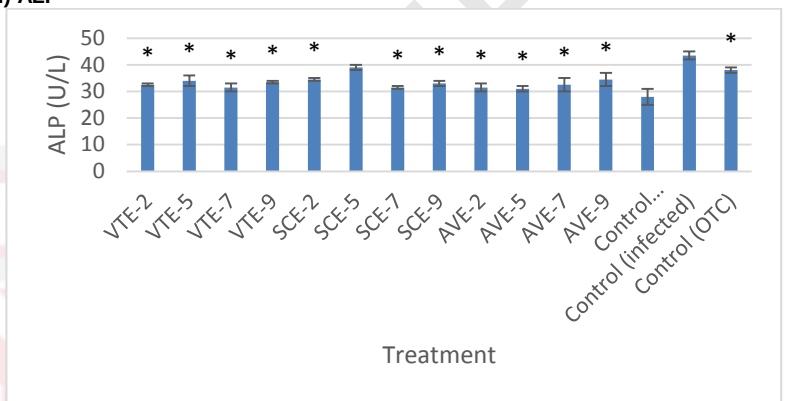


A16 Haematological parameters of *Oreochromis* sp. (post-challenge) fed &VTE, VTE, SCE, AVE and Control diet. Data are expressed as the mean ± SEM. Statistical differences (P<0.05) from the control (infected) group are indicated by asterisks. RBC = red blood cell, Hb = haemoglobin, WBC = white blood cell, PCV = packed cell volume, MCV = mean corpuscular volume, MCHC = mean cellular haemoglobin content, P. protein = plasma protein

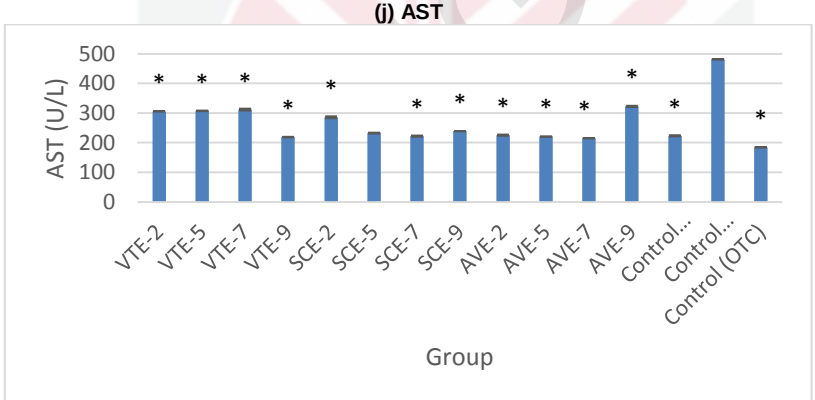
(h) ALT



(i) ALP



(j) AST



A17 Biochemical parameters of *Oreochromis* sp. (post-challenge) fed &VTE, VTE, SCE, AVE and Control diet. Data are expressed as the mean ± SEM. Statistical differences (P<0.05) from the control (infected) group are indicated by asterisks. ALT = alkaline aminotransferase, ALP = alkaline phosphatase, AST=aspartateaminotransferase

BIODATA OF STUDENT

Sharifah Raina binti Manaf was born on 22 Novermber 1986 in Kelantan, to Mr. Manaf bin Daud and Mrs Sharifah Zainun binti Mohd Salleh. She obtained her lower secondary and higher secondary school certificate from SMK Zainab (1), Kelantan. She furthered her studies and awarded a Matriculation Science in 2005 from Perlis Matriculation College. She continued her degree in Agrotechnology (Aquaculture) in 2008 and her Master Science of Aquaculture in 2010 from Universiti Malaysia Terengganu, Terengganu.

Her career started as a substitute teacher at SK Demit, Kelantan in 2008 for 3 months. After that, she work as research assistant at Institute Tropical Agriculture, UPM, Selangor. Her area of research at that time was fermented of sago. Currently she is under Young Scheme (Tenaga Pengajar Muda), Faculty of Plantation and Agrotechnology, Shah Alam, Selangor.

Sharifah Raina is married with 1 children. Her publications, seminar, workshops and conference attended and research carried out outlined below:

LIST OF PUBLICATIONS

Sharifah Raina, M. Hassan, M.D., Abdul Razak, A., Noordin, M.M., Ruhil Hayati, H., Kumari Geetha, M., Nora Faten Afifah, M., Rashidah, A.R. and Nur Hidayahanum, H. (2016). The effects of *Vitex trifolia*, *Strobilanthes crispus* and *Aloe vera* Herbal-mixed Dietary Supplementation on Growth Performance and Disease Resistance in Red Hybrid Tilapia (*Oreochromis* sp.). *Journal of Aquaculture Research and Development* 7: 1-6.

Sharifah Raina, M., Hassan, M. D., Ruhil Hayati, H., and Nur Hidayahanum, H. Antimicrobial Activity of the Selected Local Plant Extracts against Aquatic Bacteria. 2012 Malaysia-Thailand Graduate Forum in Life Science, Food Science and Agriculture. 12-14 December 2012. Mahidol University, Bangkok, Thailand. Oral presenter.

Sharifah Raina, M., Hassan, M. D., Ruhil Hayati, H., and Nur Hidayahanum, H. *In vitro* studies of selected local plants against freshwater food fish pathogens and toxicity in Tilapia (*Oreochromis* sp.). Postgraduate Colloquium Series 2. November 2013. Bilik Jemaah, Faculty of Veterinary Medicine, UPM. Oral presenter

Ruhil Hayati, H., Hassan, M. D., Ong, B. L., Abdelhadi, Y. M., Nur Hidayahanum, H. and **Sharifah Raina, M**. Characterization, antibiotic sensitivity and plasmid profiles of Aeromonas hydrophila gp. 2 isolates in diseased freshwater fish. Postgraduate Colloquium Series 2. 12 December 2013. Bilik Kuliah 12, Faculty of Veterinary Medicine, UPM.

Ruhil Hayati, H., Hassan, M. D., Ong, B. L., Abdelhadi, Y. M., Nur Hidayahanum, H. and **Sharifah Raina, M.** Histopathological study of Oreochromis sp. experimentally infected with Aeromonas hydrophila and detection of 10 virulence genes by PCR. Postgraduate Colloquium Series 2. 19 December 2013. Bilik Kuliah 12, Faculty of Veterinary Medicine, UPM.

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Ruhil Hayati, H., Hassan, M. D., Ong, B. L., Abdelhadi, Y. M., Nur Hidayahanum, H. and **Sharifah Raina, M**. Characterization, Pathogenicity and Antibiotic Sensitivity Profiles of *Aeromonas* spp. Isolated from Motile Aeromonads Septicemia (MAS) – Like Disease in Freshwater Fishes.

Simposium Biologi Kebangsaan 2013. 28-30 May 2013. Thistle Port Dickson Resort.

Hassan, M.D., **Sharifah Raina, M**., Abdul Razak, A., Noordin, M., Ruhil Hayati, H., Nur Hidayahanum, H., Nora Faten, A.M., Hazer, M. and Kumari Geetha, M. Effect of Diets Supplemented with Simpleleaf Chastetree (Vitex trifolia) Extracts on Growth Performance and Histology of the Red Hybrid Tilapia (Oreochromis sp.). International Postgraduate Conference on Biotechnology 2014. August 2014. Global Square Building, Soka University, Japan. Poster presenter.

Ruhil Hayati, Hamdan., Hassan, M. D., Ong, B. L., Abdelhadi, Y. M., Nur Hidayahanum, H., **Sharifah Raina, M**., Nora Faten, A.M., Hazer, M., Kuttichantran, S. and Kumari Geetha, M. Tissue Detection of Aeromonas hydrophila Type 2 in Experimentally Infected Oreochromis sp. using Immunohistochemical Assay. International Postgraduate Conference on Biotechnology 2014. August 2014. Global Square Building, Soka University, Japan.

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Ruhil Hayati, Hamdan., Hassan, Mohd. Daud., Nur Hidayahanum, Hamid., **Sharifah Raina, Manaf**., Nora Faten, Afifah Mohamad., Kuttichantran, Subramaniam and Kumari Geetha, M. Virulence Genes Detection of Aeromonas hydrophila and Histopathological Study of Its Experimentally Infection to Oreochromis sp. National Postgraduate Seminar 2014. 10 September 2014. Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

Ruhil Hayati, H., Hassan, M.D., Ong, B.I., Abdelhadi, Y.M., Nur Hidayahanum, H., **Sharifah, R.M**., Nora Faten, A.M., Kuttichantran, S. and Milud, Alsaid. 2015. Virulence Genes Detection of Aeromonas hydrophila Originated from Diseased Freshwater Fishes. *Advances in Environmental Biology,* 9(22): 22-26.

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