PERFORMANCE OF MYCELIAL BIOMASS FROM THE MUSHROOM *Ganoderma lucidum* AS FEED ADDITIVE ON GROWTH AND QUALITY OF RED HYBRID TILAPIA (*Oreochromis spp.*)

GREMA YERIMA

FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2020

PERFORMANCE OF MYCELIAL BIOMASS FROM THE MUSHROOM *Ganoderma lucidum* AS FEED ADDITIVE ON GROWTH AND QUALITY OF RED HYBRID TILAPIA (*Oreochromis spp.*)

GREMA YERIMA

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE BIOTECHNOLOGY

INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITI MALAYA
KUALA LUMPUR

2020

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **GREMA YERIMA**

Registration/Matric No: **SOC180002**

Name of Degree: **MASTER OF SCIENCE BIOTECHNOLOGY**

Title of Dissertation ("this Work"):

PERFORMANCE OF MYCELIAL BIOMASS FROM THE MUSHROOM *Ganoderma lucidum* AS FEED ADDITIVES ON GROWTH AND QUALITY OF RED HYBRID TILAPIA (*Oreochromis spp.*).

Field of study: **BIOTECHNOLOGY**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this work;
(2) This work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the tittle of the work and its authorship have been acknowledged in this work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitute an infringement of any copyright work;
(5) I hereby assign all and every right in the copyright to this work to the university of Malay ("UM"), who henceforth shall be owner of the copyright in this work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this work, I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined UM.

Candidates Signature Date: 01/07/2020

Subscribed and solemnly declared before,

Witness's Signature Date:01/07/2020

Name:
Designation:

PERFORMANCE OF MYCELIAL BIOMASS FROM THE MUSHROOM *Ganoderma lucidum* AS FEED ADDITIVE ON GROWTH AND QUALITY OF RED HYBRID TILAPIA (*Oreochromis spp.*)

ABSTRACT

The present study describes the potentiality of dietary supplement of mycelial biomass of *Ganoderma lucidum* (MBGL) as functional feed additives formulated along with other feed ingredients at different inclusion levels (5g/kg diet, 10g/kg diet and 15g/kg diet). A hundred and twenty (120) red hybrid tilapia were used for this experiment in duplicates treatment. Fifteen fish were allocated to each tank with an average weight of 17.0g. A six (6) week feeding trial was conducted to evaluate the effect of GL biomass on red hybrid tilapia body composition, organosomatic index, as well as hematological indices. At the end of the feeding trial, three fish were randomly selected in each tank for fillet body composition study and organosomatic indices (condition factor, hepatosomatic indices, and viscerosomatic indices). Blood samples were collected from 7 fish for hematological studies from each group of diet. The result obtained from this study shows that the dietary supplement of GL biomass has a significant ($P<0.05$) influence on red hybrid tilapia organosomatic indices. The inclusion of this diet as feed additives has no adverse effect on the hybrid tilapia hematological indices. Based on the findings of this study, the mycelial biomass of *Ganoderma lucidum* can be used as a functional feed additive to improve aquatic productivity in aquaculture. The optimum supplementation level suggested in this study is 10g/kg diet which is considered sufficient to meet the nutritional requirement of hybrid tilapia.

Keywords: *Ganoderma lucidum*, red hybrid tilapia (Oreochromis sp), body composition, organosomatic indices, hematological indices.

PRESTASI BIOMAS MYCELIAL DARI MUSHROOM *Ganoderma lucidum* SEBAGAI TAMBAH MAKANAN TENTANG PERTUMBUHAN DAN KUALITI TILAPIA HYBRID MERAH (*Oreochromis spp*.)

ABSTRAK

Kajian ini menggambarkan potensi suplemen biomassa mycelial *Ganoderma lucidum* (MBGL) sebagai bahan tambahan makanan berfungsi yang diformulasikan bersama dengan ramuan makanan lain pada tahap kemasukan yang berlainan (diet 5g / kg, diet 10g / kg dan diet 15g / kg). Satu ratus dua puluh (120) tilapia hibrid merah digunakan untuk eksperimen ini dalam rawatan pendua. Lima belas ikan diperuntukkan kepada setiap tangki dengan purata berat 17.0g. Satu percubaan makan selama enam (6) minggu telah dijalankan untuk menilai kesan biomass GL pada komposisi badan tilapia hibrid merah, indeks organosomatik, serta indeks hematologi. Pada akhir percubaan makan, tiga ikan dipilih secara rawak dalam setiap tangki untuk kajian komposisi badan fillet dan indeks organosomatik (faktor keadaan, indeks hepatosomatic, dan indeks viscerosomatik). Sampel darah dikumpulkan dari 7 ikan untuk kajian hematologi dari setiap kumpulan diet. Hasil yang diperolehi daripada kajian ini menunjukkan bahawa makanan tambahan GL biomass mempunyai pengaruh signifikan ($P < 0.05$) pada indeks organosomatik tilapia hibrid merah. Kemasukan diet ini sebagai bahan tambahan makanan tidak memberi kesan buruk kepada indeks hematologi tilapia hibrid. Berdasarkan penemuan kajian ini, biomassa mycelial *Ganoderma lucidum* boleh digunakan sebagai bahan tambahan makanan berfungsi untuk meningkatkan produktiviti akuatik dalam akuakultur. Tahap suplemen optimum yang dicadangkan dalam kajian ini adalah diet 10g / kg yang dianggap mencukupi untuk memenuhi keperluan pemakanan tilapia hybrid.

Kata kunci: *Ganoderma lucidum*, nila hibrida merah (Oreochromis sp), komposisi badan, indeks organosomatik, indeks hematologi.

ACKNOWLEDGEMENTS

In the Name of ALLAH the most beneficial and the most merciful. I would like to express my gratitude to Almighty Allah for giving me wisdom and the opportunity to be in Malaysia and to witness the end of my study, for sure glory be Allah. I would like to express my sincere gratitude to my supervisors in person of Dr. Norhidayah Mohd Taufek, and Dr. Wan Abd Al-Qadr Imad Bin Wan Mohtar for giving me the opportunity to acquire double knowledge (Aquaculture and Fungal Biotechnology). Your kind courage, assistance, suggestion, advice, personal and financial commitment toward success of this work has built lasting confidence in me and memories that would never be disremembered. Eloquently, I am highly thankful to the Yobe state government of Nigeria. Under the able leadership of Alhaji Dr. Ibrahim Gaidam **FCNA, FCPA** for giving me a scholarship award to study at the best Malaysian University (University of Malaya). I am very much grateful to all my course lecturers during my course work program for drilling me with vast knowledge in the field of modern biotechnology. Indeed, your message is reliable in solving sustainable development goals, am very thankful you all. I would like to appreciate the effort of the entire staff of the Institute of Biological science for being helpful and guide giving whenever required. A huge appreciation goes to my classmate and fellow research student for being together on the field of knowledge sharing, especially and specially Jaganath, Kumeera, and Janathu for working energetically round the clock, being together with you give privilege and a lesson for me. I thank you all and wishing you the best of success in your feature endeavor.

Finally, I would like to thank my entire family, friends, and well-wisher for your courage, word of advice, and prayers to see my success in this journey. Lastly, I would like to appreciate my wife and my children for your patients to stay and manage throughout my absence.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

CF	:	Condition factor
DCP	:	Di-calcium phosphate
FM	:	Fishmeal
GL	:	*Ganoderma lucidum*
Hg	:	Hemoglobin
Ht	:	Hematocrit
HSI	:	Hepatosomatic indices
MB	:	Mycelial biomass
MBGL	:	Mycelial biomass of *Ganoderma lucidum*
MCH	:	Mean corpuscular volume hemoglobin
MCHC	:	Mean corpuscular volume hemoglobin concentration
MCV	:	Mean corpuscular volume
PCV	:	Packed cell volume
RBC	:	Red blood cell
VSI	:	Vicerosomatic indices
WBC	:	White blood cell

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Introduction.

The aquaculture industry is among one of the most rapidly growing sectors in many parts of developed and developing countries compared to other food production sectors (Fazio et al., 2019). This is to make up protein demand for human consumption (El-Naby et al., 2019). Fish contribute to human nutrition by providing approximately 20 % of protein, an increase in the human population has increased protein demand, especially in developing countries. Apart from protein, fish also provides beneficial oil, such as Omega 3 and 6 (polyunsaturated fatty acid). Besides being a source of protein and fat, fish provide micronutrients, which helps in eradicating or reducing disease-related to micronutrient deficiency. Fish consists of micronutrient which is not abundant in other plants and mammals; including iodine, zinc, calcium, selenium, magnesium, and vitamin D (Mohanty et al., 2017; Taufek et al., 2016).

Aquaculture is expected to emerge as a prime source of fish in the year coming 2030 due to the customer demand and depletion of wild fish caused by fisheries (Pauly et al., 2017; Fazio et al., 2019).

Red hybrid tilapia (*Oreochromis spp.*) is one of the promising aquaculture fish of this century due to their unique characteristics such as faster growth, high market value, and early sexual maturity (Fitzsimmons, 2010).

Rapid growing of aquaculture increases mass production awareness to farmers worldwide; as a result of expanding production scale, now aquaculture is facing a serious challenge including infectious disease which poses a significant challenge by causing heavy losses to farmers. Substantially, vaccines, antibiotics, and other chemotherapeutics agents are widely used to prevent and control parasitic, viral, bacterial, and fungal diseases. Unfortunately, most of these chemotherapies found to be ineffective in

conferring protection on their own due to the resistance development (Ahmad et al., 2011).

Consumption of fish and other aquatic organisms tainted with antibiotics influences the development of adverse drug reaction or antibiotic-resistant bacteria in humans. Hence, to avoid the antimicrobial resistance developments in human, the application of antibiotics has been prohibited in the aquaculture industry (Aly et al., 2014). The use of antibiotics as prophylaxis in aquaculture has led the public to realize that resistant bacterial species are eventually becoming pathogenic to humans and other living organisms. Due to the consequences of microbial resistance development, many countries banned the use of antibiotics in aquaculture. (Dawood et al., 2015). A worldwide effort has been raised to minimize or eliminate the use of antibiotics in aquaculture industries. This effort was achieved by taking serious action in Europe by the European Parliament and Council Regulation (EC)_No1831/2003) in January 2006 (Koh et al., 2016).

Therefore, an alternative, inexpensive, and effective substitute is needed to reduce, replace, or eliminate the use of antibiotics and other chemotherapy due to their biological consequences to both humans and animals.

Feed additives are ingredients including minerals, vitamins, fatty acids, amino acid, pharmaceutical, fungal products, and sterols, used in animal feed to influence the physical and chemical properties of a feed, mainly to improve the performance of aquatic organisms (Dawood et al., 2018). Commercially available additives including; preservatives, pellet binders, antimicrobial compounds, and antioxidants. Other additives that directly improve aquatic performance and productivity are; prebiotics, probiotics, enzymes, mushroom, plant and animal-derived extracts, and acidifiers (Caipang et al., 2019; Dawood et al., 2018).

For this and many reasons, there is a need to develop an alternative, inexpensive and effective substitute to replace the use of antibiotics in aquaculture to enhance the normal body functioning of fish. Feed additives contain a variety of nutrients that are essential for fish health, growth performance, biochemical response, feed utilization, and whole-body composition.

Ganoderma lucidum (GL) were used in this study as an alternative to antibiotics and other chemotherapeutics agents, as a medicinal mushroom GL has a promising history on several disciplines. This has demanded our attention to use this natural product as feed additives under aseptic mass production. *Ganoderma lucidum* is a medicinal mushroom containing a variety of bioactive compounds that can improve production in aquaculture. Previous studies reported that *Ganoderma lucidum* approximately consists of 10.54% moisture, 5.93% ash, 17.55% protein, 2.60% lipid, 30.25% crude fiber, 33.13% carbohydrate, 23.52% nitrogen (Shamaki et al., 2012). It also contains other bioactive compounds such as triterpenoid, flavonoid, lignans, polysaccharides, peptidoglycans, sterol (lanosterol, ergosterol, and ergosterol peroxide) and different classes of amino acid (Martínez-Montemayor et al., 2019).

The use of *Ganoderma lucidum* and its preparation are not adequate due to the difficulties of mass production. There is limited research on the extraction from mycelium culture as most of the research focuses on extraction from the fruiting body. Under this research, mass production of GL biomass was carried out using mycelia culture under repeated batch fermentation. Through this method, the fermentation time will be reduced from 10days to 5days (Wan et al., 2016). Most of the nutritional and therapeutic potential of *Ganoderma lucidum* were studied *in-vitro* and few *in vivo*, and further trial is required to evaluate the nutritional potential of *Ganoderma lucidum* fully (Deepalakshmi et al., 2011).

On overview, in this study the biomass derived from mycelium of *Ganoderma lucidum* was used as functional feed additives, formulated along with other feed ingredients in three different concentrations; i.e.: 5g/kg, 10g/kg and 15g/kg diet respectively. A hundred-twenty fish weighed approximately 17g were used with 15 fish per group in duplicate treatments. Six weeks of feeding trials were conducted to evaluate the effect of MBGL on red hybrid tilapia organosomatic indices, body composition and hematological indices of the fish.

1.2. Objectives.

1. To evaluate the nutritional composition and impact of mycelial biomass of *Ganoderma lucidum* (MBGL) on red hybrid tilapia body composition.

2. To determine the influence of dietary mycelial biomass of *Ganoderma lucidum* (MBGL) on red hybrid tilapia organosomatic indices

3. To assess the impact of dietary mycelial biomass of *Ganoderma lucidum* (MBGL) on red hybrid tilapia hematological indices

1.3. Hypothesis.

a. *Ganoderma lucidum* biomass can be used as a feed additive in tilapia culture without adverse effect on body composition, organosomatic indices and hematological response.

b. The parameters under body composition, organosomatic indices as well as hematological response considerably varies due to the variation in dietary concentration.

CHAPTER 2: LITERATURE REVIEW

2.1. Global tilapia production.

To date, tilapia consists of over 60 species, among which ten (10) were used as food fish. They were originated from lake tropical Africa to the Nearest-East. The typical environment for their survival is nearby lakes, rivers, and other small water bodies. Some of them can withstand and produce in salty water up to 10%, which is three times in the concentration of normal seawater. In a natural environment, some species of tilapia feed on vegetables and algae. Tilapia is the essential aquaculture fish of the 21st century due to their unique characteristics such as; high market value, adaptation to poor water quality, ability to withstand water temperature around 21-29^{o}c, early sexual maturity, and faster growth. Tilapia can rapidly grow and attain a marketable size up to 250-450g within 8months of culture, even when fed with a plant-based diet (Fitzsimmons, 2010).

Culturing tilapia under the intensive system are highly vulnerable to stress condition; resulted from the fluctuation of water quality, poor management, and disease from naturally occurring microorganism. Antibiotics and other chemotherapeutics agents are commonly used to control the risk associated with these factors. However, the abuse of antibiotics has led to the development of antibiotic-resistant bacterial strain (Amin et al., 2019).

According to the Food and Agriculture Organization of United Nations (FAO) global tilapia production was estimated up to 6.5 million MT in the year 2018, and foreseen to reach 7.3 million MT by 2030 (Srichaiyo et al., 2020), top world tilapia producers are; China with 1.78MT, Indonesia 1.12MT, and Egypt 0.88MT. However, Vietnam, Bangladesh, and Philippines are other growing leading producers (Jansen et al., 2019). Tilapia is the second farmed fish among groups of finfish, where its production escalated up to 5,898,793MT in the year 2016 (Abdel-Ghany et al., 2019).

2.2. Nutritional requirement of tilapia.

The nutrient requirement of red hybrid tilapia *(Oreochromis spp)* is comparable to catfish in that they can tolerate high dietary fiber and carbohydrate than other types of fish cultured (Mjoun et al., 2010). To achieve faster growth in aquaculture at low input, a good quality feed must be prepared. Slightly variation may occur in the same culture growth due to physiological variables such as sex. However, nutrient variability exists among species of tilapia primarily affected by the growth of the fish (Mjoun et al., 2010). Tilapia can easily digest and assimilate nutrients into flesh (Mehana et al., 2015). Feed additives contain functional ingredients such as vitamins, amino acids, fatty acids, and minerals, which can significantly improve tilapia growth performance, feed utilization, and whole-body composition (M. H. Ahmad et al., 2011). Feed additives are an excellent remedy to increase the efficiency of feed to control tilapia, shrimp and other fish diseases (Mehana et al., 2015).

Protein is considered as major dietary nutrient suitable for enhancing fish performance. However, excess protein in fish whole diet may be wasteful which could increase unnecessary expenses (Bahnasawy, 2009).

Protein requirement of hybrid tilapia is considered as one of the most crucial diets among the nutritional needs of tilapia, which received considerable attention during complete fish feed formulation. The dietary protein requirement of tilapia, for maximum growth appears to be in percentage and likely to be higher than that of monogastric terrestrial animals such as poultry (Van Norren et al., 2009).

Dietary carbohydrate enhances tilapia growth performance and helps in improving the immune system of fish (S. Li et al., 2018). Starch from carbohydrates is a crucial element enhancing the quality of feed in aquaculture due to its binding properties (Sørensen et al., 2010). Specifically, there are no appropriate carbohydrate requirements of fish; however,

it helps in reducing formulation cost also by reducing protein and lipid catabolism for energy (Roberson, 1990). Increase in dietary carbohydrate of fish significantly promote glycolysis and reduced inhibition of gluconeogenesis. Imbalance regulation of gluconeogenesis and glycolysis would lead to glucose intolerance in fish (Panserat, 2009).

Dietary lipid is one of the essential ingredients that provide energy, phospholipids, essential fatty acids, sterol & fat-solute vitamins. Together they maintain structural components of cells membrane (Wong et al., 2012). An increase in lipid improves the efficiency of feed (Chatzifotis et al., 2010). However, excessive use of dietary lipid affects growth performance and causes abnormal fat deposition (Mohanta et al., 2008).

2.3. Feed and feeding practice in aquaculture.

In the intensive system of the fish farming highest cost of production comes from a feed, in which it approximately account for 60 – 80% of total production cost; however, in the semi-intensive system of fish farming feed and fertilizer account for 30 – 60% of total production cost (Hasan, 2010). Farmers believed that overfeeding fish could improve growth performance within a shorter period without realizing utilization efficiency. This wrong perception mostly released out by manufacturers to enable the use of more feed than required. High-quality feed usually contains high protein, provided to aquaculture industries without knowing fish protein requirement, and this gradually led to wastage of feed. Lack of adequate feeding strategy unlikely knock down productivity (Craig et al., 2017). To enhance suitable production, farmers must improve feed conversion ration by providing an appropriate quantity of feed per time, feeding duration, and time of feeding termination at all costs. It has been understood that quality feed not necessarily, in turn, provides a high profit; instead, it gives feed management. It has been established that better feed management reduce feed cost approximately by 15-20% (Rola et al., 2007).

2.4. Antibiotics in aquaculture.

Antibiotics are substances which can inhibit or kill microorganism, since from their discovery by Fleming in 1928. This makes them essential drugs of choice in treating both human and animal diseases. Antibiotics originally developed from synthetic and natural sources. Antibiotics remain sufficient to treat bacterial infectious disease and remain safe to their host (non-toxic). Antibiotics are used in aquafeed, as therapeutics, metaphylactic or as prophylactic (Burridge et al., 2010).

Antibiotics in aquaculture were adopted frequently decades ago until, in this recent year, it received public attention. Complex investigations have been made to access environmental risk associated with antibiotics used, being one of the essential therapies among groups of pharmaceuticals. However, there is still a lack of adequate understanding of the significance of resistance development in some bacterial strain (Kümmerer, 2009). Antibiotic has been used to control the effect of unwanted microbial growth. However, some organisms like *Pseudomonas aeruginosa* developed resistance ability against penicillin G. during cell division, this termed primary resistance (vertical resistance transfer). Other resistance may occur during therapy, i.e., during contact of microbial organism with antibiotics, this termed secondary resistance and the resistance that occurs through gene transfer between microorganism termed plasmid-mediated resistance or horizontal resistance transfer. The effect of resistance development can reach the environment through the aquatic and terrestrial organisms, and finally, reach humans through the consumption of contaminated organism or by drinking water tainted with antibiotics (Kümmerer, 2009).

Antibiotics received considerable attention due to its effects on the environment and aquatic ecosystem, it has been reported that trimethoprim and sulfadiazine inhibit essential organisms such as *Phaeodactylum tricofnutum* (algae) and *Brachomus*

koreanus (rotifers), sulfamethoxazole and norfloxacin inhibit brain acetylcholinesterase activity in some species of fish such as goldfish (Du et al., 2019).

2.4.1. Effects of antibiotics in aquaculture.

The intestinal tract of fish harbor microbiota useful for nutrition, digestion and disease control (Navarrete et al., 2008). The essential processes of microbiota in fish involves in epithelial proliferation, nutrient metabolism & innate immune response (Navarrete et al., 2010). Possible changes may occur to gastrointestinal microbiota as a result of antibiotics treatment. To enhance safety in fish, it is essential to establish an understanding of how antimicrobial compounds modify fish gastrointestinal microbiota (Kerry et al., 1997).

Public risk associated with antibiotics exposure depends on the duration and quantity of substances being consumed. Food derived from animal origin contaminated with antibiotics resistant bacteria are potential source of health threat to public, some resistant organism transfers their resistant gene to other organisms through conjugation. Pathogens that pass through this chain may not respond to antibiotics treatment, hence resistant organism served as reservoir of resistance gene and ultimately end up as a threat to public (Martinez et al., 2008).

2.5. Feed additives in aquaculture.

Feed additives are ingredients, including minerals, vitamins, fatty acids, amino acid, pharmaceutical, fungal products, and sterols. Feed additives are used in animal feed formulation to influence the physical and chemical properties of feed and to improve the quality and performance of the aquatic organism (Dawood et al., 2018). Substances added to feed resulting in; preservation, flavoring, enhancing and improving the appearance of feed is referred to as additives (Caipang et al., 2019).

Aquaculture depends solely on the use of a balanced nutritional diet to lower the cost of production and improve productivity. Quality well balance feed depends on types of feed ingredients and additives considered during formulation, i.e., the mixture of both organic and inorganic substances. The feed may vary based on the component of raw materials considered during formulation; additives added to feed during feed preparations are to improve feed quality, and to enhance health benefit with the aim to improve productivity. Non-nutritive ingredients, including antioxidants, probiotics, immunostimulants, and antibiotics, are used to enhance the growth of fish as well as water treatment. The use of these compounds in aquaculture increased the cost of production. To limit escalated cost of production majority of aquafeed companies turned into the application of functional feed additives, which include prebiotics, probiotics, enzymes, phytogenic compounds, organic acid, immune stimulant, mycotoxin binder, and yeast products (Bharathi et al., 2019).

2.5.1. Probiotics.

According to World Health Organization (WHO) probiotics are defined as "living organisms when added in optimum amount can provide a health benefit" (Ringø, 2020). Probiotics involve the application of entire microorganisms or the use of a beneficial component of their body, which can often provide a health benefit to the host. Probiotics are often active in their environment as well as in host body (S. Liu et al., 2020), also defined as live microorganism which has a beneficial effect on a host body through modifying host intestinal microbiota. It could improves the nutritional value of feed and improves host response to disease (Romero et al., 2012). Probiotics prevent multiplications of the pathogenic organism in the fish gut, improve digestion, water quality, and enhance the fish immune response (Bharathi et al., 2019). Microorganisms commonly used as probiotics include *Enterococcus, Micrococcus, Lactobacillus, Lactococcus, Cyanobacterium, Streptococcus* and *Weissella.* (Encarnação, 2016). In

humans' probiotics could inhibit/lowering cancer cell proliferation hence describe as healthy bacteria or friendly bacteria (Bharathi et al., 2019). Probiotics that have been used in aquaculture are summarized in Table 2.1. follow:

Table 2. 1: Important probiotics organism used in aquaculture.

Probiotics	**Importance**	**References**
Enterococcus faecium	Improved growth performance and enhance the immune response in tilapia.	(Yousefi et al., 2018)
Bacillus coagulans and Rhodopseudomanas palustris	Promote growth rate and increase weight gain in tilapia.	(Xuxia Zhou et al., 2010)
Streptococcus faecium	Improve growth, protein and lipid content in Nile tilapia.	(M.-J. Kim et al., 2006)
Bacillus cereus	Supplementation of 0.5g/kg in diet improve growth performance of juvenile common dentex.	(Hidalgo et al., 2006)
Arthrobacter enclensis	Increase survival rate in shrimp.	(S. Liu et al., 2020)

2.5.2. Prebiotics.

Prebiotics are non-digestible ingredients that are beneficially added to stimulate growth, improve the activity of gut microbes and attempt to improve host health (Ebru et al., 2016). Prebiotics used in aquaculture includes mannan-oligosaccharide, Oligofructose, Oligosaccharide, Inulin, Fructooligosaccharide, Galactooligosaccharide, α, & β-glucan. Prebiotics can be associated with the following criteria; (a) Should be beneficial to fish health. (b) Must be resistant to the fish gut. (c). Should be fermentable by fish microbiota (Bharathi et al., 2019). Previous studies stated that prebiotics has beneficial natural effects on fish feed utilization, growth performance, intestine microbiota, carcasses, immunity, and disease-resistant ability. Prebiotics improved growth performance (weight gain & specific growth rate), modulate intestinal microbiota, activate lysozyme activity, intestinal antioxidant and serum complement (Z. Li et al.,

2019). Prebiotics play a significant role in maintaining homeostasis between both host cells and microbiota (Zou et al., 2016). Prebiotics commonly used in aquaculture are summarized in table 2.2 below.

Table 2. 2: Some common prebiotics used in aquaculture.

Prebiotics	**Importance**	**References**
Fructo oligosaccharide	10g/kg in feed increases fish feed intake and improve digestibility.	(Grisdale-Helland et al., 2008)
Inulin	Increase magnesium, iron, RBC, and increase lysozyme activities in Nile tilapia.	(Tiengtam et al., 2015)
Mannan oligosaccharides	0.4 % as a diet increases intestinal muscle, fold and thickness.	(Yuji-Sado et al., 2015)
Fermacto prebiotics	Improved fish growth at 3g/kg in carp.	(Mazurkiewicz et al., 2008)

2.5.3. Mushroom.

Mushroom contains different varieties of bioactive compounds including polysaccharides such as chitin, α and β-glucans, hemicellulose, xylans, galactans and mannans (Kalač, 2009). Mushroom contains antitumor, antiviral, antimicrobial, immunostimulant, and antioxidants properties. Due to this awareness, mushroom gradually gets acceptance to be used in aquaculture (Van Doan, Hoseinifar, Esteban, et al., 2019). Majority of mushrooms have a different chemical constituent; a polysaccharide derived from this fungus belongs to β-glucan group. Pancreas digestive enzymes do not hydrolyze β-glucan glycosidic bond. Therefore, mushroom polysaccharides resist stomach acid hydrolysis and finally remain indigestible (Van Doan et al., 2016).

The Nondigestible mushroom polysaccharide can serve as prebiotics (Singdevsachan et al., 2016). Mushroom polysaccharide not only functions as prebiotics but rather used to treat many life threatening ailments (Thatoi et al., 2014). There is various published

and ongoing research regarding the use of mushroom as prebiotics as well as addressing public awareness on the general advantages of mushroom in aquaculture industries (Zou et al., 2016). Table 2.3. summarized the list of mushrooms previously used in aquaculture.

Table 2. 3: Species of mushroom commonly used in aquaculture.

Mushroom	**Importance**	**References**
Ganoderma lucidum	β-glucan derived from *G. lucidum* increases weight gain, survival rate, feed intake and specific growth rate in carps.	(Chithra et al., 2016).
Pleurotus florida	This species enhances bactericidal, phagocytic, respiratory burst (RB), lysozyme activities and stimulate superoxide anion production in carps.	(Kamilya et al., 2006).
Agricus bisporus	Used to improved growth performance in carps.	(Zou et al., 2016).
Coriolus versicolor	Used to increase RBC, WBC, hemoglobin, ESR, total protein, blood urea, resistant to *A. hydrohila.*	(Chang et al., 2013; Harikrishnan et al., 2012a).
Lentinula edodes	It increases hematocrit, serum lysozyme, total leucocytes, phagocytic activity, myeloperoxidase activities and IgM and resistant to *L. garvieae*.	(Baba et al., 2015).
Hericum erinaceum	Improved resistance ability against *P. dicentrarchi* and improved immune response.	(Harikrishnan, Kim, et al., 2011a).
Phellinus linteus	Improved growth performance and provide resistance against *Vibrio anguillarum* juvenile flounder	(M.-J. Kim et al., 2006).
pleurotus ostreatus	It increases growth, lysozyme activities and hematocrit of catfish. Methanolic extract of *P. ostreatus* increases specific growth rate, phagocytic, lysozyme & myeloperoxidase and resistance to *A. hydrophila.* Polysaccharide from this species increase Histosomatic index in tilapia	(Ahmed et al., 2017; Bilen et al., 2016; Katya et al., 2016).

Table 2.3. Continued.

Pleurotus sajor-caju	The stalk is used to improved growth rate, phagocytic, lysozyme, and myeloperoxidase and resistance to *A. hydrophila* in rainbow trout.	(Van Doan, Hoseinifar, Esteban, et al., 2019).
Innotus obliquus	Antiprotease, increase lysozyme activities, production of reactive oxygen and nitrogen, myeloperoxidase and resistance to *Uronema marinum* in olive flounder.	(Harikrishnan et al., 2012b).

2.6. Ganoderma.

There are various species of *Ganoderma,* mainly use for medical purpose including; *Ganoderma lucidum*, *Ganoderma luteum, Ganoderma atrum, Ganoderma appalanatu, Ganoderma austral, Ganoderma capense, Ganoderma tropicum, Ganoderma tenue* and *Ganoderma sinense* (Deepalakshmi et al., 2011). Among all, *Ganoderma lucidum* is the species of interest for this research. As it is valued empirically throughout the world as a medicinal and food product. The mushroom provides a wide variety of bioactive compounds that were used in medicine, and these compounds are considered as active and effective against several life-threatening diseases in both humans and animals. Approximately it has been estimated that there are over 1.5million species of fungi worldwide, among which only 82,000 were discovered. Among the known species, 5,000 of them belong to the macro-fungi and considered edible. Species of fungi from basidiomycete are of utmost importance due to interest enacted on them because of the presence of bioactive compounds (Deepalakshmi et al., 2011).

2.6.1. The nutritional constituent of *Ganoderma lucidum.*

The nutritional composition of several species of mushroom has been documented in many laboratories around the globe, the nutrient composition of locally produce mushroom remains speculative. However, the nutritional balance is affected by several

factors such as; growth, strain, cultivation method, stage at which harvested, and proportion of fruiting bodies. *Ganoderma lucidum* consists of protein, crude fat, starch, and reducing sugar, although this constituent may vary from strain, origin, cultivation and extraction procedures. Carbohydrate composition derived from crude *G. lucidum* contain d-glucose, d-galactose, d-xylose, d-mannose, d-GlcNac, I-fucose and d-rhamnose at different concentration. Mushroom contains a high amount of chitin, nitrogen, protein, total carbohydrate, lipid, and ash (Deepalakshmi et al., 2011).

Total carbohydrate can be divided into reducing sugar and dietary fiber which consist of soluble polysaccharide and crude fiber (Ulziijargal et al., 2011). Polysaccharide derived from *Ganoderma lucidum* was previously used as a dietary supplement on freshwater prawn at different concentrations level (0, 1.0, 1.5, 2.0, & 2.5g/kg). A 90days feeding trial was conducted to evaluate prawn growth performance, fillet composition, the activity of digestive enzymes, antioxidants, and finally, metabolic enzyme activities. At the end of the experiment, all the above parameter's highest performance obtained from the highest diet. i.e., 2.5g/kg produce better result in comparison to their competitive groups (Mohan et al., 2016). According to Shamaki et al., (2012), the proximate composition of *Ganoderma lucidum* (fruiting body) consists of moisture 10.54%, Ash 5.93%, protein 17.55%, lipid 2.60%, crude fiber 30.25%, carbohydrate 33.13%. *Ganoderma lucidum* also contain other bioactive compounds such as triterpenoid, flavonoid, lignans, polysaccharides, peptidoglycans, sterol (lanosterol, ergosterol, and ergosterol peroxide) and different classes of amino acid (Martínez-Montemayor et al., 2019).

2.6.2. *Ganoderma lucidum* biomass as feed additives in aquaculture.

The use of feed additives serves as an alternative to antibiotics and other chemicals used to control fish diseases. The use of an antimicrobial agent in controlling fish disease

becomes worrisome. Extensive usage of antibiotics in aquaculture has created problems for the industry and increase opportunities for developing suspicious meat/products. There is an ongoing global effort to reduce the use of antibiotics in the aquaculture industry due to the evidence of accumulating unrestricted detrimental effects on fish, human health, other terrestrial animals, and the environment. It has been proven that *Astragalus radix* and *Ganoderma lucidum* are every effective in enhancing the immune response of carp fish (Yin et al., 2009). Supplementation of *Ganoderma lucidum* and *Astragalus* extract (0.5%) for five weeks, has prevented the fish against respiratory burst activity, lysozyme activity, phagocytosis, circulatory antibody and prevent fish against *A. hydrophila* infection (Yin et al., 2009).

2.7. Body composition of fish.

Body composition is defined as an indicator of fish physiological condition which involves an analysis of water, fat, ash, and protein content. It is of significant concern in aquaculture because it affects fish growth, appetite, and feed utilization efficiency (Breck, 2014). The fish whole-body proximate composition considerably varies among different species of fish, and it largely depends on the type of feed used to fed the fish. This, in turn, remains as primary determinate for fillet nutrient and fillet quality and quantity (Teame et al., 2016). To meet the standard requirements, it is necessary to evaluate proximate fillet composition including ash, protein, lipid, and moisture to ensure that they fit standard requirements set by food regulations and for commercial specifications (H. E. Mohamed et al., 2010).

Chemical proximate composition of freshwater fishes is valuable for the nutritionists to determine the total level of fat and protein. In general fish live body weight composition made up of 70-80% water, 20-30% protein 2-12% lipid (Naeem et al., 2017). Fillet proximate composition is an essential aspect as a way to broadly defines fish body

nutrition, and it is closely associated with consumer nutritional attribute (Grigorakis, 2017).

Growth of fish is characterized by changes in size and tissue composition, to ensure safety and product quality. The study of body composition is interestingly increasing among aquaculture nutritionist and genetic improvement. Body composition influence by size and species of fish. This is considered an important variable in the fish processing industry (Furuya et al., 2019). In aquaculture, the study of fish carcasses and fillet quality, gained considerable attention among both consumers and aquaculture industries because it is directly related to the human health nutrition (Sahu et al., 2017). The proximate composition of fish may significantly vary, depending on the season, experimental diet, sex, and age. Fish are poikilothermic organisms whereby changes in water parameter can adversely affect their growth, body composition, and productivity (Mubarik et al., 2019).

Tilapia exhibit sexual dimorphic growth in which male grow faster and more significant than female, therefore, several variables can affect the overall chemical composition of fillet (Biró et al., 2009). Overall, body composition of fish is affected by both endogenous and exogenous factors. Exogenous caused by a diet of fish while endogenous factors including genetic, linked to sex, age, and size. Various studies have relatively carried out to examine the effects of pH, temperature, salinity and oxygen concentration on proximate composition of fish (F. A. Mohamed et al., 2016).

During frequent feeding, the protein content of carcasses will be decreased slightly while lipid content increases. Tilapia lipid content is directly related to dietary fat content, and protein content remains stable or more when feeding with different dietary supplement (Alwan et al., 2017).

Dietary supplement, age or body size have a definite outcome on fish general body composition. Changes to these factors can affect physical dimensions such as length,

weight, or mass of the whole body and to other respective tissue and organs of the body and eventually affect body protein, lipid, and other body chemical constituent (Ihie et al., 2018).

2.7.1. Organosomatic indices.

2.7.1.1. Condition factor.

Condition factor is an indicator for determining the general health of fish in biology since the discovery of the method at the beginning of the 20th century. Condition factor reflects the biological and physical circumstances of a fish and serves as a physiological measurement of fish concerning their general welfare. It can be used to compare two conditions of two living populations under different climate, stocking density, feeding density, and other conditions (Ighwela et al., 2011).

Factors such as feeding intensity and growth of fish during development influence CF. It also provides information on the physiological variation of fish and is used to compare growth variation among the various living population of fish (Ighwela et al., 2011). CF of fish decrease with increasing in length (Froese, 2006). Therefore, CF can be used to determine feeding activity of fish whether it is making adequate utilization (Gomiero et al., 2008). The condition factor of fish remains very useful in aquaculture as a mathematical model for determining fish growth in relation to their environment under which they were cultured. CF gives an objective and practicable outcome to describe and estimate fish growth between sampling intervals (Moslen et al., 2017). According to Maina et al., (2019) male fish has relatively higher CF than the female counterpart. Two growth condition were noticeable for adequate evaluation of CF (isometric and allometric growth) due to nutritional intake, water quality, habitat, stocking density, sex and stocking time (Saha et al., 2019).

Allometric growth is associated with poor conditions, which suggests that fish become slimmer due to an increase in weight. However, isometric growth suggests appropriate growth conditions and fish become relatively deeper-bodied due to an increase in length (Maina et al., 2019). In tilapia, it has been confirmed that tilapia fed with farm-made diet shows more isometric growth. Fish becomes comparatively deeper-bodies as it increases in length (Anani et al., 2016). Besides, according to Keri et al., (2011) dietary maltose significantly influence tilapia condition factor.

2.7.1.2. Hepatosomatic indices.

Hepatosomatic indices of fish are associated with liver energy reserves & metabolism activity. The liver is the most significant organs for absorption and storage of lipid-derived from feed representing a vital role in metabolism (Magalhães et al., 2012). Usually, when feed is available at optimum, it becomes favorable to increase HSI value. The daily increase in body weight is related to the increase in HSI. The liver performs a variety of physiological functions, including converting sugar into glycogen, detoxification of toxic substances, destroy old red blood cells, and act as hemopoietic organs (Morrison, 2017). A healthy liver serves as the potential organ for fish growth. HSI provides information about the condition of the liver, body weight, and body energy reserve in fish. It also provides information on fish health conditions, quality of water on which the fish were cultured. As well as the status of energy stored in fish and a good indicator of fish feeding condition (Jan et al., 2016).

Higher HSI indicates fish are overgrowing under a favorable situation. Lower HSI shows fish are not growing due to environmental problems (Morrison, 2017). Hepatosomatic indices play an important role in aquaculture for understanding fish metabolism, digestion, absorption, synthesis & secretion of digestive enzymes and carbohydrate metabolism. Hepatosomatic indices decrease during post-spawning and

significantly increases during the resting phase, where changes in HSI indicate reliable physiological changes, especially metabolic activities centered in the liver. However, according to Singh et al., (2017) a decrease in HSI also has a relation to metal toxicity on fish.

2.7.1.3. Vicerosomatic indices.

Vicerosomatic indices are used to determine fish health status in which an increase in VSI may be due to the role bioactive compounds added into the feed as additives (Sogbesan et al., 2017). VSI of fish increases with an increase in dietary lipid hence; digestible feed calories are discarded through viscera. Excessive increase in lipid content can negatively affect the quality of fish through the degradation of fillet by lipid oxidation (Yıldız, 2004). It may vary among the living populations of fish due to stocking density (Ni et al., 2016) and proved to be significantly increased with an increase in dietary carbohydrate level (M. Ahmad et al., 2012).

The study of viscerosomatic and hepatosomatic indices play a significant role, especially to fish metabolism, digestion, absorption, carbohydrate metabolism, synthesis, and secretion of digestive enzymes. (Ighwela et al., 2014).

2.8. Serum biochemistry.

Blood parameters are fundamental in aquaculture for diagnosing the functional and structural status of fish exposed to different toxicants, changes in fish serum biochemistry indicate the occurrence of possible alterations in metabolism and biochemical processes (Fırat et al., 2011). Fish serum reflects the biochemical status of body metabolism, and there are factors that responsible for affecting these processes, such as environmental stressor and heavy metals. Therefore, together they could alter biochemical parameters in fish. Heavy metal, like silver (Ag), significantly affect fish general health conditions which, resulted in severe mortality. High quantity of indigestible ingredients; such as

starch, fiber, and antinutritional substances in a feedstuff expected to negatively affect protein, energy digestibility and influences the serum biochemical properties (Öner et al., 2008). Blood serves as a pathophysiological reflector of the entire body.

Biochemical parameters are an indicator of fish health and physiological response in that it defines various stressors. An increase in the dietary protein level of a diet can increase serum protein when anabolic responses surpassed catabolic response (Rathore et al., 2018). The decrease in serum total protein below normal range relates to liver dysfunction, which also suggests that a reduction of serum total protein is attributed to increasing in stressor exposed by fish (Abdelkhalek et al., 2017). Serum total protein plays a vital role in maintaining healthy functioning and metabolic activity of osmotic pressure, plasma colloid and transport of material into various part of the body in fish (Zhu et al., 2017).

2.9. Hematological indices.

Fish hematological studies were in existence since 1943 (Field et al., 1943). Since that time literature regarding fish hematology are increasing, techniques and the knowledge of blood analysis are progressively improving (Fazio, 2018; Lorenz et al., 2018; Pula et al., 2018). Fish hematology is essential for analyzing/diagnosing blood-related disease and is a good indicator of a pathological condition of fish. Analysis of blood morphology facilitates the diagnosis of illness, and this can serve as a prognosis indicator of fish pathology. Blood analysis help to identify fish disease quickly and effectively (Weinert et al., 2015). Recent studies reported that dietary supplement like lycopene might be helpful in abrogation of toxicity by changing the hematological condition and antioxidant of fish (Yonar et al., 2020).

Probiotics bring about changes to fish hematological parameters, such as activation of innate immune response and changes to whole blood count within 1-6 weeks of

supplementation. This shows that administration of mixed diet with probiotics can effectively minimize mortality and restore altered hematological parameters in fish (Harikrishnan, Kim, et al., 2011b).

Musa Creek (2012) has reported hematological parameters based on sex on yellowfin seabream; female fish RBC Count was higher than male fish. However, Hct, MCV, MCH, MCHC, and differential count of leukocyte did not show a significant difference between male-female fish (Motlagh et al., 2012).

Hematological studies considered as the most dynamic parameter for assaying fish physiological and pathological changes. This method adopted by fish biologist as the most efficient method in many parts of the world (Gabriel et al., 2011). However, qualitative and quantitative variation suggests the significance of the result regarding fish diagnosis (Martins et al., 2004). Numerous studies have been carried out to evaluate the normal range of blood parameters, whereby; many physiologists and pathologists have established this effort (Rambhaskar et al., 1987; Xiaoyun Zhou et al., 2009). Most of the recent literature indicates that it's complicated to interpret the normal range of fish blood parameters; this is due to the variety of factors that may cause variation at a point. Both external and internal factors including sex, stocking rate, size and environmental factors such as temperature, pH, DO influence fish hematology (Karimi et al., 2013).

Esin et al., (2015) suggested that fish fed with mushroom (*Lentinula edodes*) enhance hematological parameters of fish (Baba et al., 2015). It has been reported that supplementation of *Innotus obliquus* into fish feed positively enhance hematology and innate immune system of fish (Harikrishnan et al., 2012a). Beneficial feed additive optimally improve fish performance and immune response in Nile tilapia (Van Doan, Hoseinifar, Chitmanat, et al., 2019).

CHAPTER 3: MATERIALS AND METHODS

3.1. Experimental diet.

Ganoderma lucidum was collected from Functional Omics and Bioprocesses Center, University of Malaya. Species were confirmed by (Supramani et al., 2019). Crude *Ganoderma lucidum* was subjected to grow into mycelium, then proceed into batch fermentation, as shown in Figure 3.1. Media used including 30% D(+)-Glucose monohydrate $C_6H_{12}O_6.H_2O$, 1g yeast extract, 0.5g di-Potassium Hydrogen Orthophosphate anhydrous K_2HPO_4, 0.5g Potassium dihydrogen phosphate anhydrous KH_2PO_4, 0.5g Magnesium sulfate $MgSO_4$, 0.5g Ammonium chloride NH_4CL in 500ml working volume for first seed culture (10days) and second seed 20% inoculum, 30% glucose, 50% media (culture duration; 10days for each seed culture). Through this method, the fermentation time was reduced from 10days to 5days (Wan et al., 2016). At the end of the second seed, the culture was filtered using Buchner funnel filtration apparatus to obtain the biomass. The biomass was dried in a compact laboratory dryer (24h) at 50°c.

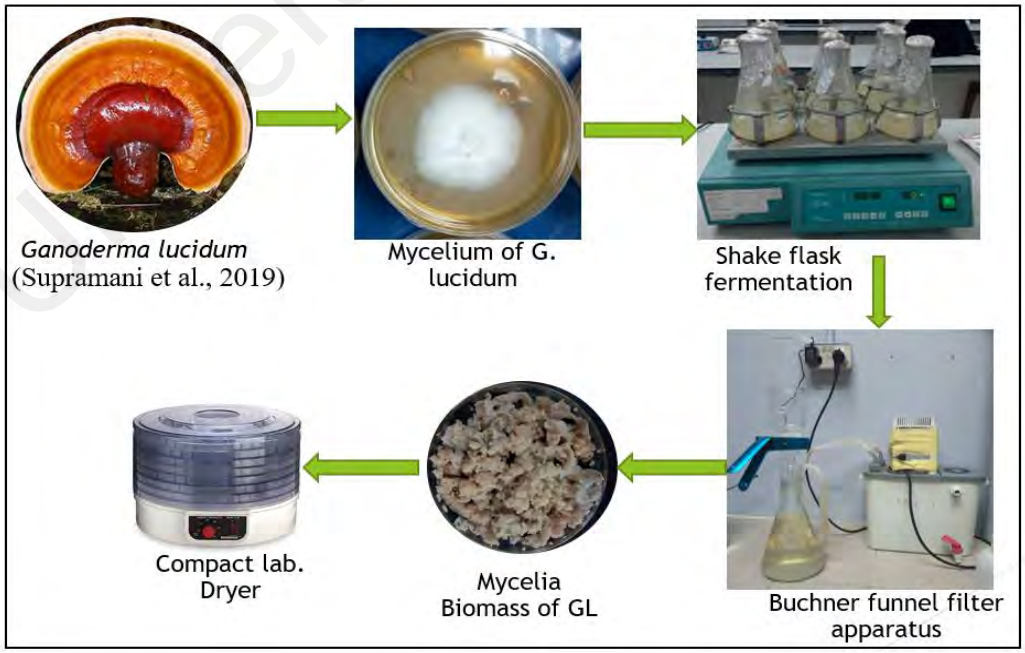

Figure 3. 1. Production of mycelial biomass of GL.

3.2. Experimental feed.

The dried biomass was collected and use as feed additives along with other feed ingredients, including fishmeal, rice bran, soybean, cornmeal, mineral pre-mix, di-calcium phosphate (DCP), fish oil, L-lysine, methionine, and vitamins pre-mix. The experimental feed was formulated using the Winfeed software version 2.8. Approximately 0.3cm diameter pellets were prepared by using a small pelleting machine, then transfer into oven for drying at 60^{o}C overnight to attain a constant weight and finally transferred into a cold room (4^{o}C) for storage (Taufek, 2016). The inclusion level of each feed ingredient was presented in Table 3.1. The proximate composition of all feed ingredients was presented in table 3.2. and proximate chemical composition of formulated feed (for each treatment) was presented in Table 3.3.

Figure 3. 2. Production of feed pellet.

Table 3. 1: Feed formulation and chemical composition of the experimental diets.

Ingredients (g/kg)	Control(g/kg)	T1 (5g/kg)	T2 (10g/kg)	T3 (15g/kg)
Fishmeal	300	300	300	300
Cornmeal	193.9	193.3	192.6	192
Rice bran	199.3	198.3	197.4	196.4
Soymeal	236.8	233.4	230	226.6
Mycelial biomass	0.0	5.0	10.0	15.0
Vitamin	2.0	2.0	2.0	2.0
Mineral	3.0	3.0	3.0	3.0
Lysine	10.0	10.0	10.0	10.0
Methionine	5.0	5.0	5.0	5.0

Table 3.1. Continued.

Fish oil	40.0	40.0	40.0	40.0
Dcp	10.0	10.0	10.0	10.0
Total	1000(g)	1000(g)	1000(g)	1000(g)

Table 3. 2: Proximate composition of dry feed ingredients.

Diet	Protein%	Fiber%	Carbohydrate%	Ash%	Lipid%
Fishmeal	54.29	14.54	5.6	23.16	2.41
Soybean meal	43.01	9.64	40.04	5.16	2.14
GL Biomass	32.23	13.80	48.38	1.14	4.45
Rice bran	11.23	19.40	55.30	5.3	8.76
Corn meal	6.64	9.81	79.23	1.73	2.60

Table 3. 3: Nutritional composition of experimental diets.

Nutrient	Control	5g/kg	10g/kg	15g/kg
Protein	30.15	33.51	35.36	31.78
Fibre	1.63	1.22	1.28	1.65
Carbohydrate	29.47	17.18	19.24	20.92
Lipid	6.29	5.25	5.26	5.82
Ash	12.00	12.20	12.61	12.59
Dry Matter	87.25	69.36	73.75	72.76
Moisture	12.75	30.64	26.25	27.24
Gross energy (kJ/g)	13.88	10.83	10.96	11.27

[1]Gross energy was calculated by multiplying the generalized physiological value of protein (19kJ), NFE (15kJ) and lipid (36kJ). Energy contributed by protein calculated as (%) of protein x 19 = x, NFE (%) x 15= y and lipid (%) x 36 = z [GE kJ/g = (X+Y+Z)/100 (Natarajan, 2006)

[2]Carbohydrate calculated as 100 – (%CP + % crude fiber, % crude ash, % moisture, % crude fat) (Bhuyain et al., 2019; N. M. Taufek et al., 2016).

3.3. Experimental fish and setup.

One hundred and twenty (120) red hybrid tilapia (*Oreochromis spp*) were purchased from a reputable hatchery (Sungai Buloh fish farm, Selangor, Malaysia), with average weight of (18-17.0g), then safely transported to aquarium laboratory in the Institute of

Biological Science, Faculty of Science, Universiti Malaya. Upon arrival, the fish were transferred into their respective tanks containing de-chlorinated water. The fish were allowed to rest for 24h before feeding. Then they were subjected to acclimatization for two weeks using the commercial feed. At the end of the acclimatization, the fish were divided into four groups in duplicate samples with 15 fish per tank.

Figure 3. 3. Red hybrid tilapia (*Oreochromis spp*.).

On starting the feeding trial, the fish were fed with one of the four experimental diets, i.e., control (without feed supplement), feed containing 5g/kg of MBGL, feed containing 10g/kg of MBGL, and feed containing 15g/kg of MBGL. Eight plastic tanks were used with a water carrying capacity of 100 liters. All the tanks were equipped with an aquarium pump and filter box to provide aeration, filtration, and internal circulation.

Water qualities were observed regularly to ensure the pH, temperature, dissolved oxygen, ammonia, and nitrate are within the normal range for tilapia culture. pH was maintained within the range of 6 to 8, ammonia and nitrate were maintained within 2.0 to 5.0mg/l, DO were maintain above 6.0mg/l and water temperature at 27-29°C (Jimoh et al., 2019b; Mishra et al., 2008; Taufek et al., 2016)

3.4. Proximate and chemical analysis of ingredients and body composition.

All the feed ingredients and experimental diets were analyzed for the proximate composition according to the Association of Official Analytical Chemist methods (AOAC, 2003).

3.4.1. Crude protein.

For crude protein analysis, the Kjeldah1 method was used. Approximately 0.15g sample was weight into Kjeldah1 digestion tube, and later 100mg of Kjeltab catalyst and 6ml of concentrated Sulphuric acid was added into each tube. The tube was placed into the FOSS Tecator Digester Auto at a starting temperature of 420ºC, for 1hour, and later allowed to cool for 15minute before the distillation process. An estimate of 80ml of de-ionized water & 50ml of sodium hydroxide were added automatically by the distillation machine and mixed thoroughly and distilled with 4% boric acid (25ml). Titration indicator were prepared using 100mg of bromocresol green dissolve in 100mg of methanol before adding 70ml methyl red in 100ml of methanol. Approximately 7-8 drops of bromocresol green and methyl red indicators were added into a conical flask containing 25ml of 4% boric acid. Finally, titration value detected by using hydrochloric acid drop by drop until constant titrate detected. All samples, including blank, were analyzed in duplicate.

The protein content of each sample was calculated using the below calculation:

$$\%\text{Protein} = \frac{W3-(W1-C)-(W5-W4-D)}{W2} \times 100 \qquad (3.1)$$

Where 870.18 is a multiplication factor to convert titer to % protein based on standardized protein factor

W1 = sample weight (g)

W2 = blank weight (g)

W3 = sample/blank titer (Mlay et al., 2007).

3.4.2. Crude lipid.

Crude lipid content of the experimental diet, feed, and fillet were determined using the Soxhlet method and extraction with petroleum ether. In the process, extraction cups were dried in an oven and 2g of the sample was added into a cellulose thimble. Then, an empty beaker was filled with 80ml of petroleum ether. The beaker, together with thimbles, was sealed using an aluminum sheet to prevent the solvent from evaporation and allowed to stay overnight. Then the thimble was removed from the beaker, and petroleum ether was poured into the extraction cup and placed into a rotatory evaporator to separate the lipid from the solvent. Subsequently, the cups were dried in an oven at 120°C for two hours and then allowed to cool off in a desiccator before weighing. The samples in the thimble were kept for crude fiber analysis (Taufek, 2016).

% of crude lipid were calculated as: $\frac{(W3-W2)}{W1} \times 100$

(3.2)

Where:

W1 = weight of sample.

W2 = weight of extraction cup initial.

W3 = weight of extraction cup final.

3.4.3. Dry matter.

Dry matter was determined by weighing empty dried crucible (W1). Approximately, 2g of the sample was added into the crucible. Both the crucible + sample were weighed (W2), then the sample was placed into an oven at 105°C for 24hours to obtained constant

weight. After 24hours, the sample + crucible was allowed to cool in a desiccator, then weighed to obtained final weight (W3).

Dry matter was calculated by using the following formula:

$$\text{Dry matter} = \frac{(W3-W1)}{W2-W1)} \times 100 \qquad (3.3)$$

Where :

W1 = weight of the empty crucible

W2 = weigh of crucible + sample

W3 = weight of crucible + sample after 105^{o}c

3.4.4. Ash.

Ash content of feed and fillet was determined by drying the samples derived from the dry matter in a muffle furnace (Naberthern) at 600^{o}c overnight. Then the sample was cooled in a desiccator and reweighted to determine the ash content, calculated using the formula as follows:

$$\text{Ash\%} = \frac{(W4-W1)}{(W3-W1)} \times 100 \qquad (3.4)$$

Where W1 = weight of empty crucible

W3 = weight of crucible + sample after drying at 105^{o}c

W4 = weight of crucible + sample after drying at 600^{o}c

3.4.5. Crude fiber.

The crude fiber was estimated using a defatted sample derived from crude lipid analysis. Fiber capsules together with lids was weighed. Approximately 0.60g of sample were weighted, then put into the fiber capsules. An extraction vessel with 350ml of 1.25% sulphuric acid was placed on a hot plate, heated to boil. Then the capsule tray along with fiber capsules containing the samples was placed in the carousel and put on the stopper to lock the capsules in place. The extraction carousel was partially lowered into the boiling reagent sufficient to immerse the samples. Gentle boiling was carried out for 30 minute and after 5 minutes of boiling the carousel was removed from the extraction vessel and washed 3 times with fresh hot water each time. Then the extraction vessel was washed and filled with 350ml of 1.25% of sodium hydroxide on a hot plate and boiled. The same procedures as sulphuric acid was repeated. Then the capsules were dried in an oven at 130ºC for 2 hours. They were then cooled off in a desiccator and weighed. The weighted sample were placed in pre-weighed and pre-dried crucibles for ashing procedure at 600ºC for 4 hours. Then cooled off in a desiccator before reweighing to determine the crude fiber content.

The crude fiber content of the diet was calculated as follows:

$$\% \text{ Crude fiber} = \frac{W3 - (W1 \times C) - (W5 - D)}{W2} \times 100 \qquad (3.5)$$

Where W1 = initial capsule weight (g)

W2 = sample weight (g)

W3 = capsule + residue weight (g)

W4 = Empty ashing crucible (g)

W5 = total ash (g)

C = blank correction for capsule solubility

D = capsule ash (g)

Nitrogen-free extract was calculated as 100 – (%CP + % crude fiber, % crude ash, % moisture, % crude fat) (Taufek, 2016).

3.5. Organosomatic Indices.

At the end of the feeding trial, 3 fish were sacrificed from each tank for organosomatic indices studies (condition factor, hepatosomatic indices and viscerosomatic indices). For the CF analysis, the fish live body weight and body length (cm) were recorded and calculated using below formula as standard. For determination of HSI, the fish were dissected, and the weight of liver and final body weight were recorded. Finally, for the VSI, the weight of whole visceral organs and final body weight were collected and calculated according to the standard given below: (Xiao et al., 2019)

1. Condition Factor = [body weight (g)/ (body length (cm))3] /$cf = w \times 100 \div L3$
2. Histosomatic index (%) = (Wet weight of liver (g)/Final weight of fish (g)X100)
3. Viscerosomatic index (%) = (viscera weight (g)/whole body weight (g) × 100)

3.6. Hematological indices.

After 42 days of the feeding trial, the fish were fasted for 24 hours prior to harvested. Blood was randomly collected from 7 fish in each tank through caudal vein puncture using 1ml syringe and 24G needles for the determination of hematological parameters. Approximately 2-3ml of blood was collected from the fish through caudal puncture and pooled together before transferring into a serum heparinized blood collection tube coated with clot activator gel for determination of serum total protein. Samples were analyzed using ADVIA 2400 clinical chemistry. Another set of blood was transferred into vacutainer blood collection tube containing EDTA (Ethylenediaminetetraacetic acid) for

hematological analysis of hemoglobin, red blood cell count, hematocrit/packed cell volume, mean corpuscular volume, mean corpuscular volume hemoglobin concentration and white blood cell count using SYSMEX XN series machine (Sysmex Kobe Japan, 1988) method.

3.7. Data analysis.

All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 22.0. The difference between means was compared by Duncan's post hoc test at 5% ($P<0.05$) probability level. Data are presented as means ± standard error of the mean.

CHAPTER 4: RESULT

4.1. Effect of mycelial biomass of *Ganoderma lucidum* on red hybrid tilapia body composition.

The fillet nutritional composition of fish fed with a dietary supplement of MBGL was summarized in Table 4.1. During the feeding trial, it was observed that tilapia body protein increases with an increase in the dietary supplement. However, fish fed with the highest diet (15g/kg) accumulated more protein (87.61) $P<0.05$ compared to control and other treatment groups. An increase in dietary supplements did not influence the body's lipid content and the ash content of the fish. The body moisture content appears to be relatively the same between control, 5g/kg diet, and 10g/kg diet. However, the moisture content is slightly higher in the 15g/kg diet (table 4.1). The energy content of the body showed no significant difference between the treatments and the control group ($P>0.05$).

Table 4. 1: Nutritional composition of fish fillet fed with experimental diets.

Nutrient (g/100g)	Control	5g/kg	10g/kg	15g/kg
Protein	81.86 ± 1.73^{b}	86.76 ± 0.49^{a}	86.77 ± 1.86^{a}	87.61 ± 2.03^{a}
Lipid	1.44 ± 0.00^{c}	1.45 ± 0.03^{c}	1.52 ± 0.02^{b}	2.05 ± 0.00^{a}
Ash	6.67 ± 0.22^{ab}	6.92 ± 0.08^{a}	6.34 ± 0.23^{b}	6.68 ± 0.08^{ab}
Carbohydrate	10.00 ± 00^{a}	4.21 ± 00^{c}	5.15 ± 00^{b}	2.92 ± 00^{d}
Fibre	0.03 ± 00^{c}	0.66 ± 02^{c}	0.22 ± 00^{c}	0.74 ± 00^{c}
Dry matter	95.27 ± 0.36^{a}	94.94 ± 0.23^{ab}	95.62 ± 0.03^{a}	94.62 ± 0.06^{b}
Moisture	4.73 ± 0.37^{ab}	4.80 ± 0.21^{ab}	4.38 ± 0.03^{b}	5.38 ± 0.06^{a}
Energy (kJ/g)	17.57 ± 00^{a}	17.63 ± 00^{a}	17.80 ± 00^{a}	17.82 ± 00^{a}

[1]The result above represent mean ± SEM of three (3) fish per tank with total of 6 fish per treatment
[2]Mean value between each treatment with different superscript are significantly different ($P<0.05$).
[3]All values were expressed in (g/100g), except for energy expressed as (kJ/g).

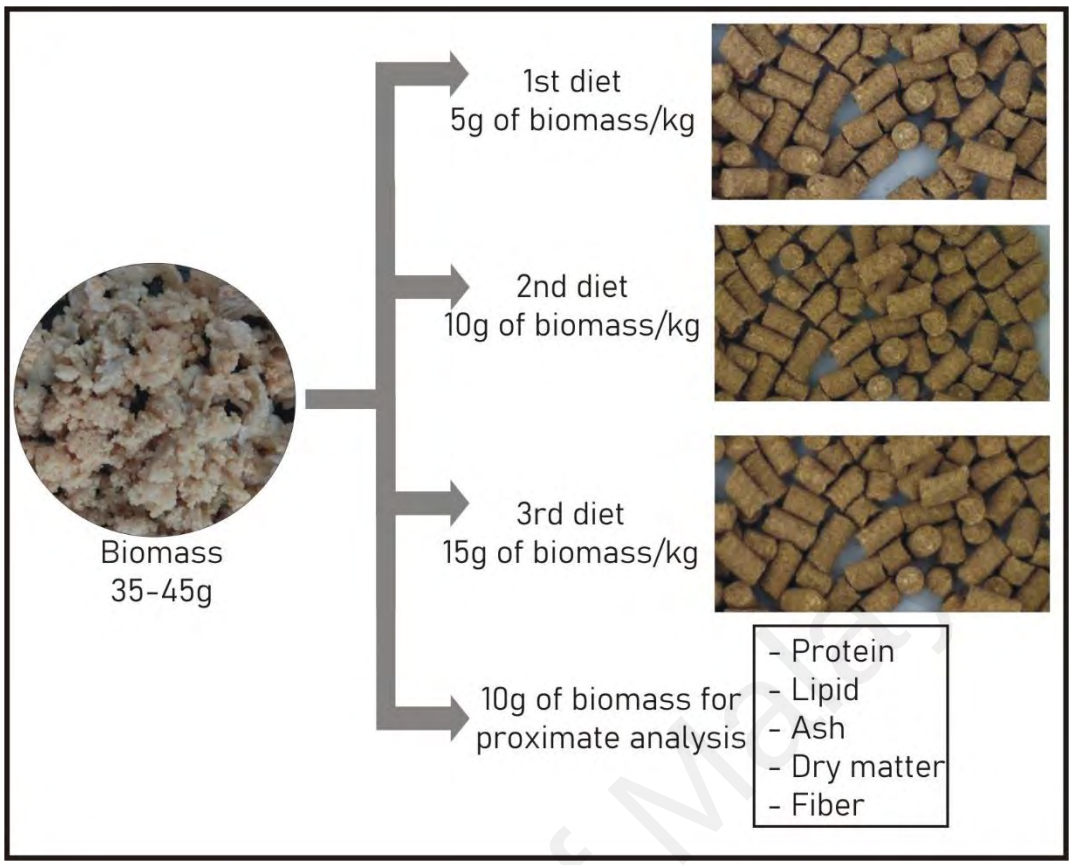

Figure 4.1.Total mycelial biomass of *Ganoderma lucidum* produced (dry weight).

4.2. **Effect of mycelial biomass of *Ganoderma lucidum* on red hybrid tilapia organosomatic indices.**

Table 4.2. represents the organosomatic indices of the hybrid tilapia at the end of the 6-weeks of feeding trial. During the feeding trial, the experimental feed was well accepted by the fish. The condition factor recorded shows that there are no significant differences between the treatment and the control ($P > 0.05$). However, the CF of all treatments are slightly higher compared to the control (Table 4.2). The hepatosomatic index showed a significant increase in all treatments ($P<0.05$) highest value was recorded in 5g/kg diet (2.51±0.50), while the same value was recorded for 10g/kg diet and 15g/kg diet (2.47±0.21) compared to control (1.97±0.06). Viscerosomatic index also showed a significant increase with an increase in the dietary supplements. The groups fed with 5g/kg and 10g/kg show significantly higher VSI (11.34 and 13.62 respectively) compared to other diets.

Table 4. 2: Organosomatic indices of fish fed the experimental diets.

Diet	Condition Factor	Hepatosomatic index	Vicerosomatic index
Control	1.60±0.08[a]	1.97±0.06[b]	8.00±1.55[a]
5g/kg	1.62±0.04[a]	2.51±0.50[a]	11.34±0.29[b]
10g/kg	1.61±0.04[a]	2.47±0.21[a]	13.62±1.15[b]
15g/kg	1.85±0.26[a]	2.47±0.30[a]	11.06±0.91[ab]

[1]The result above represent mean ± SEM of three (3) fish per tank with a total of 6 fish per treatment
[2]Mean value between each treatment with different superscripts are significantly different (P<0.05).

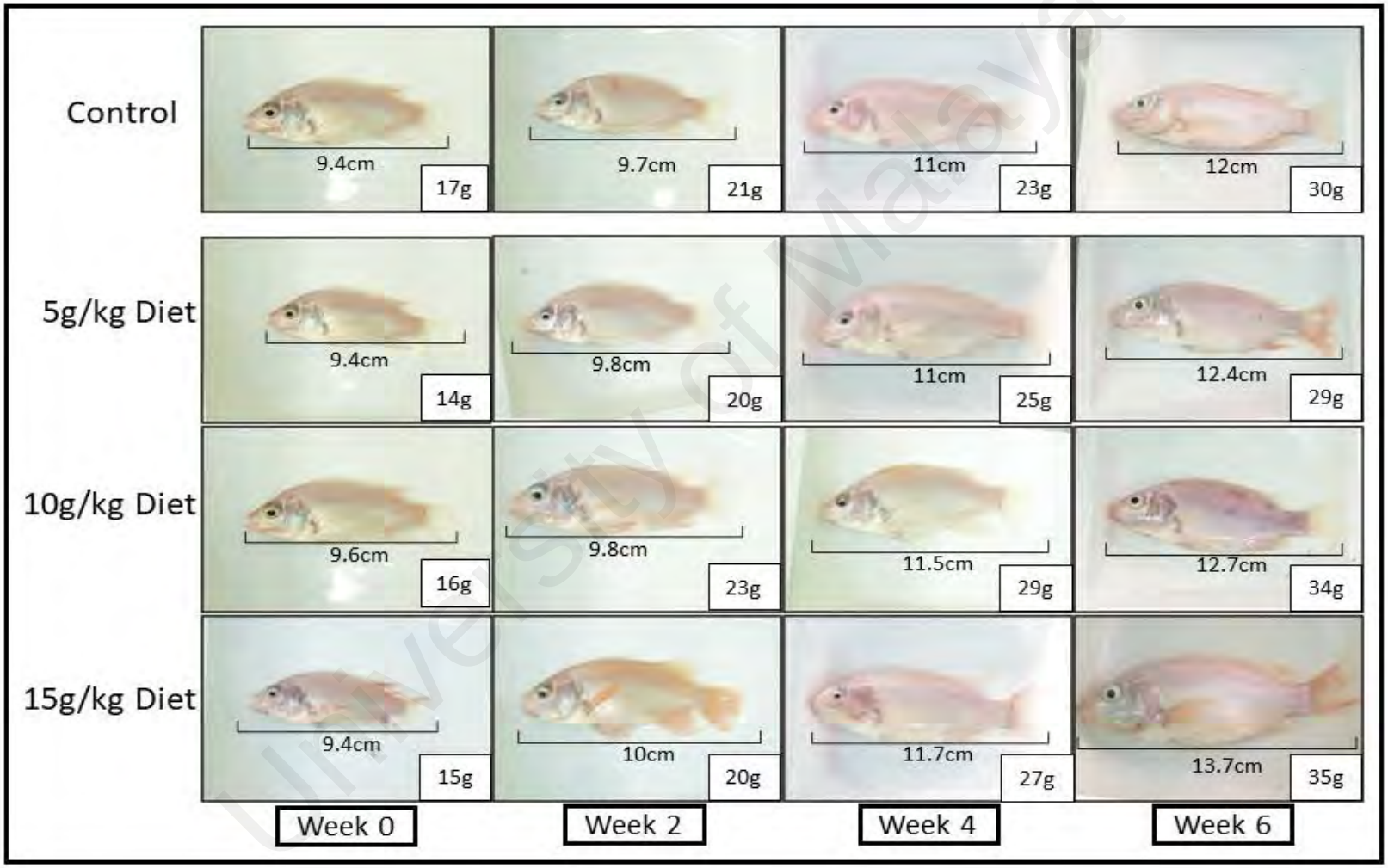

Figure 4.2. Weight and the total length of fish every two weeks for a duration of 6 weeks feeding trial.

4.3. **Effect of mycelial biomass of *Ganoderma lucidum* on red hybrid tilapia hematological indices.**

Table 4.3. represents the hematological parameters of hybrid tilapia after six weeks of feeding trial. Hemoglobin (HGB) concentration significantly increases with an increased in dietary supplement up to 5g/kg and 10g/kg diet (6.43±0.09, 6.23±0.33 g/dl) and decreases as the concentration increase up to 15g/kg (5.58±0.11 g/dl) compared to control (5.75±0.35) . Packed Cell Volume (PCV) does not shows significant different in between treatments (*P<0.05*) 5g/kg diet (35.00±0.58 %), 10g/kg diet (33.50±0.58 %), and 15g/kg diet (31.50±2.06 %). However, it is significantly higher than control (30.00±1.73 %). On the other hand, red blood cell count (RBC) shows no significant difference across all the diets. Mean corpuscular volume (MCV) was significantly higher in 10g/kg diet. There was no significant difference between the treatment and control (P>0.05) in mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH). White blood cell counts significantly increase (*P<0.05*) with an increase in dietary supplements. Highest WBC were recorded from 5g/kg diet (164.30±2.79), 15g/kg diet (161.85±22.10) and 10g/kg diet (143.13±13.27) compared to control (133.88±13.92). All the values reported here are within the normal range of healthy tilapia (Hrubec et al., 2000).

The total serum protein in the group fed with 10g/kg diet is slightly higher (3.40±0.23) compared to control (3.20±0.06), 5g/kg diet and 15g/kg diet showed a slight decrease (3.16±0.07, 3.10±0.04) compared to control. However, there is no significant difference between all groups (P>0.05). Interestingly all values are within a reasonable range of healthy hybrid tilapia (2.3-3.6 g/dl) (Hrubec et al., 2000).

Based on the fish body nutrient, organosomatic indices and hematological indices, this study suggests that the inclusion of 10g/kg diet is sufficient to improve red hybrid tilapia general wellbeing.

Table 4. 3: Hematological indices of red hybrid tilapia fed with the experimental diets.

Parameters	Control	5g/kg	10g/kg	15g/kg
HGB (g/dl)	5.75±0.35[ab]	6.43±0.09[a]	6.23±0.33[a]	5.58±0.11[ab]
PCV/HTC (%)	30.00±1.73[ab]	35.00±0.58[a]	33.50±0.58[a]	31.50±2.06[a]
RBC (10^{12}/L)	2.04±0.14[b]	2.47±0.04[a]	2.15±0.17[b]	2.08±0.13[ab]
MCV (Fleurence)	148±1.73[b]	141.25±0.25[b]	155.50±3.18[a]	149.25±1.31[b]
MCH (pg)	28.20±0.34[a]	26.05±0.38[a]	28.78±1.02[a]	26.65±1.54[a]
MCHC (%)	19.08±0.11[a]	18.45±0.26[a]	18.53±0.36[a]	17.83±0.89[a]
WBC (10^9/L)	133.88±13.92[b]	164.30±2.79[a]	143.13±13.27[b]	161.85±22.10[a]
SERUM TOTAL PROTEIN(g/L)	3.20±0.06[a]	3.10±0.04[a]	3.40±0.23[a]	3.16±0.07[a]

[1]The result presented above as mean ± SEM of seven (7) fishes/tank in duplicate group.
[2]Mean value in the same row with different superscript are significantly different ($P<0.05$).
[3]HGB (hemoglobin), PVC (packed cell volume), HTC (hematocrit), RBC (red blood cell), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), WBC (white blood cell).

CHAPTER 5: DISCUSSION

Based on the nutritional composition of the mycelial biomass of *Ganoderma lucidum*, it was found to contain 32.23% protein, 13.80% fiber, 48.38% carbohydrate and 4.45% lipid content. Following the feeding trial, it was observed that the supplementation of MBGL into tilapia feed could improve the fish body composition, organosomatic indices, as well as hematological indices of the fish. Mycelial biomass of *Ganoderma lucidum* can be used as feed additives in tilapia feed because tilapia tolerate high dietary fiber and carbohydrate than other types of fish cultured (Mjoun et al., 2010). Hence, they can well digest and assimilate the nutrient into flesh (Mehana et al., 2015).

5.1. Effect of mycelial biomass of *Ganoderma lucidum* on red hybrid tilapia body composition.

The nutritional composition of fillet remains a vital parameter of concern in fish nutrition studies due to their imaging importance association with product quality. The fillet protein of the hybrid tilapia might be affected by an increased in the dietary supplement at each phase of the experiment. This indicates that protein synthesis is presumably promoted due to the supplement (Van Norren et al., 2009; Xiao et al., 2019). The result from this study correspond with previous studies that use the mushroom as a dietary supplement on tilapia fingerlings. This, in turn, has increased fillet protein with an increase in the dietary supplement (Din et al., 2012). An increase in protein content of fishes is due to the presence of considerable amount of a protein in a diet (Muttharasi et al., 2019). Several dietary supplements significantly increase body protein and this was studied by Obasa et al., (2011) and Sotolu, (2009). It has been reported that commonly used dietary supplements effectively improved the nutritional value of fish meat (Godoy et al., 2019). On the other hand, the ash content of the fillet did not vary considerably between the treatment and control concerning their nutritional intake.

The lipid content of mycelial biomass provides energy, phospholipids, fatty acid, sterol and fat-soluble and vitamins to tilapia, and it also help in maintaining structural components of a cell membrane in fish (Wong et al., 2012). An increase in lipid was observed in 15g/kg diet. The increase in the lipid content of this group might be due to the role of MBGL. This increase in lipid deposition was similar to previous studies that use lysine as a dietary supplement for Nile tilapia (Michelato et al., 2016). However, there are no differences between the remaining treatments and control. The relationship between tilapia body lipid and carbohydrate intake in feed has been reported, and optimal dietary protein in aquaculture was studied by Jiang et al., (2016); Nwanna, (2016); Ogunji et al., (2000); Rahimnejad et al., (2015); J. Wang et al., (2016).

The carbohydrate content was calculated by subtraction and appear to be higher in control compared to the treatment due to the lower content of total protein. Moisture and dry matter were not affected by the dietary supplement both between and within the treatment group. The energy content of the body showed isocaloric energy retention. There is no significant difference between the treatments and control. The energy content recorded in this study is comparable to that of De Silva et al., (1989) who recorded 17kJ/g energy content in tilapia and Muttharasi et al., (2019) who recorded 16kJ/g in *Cyprinus carpio*. However, this is higher than studies by Hua et al., (2019) who recorded 7.0kJ/g in Nile tilapia after supplementation of 1.3% lysine for 12 weeks. This reveal that optimum protein-energy ration significantly varies between each species (Ogunji et al., 2000). A greater proportion of energy mostly used in metabolism and less amount used for growth (Azevedo et al., 2004; Hua et al., 2019).

Interestingly, throughout this feeding trial (42 days), no mortality was observed (10% survival) which might be due to the role of mycelial biomass of *GL* by stimulating the activity of the immune system. The result obtained from this study agrees with Gurusamy (2019), who recommended that feed enriched with mushroom might increase disease

tolerance capacity in fish. An increase in fish survival rate indicates adequate utilization of diet enriched with mushroom supplements and can be used as an alternative to hazardous chemotherapeutics agents in aquaculture (Chelladurai et al., 2019).

5.2. Effect of mycelial biomass of *Ganoderma lucidum* on red hybrid tilapia organosomatic indices.

Condition factor is an essential parameter for fish health, growth, and general wellbeing. It is one of the standard practices used in aquaculture and fisheries to evaluate the response of fish to dietary supplements (Anani et al., 2016). The condition factor recorded in this study showed a slight increase in all treatment compared to control. However, there is no significant difference between the control and the treatment. The result obtained shows similarities to the condition factor of fish fed with dietary supplements of other species of mushroom (*Pleurotus eryngii*) (Safari et al., 2018). Fish CF of greater than 1.0 indicates the good wellbeing of fish fed with experimental diets (Datta et al., 2013). A condition factor >1.0 indicates isometric growth and good health (Anani et al., 2016). The result of this study shows conformity with the findings of Datta et al., (2013) and Ighwela et al., (2011). According to Jimoh (2019) condition factor greater than 1.0 indicates a favorable response of fish to dietary supplement (Jimoh et al., 2019b). All condition factors recorded in this study were above 1.0. This signifies that quality feed was fed to fish throughout the feeding trial and indicate good health and wellbeing (Datta et al., 2013). Variations in condition factor may occur due to sex. However, this does not apply to this study due to mix sexes were used throughout the experiment time; other variations may occur due to water quality (Ighwela et al., 2011). In this study, all water parameters were maintained within standard limit (Jimoh et al., 2019a; Taufek et al., 2016; Winfree et al., 1981).

Similar to condition factors, hepatosomatic index and viscerosomatic index are all potential indicators for the general wellbeing of fish (Ighwela et al., 2014). In this study,

the mean values of HSI and VSI were all within the normal range, with no lower irregularities observed among the treatments. The result obtained from this study corresponds with the work of Ahmad (2012), where HSI and VSI considerably increased with an increase in the dietary supplement (M. Ahmad et al., 2012; Ighwela et al., 2014). Moreover, this result contradicted to the findings of Amoah (2008) who reported that liver increase in size with a decrease in carbohydrate level, lower than 20 % in the whole diet (Amoah et al., 2008). Hepatosomatic indices recorded in this study showed that all treatments are significantly higher compared to control. However, there is no significant difference between and within the treatments. Viscerosomatic indices showed a significant increase in all treatment compared to control. Group fed with 10g/kg diet shows a significant difference within the treatment. However, there is no significant difference between 5g/kg diet and the 15g/kg diet. Interestingly, all values recorded were within the normal range of healthy tilapia (Ighwela et al., 2014).

5.3. Effect of mycelial biomass of *Ganoderma lucidum* on red hybrid tilapia hematological indices.

Blood – is a fluid found in body circulation, which functions involved in the transportation of nutrients, respiration, thermoregulation, oxygen supply and defense. Hematological studies are progressively increasing and taken into consideration in fish nutrition studies because they can facilitate the evaluation of fish response to nutritional changes, disease, and water quality changes (Fazio, 2018; Xiao et al., 2019). Changes in hematological parameters are usually the first detectable response in relation to fish environment, and nutritional uptake (Dobšíková et al., 2013). In this study a significant increase in Hb and PCV were detected in the first and second diet (5g/kg and 10g/kg diet) compared to control ($P<0.05$). In contrast, the 15g/kg diet is 0.17g/dl lower in hemoglobin concentration compared to control. Reduction in hemoglobin in 15g/kg diet might be as a result of blood clotting that occurs during sample collection, where the sample was

recollected from the same fish for re-analysis. However, all the values are still within the normal range of healthy tilapia (Bittencourt et al., 2003).

Hemoglobin is a red blood cell protein that is responsible for carrying oxygen in circulation. In addition to that it also carries CO_2 out of the body cells to the lungs then releases to the atmosphere, low hemoglobin makes this process difficult to accomplish a variety of functions (Fazio, 2019). However, a high level of hemoglobin is an indication of polycythemia, a blood disease that makes the body producing more red blood cells causing blood to be thicker, rapid clotting leading to heart attacks and other heart-related disorders (Docan et al., 2018). An increase above the normal range of hemoglobin in fish is subjected to stress and low oxygen tension. Stress condition brings about various physiological changes and alterations of whole blood composition (Mlay et al., 2007).

The percentage of packed cell volume and hemoglobin concentration is an indicator of body oxygen transport in fish. In order to evaluate the general health status of fish, it is possible to establish and know the relationship between oxygen concentration available and total volume of a red cell count (Sebastião et al., 2011).

The concentration of Packed Cell Volume (PCV) is significantly higher in all treatments compared to control. This indicates that dietary supplements could influence on tilapia blood production. Several previous studies showed that fish fed with mushroom or herbal extract increases the WBC, RBC, HB and PCV Harikrishnan et al., (2009), (2010); Harikrishnan, Balasundaram, et al., (2011); Harikrishnan et al., (2012a); Harikrishnan, Kim, et al., (2011b); J.-S. Kim et al.,(2011). A decrease in PCV suggests loss of red blood cells, destruction of cells, failure of the bone to produce blood and infection which could cause a significant reduction in RBC, Hb and PCV (Clauss et al., 2008). Data obtained from this study corresponds with information published by Hrubec (2000), who evaluated the reference interval of healthy hybrid tilapia (hematology and

plasma biochemistry), the average packed cell volume of hybrid tilapia is at a range of (27 – 37 %) (Hrubec et al., 2000).

Red blood cell count is one of the vital blood parameters used to evaluate the total number of red blood cells. Usually expressed in (10^9/L), it is important because red blood cells contain hemoglobin that carries oxygen to the whole part of body tissues and organs (Docan et al., 2018). Recently, it has been confirmed that feed additives significantly enhanced total protein, erythorcytes (RBC) and hemoglobin concentration in fish (Ayyat et al., 2020).

The level of red blood cells obtained from this study indicates that all treatments are within the normal range, with little variations observed. However, the best result is obtained in the 5g/kg diet group with (2.47 10^{12}/L). An increase in circulating RBC is associated with reservoirs release i.e., spleen contraction or cell division in circulating cells, and it might also occur as a result of oxygen tension (Ribas et al., 2016). The result of this study shows similarities to that of Docan (2018), who worked the on uses of hematological parameters as a tool for diagnosing fish health status. Ashraf (2008) stated that the amount of oxygen received by body tissue depends on the maturity of RBC and Hb. Similar to this study, a significant increase in RBC were recorded as a result of dietary supplement of Ginseng herbs on tilapia (Goda, 2008).

Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) are used to diagnose anemia and causes of anemia (a condition where there is a low amount of red blood cells). MCV is the average size of red blood cells, MCH is the amount of hemoglobin per red blood cells, and MCHC is the amount of hemoglobin relative to the size of blood cells (per red blood cell), i.e., hemoglobin concentration. In this study, MCV proved to show an increase in trend with an increase in the dietary supplement. However, MCH and MCHC

demonstrated a slight decrease in treatment tanks compared to control although all parameters are within the normal range of healthy fish (Hrubec et al., 2000).

White blood cell (WBC), also known as leukocytes, is an essential part of the immune system, which plays a significant role in protecting the body against infectious organism from invading the body. They are usually originated from bone marrow and circulating through the bloodstream. A high increase of WBC occurs due to a variety of factors such as stress, inflammation, infection, trauma, or allergy. However, low WBC makes the body unable to confer protection against foreign invaders. Data obtained from this study indicate that WBC count in all the treatments was significantly higher compared to control, and within the normal range. This result attributed to an optimum health condition (P. Kumar et al., 2018; Xiao et al., 2019).

An increase in WBC observed from this study indicates that the fishes responded to the dietary supplement (MBGL). A dietary supplement that slightly elevates WBC values has a relation to immunostimulant modulatory activity (Kumala et al., 2018). Monitoring the WBC profile reflects a general immune system of fish (Baba et al., 2015). Several data were published indicating that natural products act as immunostimulants and increases the total WBC (Abdel-Tawwab et al., 2010; Jian et al., 2004; S. Kumar et al., 2013; Selvaraj et al., 2005). Another study, by Binaii et al. (2014) reported that an increase in WBC were noticed in juvenile beluga after supplementation of 3%, 6% and 12% of plant extract as dietary supplements. WBC obtained from this study indicates that 5g/kg and 15g/kg diets showed higher values compared to 10g/kg diet and control. This finding is very similar to that of Esin (2015) who uses mushroom (*Lentinula edodes*) as a dietary supplement on rainbow trout, whereby WBC significantly increases between 1% - 2% supplement after six-weeks of feeding trial (Baba et al., 2015).

Throughout the feeding trial there is no mortality observed; hence this agrees with Valladão (2019), who stated that "giving a dietary supplement to fish for a more

particulate period is a common strategy to prevent common challenges that may occur, in as long as it does not appear to alter the morphological and physiological appearance of the fish" (Valladão et al., 2019).

Blood parameters are an appropriate indicator of fish health. However, variations may occur due to temperature, culturing season, and nutritional intake of fish. All of the above factors can cause fluctuation of fish biochemical parameters (Shahkar et al., 2018). Serum total protein is a test used to measure the total amount of protein in the blood mainly two major groups of protein (albumin and globulin). An increased in serum total protein indicates the enhancement of mineral absorption especially protein and reflects protein synthesis in liver tissue due to increased dietary protein concentration (Norag et al., 2018). Various studies have proved that supplementation of prebiotics and probiotics into tilapia feed can significantly improve tilapia total serum protein, albumin, and globulin (Abou-El-Atta et al., 2019; Van Doan et al., 2016).

The serum total protein recorded from this study shows a slight increase with an increase in dietary protein, up to 10g/kg diet. However, 5g/kg diet and 15g/kg diet showed a slight decrease compared to control but were not negatively affected. All data provided herein indicates there is no overlap above the normal range of hybrid tilapia total serum protein, all values recorded are within the normal range of healthy tilapia (2.9-6.6g/dL) (Hrubec et al., 2000). From the proximate analysis, the feed given to the fish consists of 31 – 35% protein (Table 3.3); this suggested that the supplementation of MBGL into tilapia feed influences the serum total plasma protein and the diet was well accepted by the fish. This has proved that the supplementation of mycelial biomass of *Ganoderma lucidum* has no adverse effect on the tilapia serum protein. This finding agrees with Xiao (2019), who worked on the impact of dietary phenylalanine in hybrid tilapia. He concluded that total serum protein is influenced by the dietary supplement (Xiao et al., 2019). A similar record was reported from another study who used valine as a dietary

supplement in tilapia feed and reported that dietary supplement could effectively boost serum total protein (Rodrigues et al., 2019).

CHAPTER 6: CONCLUSION

6.1. Conclusion.

The potentiality of mycelial biomass of *GL* has been evaluated in this study after 42 days of feeding trial. The MBGL consists of a variety of bioactive compounds suitable for enhancing aquatic productivity in aquaculture, including protein 32.23%, lipid 4.45%, fiber 13.80%, ash 1.14%, and carbohydrate 48.38% (Table 3.3).

The result of the condition factor recorded in the current study indicates isometric growth in all fish. This signifies the quality of feed given to the fish throughout the feeding trial, which in turn, has improved the fish general wellbeing. Hepatosomatic index and viscerosomatic index of the fish were also significantly enhanced in 10g/kg diet group fish. This confirmed that feed absorption and feed utilization are at optimum deliberation.

The study of hematology provides accurate understanding helpful in identifying the general health condition of fish. These parameters remain as essential tools for effective and sensitive monitoring of fish health. Throughout this study, no mortality was observed. This might be due to the role of mycelial biomass of GL as feed additives, and this suggest that this supplement has no adverse effect on the fish hematological parameters.

Majority of mushrooms used in aquaculture demonstrate the effect of using fruiting bodies on improving fish health and productivity; however, in this study, we used biomass derived from the mycelium of *Ganoderma lucidum*. In addition to that, many studies indicated that the utilization of mushroom in aquafeed is influenced by many factors, including dosage, solubility, the molecular weight of the substance, and physicochemical properties. In this study the fish adequately accepted the supplementation and has no adverse effect to the fish body composition, organsomatic index as well as hematological indices. Hence the addition of 5g/kg supplementation of MBGL is sufficient to improve growth, and health status of red hybrid tilapia.

Further studies are suggested to evaluate the effect of mycelial biomass of *Ganoderma lucidum* on tilapia fertility. This is suggested as a result of the spawning that was observed at the end of feeding trial. The spawning was observed in all the treatments including control; although it was much higher in the treatment group specifically 10g/kg diet group compared to the control. Considering the age of the fish this phenomenon requires further studies to evaluate and established the cause of the spawning, it might be due to the dietary supplement or the culture condition.

A 100% survival rate was achieved in this study. There is a need to fully investigate the immune response of the fish, to enable confirmatory evaluation of the role of MBGL against phagocytic rate, serum lysozyme activity, respiratory burst activity, and Glutathione peroxidase activity. If possible, challenge test with some pathogenic organism also could be conducted to investigate the alternative complement activity and superoxide dismutase activity. Complete white blood cell counts also is suggested to evaluate the range of small and large lymphocytes, neutrophils, monocytes, eosinophils, thrombocyte-like cell (TLC) and thrombocyte

Complete plasma biochemistry profile could be further studied including albumin, globulin, creatinine, ammonia, total bilirubin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), sodium, chloride, magnesium, phosphorus, glucose, and cholesterol to evaluate how minerals are get accumulated into the body of the fish.

Finally, histological studies particularly how tissues and organs proliferation occur as a result of dietary supplement of *Ganoderma lucidum* in proportionate to growth performance could be further studied.

In conclusion, mycelial biomass of *Ganoderma lucidum* can be used as functional feed additives to improve aquatic productivity. However, the best result is anticipated upon prolong administration at a required concentration under aseptic condition. By doing this, it can enable farmers to improve growth performance, feed utilization, body composition,

biochemical effect, as well as hematology of the fish. Alternatively, mycelial biomass of *Ganoderma lucidum* can be used in commercial production to reduce mortality by enhancing the fish's immune system.

REFERENCES

Abdel-Ghany, H. M., El-Sayed, A.-F. M., Ezzat, A. A., Essa, M. A., & Helal, A. M. (2019). Dietary lipid sources affect cold tolerance of Nile tilapia (*Oreochromis niloticus*). *Journal of Thermal Biology, 79*(19), 50-55.

Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A., & Shalaby, A. M. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticu*s (L.). *Aquaculture, 298*(3-4), 267-274.

Abdel-Tawwab, M., Ahmad, M. H., Seden, M. E., & Sakr, S. F. (2010). Use of green tea, Camellia sinensis L., in practical diet for growth and protection of Nile tilapia, Oreochromis niloticus (L.), against Aeromonas hydrophila infection. *Journal of the World Aquaculture Society, 41*(10), 203-213.

Abdelkhalek, N. K., Eissa, I. A., Ahmed, E., Kilany, O. E., El-Adl, M., Dawood, M. A., . . . Abdel-Daim, M. M. (2017). Protective role of dietary spirulina platensis against diazinon-induced Oxidative damage in Nile tilapia; Oreochromis niloticus. *Environmental Toxicology and Pharmacology, 54*(17), 99-104.

Abou-El-Atta, M. E., Abdel-Tawwab, M., Abdel-Razek, N., & Abdelhakim, T. M. (2019). Effects of dietary probiotic Lactobacillus plantarum and whey protein concentrate on the productive parameters, immunity response and susceptibility of Nile tilapia, Oreochromis niloticus (L.), to Aeromonas sobria infection. *Aquaculture Nutrition*. *25*(6), 1367-1377.

Ahmad, M., Qureshi, T., & Singh, A. (2012). Effect of dietary protein, lipid and carbohydrate contents on the viscera composition and organ Indices of Cyprinus carpio communis fingerlings. *African journal of Biotechnology, 11*(33), 8361-8366.

Ahmad, M. H., & Abdel-Tawwab, M. J. A. (2011). The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. *314*(1-4), 110-114.

Ahmed, M., Abdullah, N., Shuib, A. S., & Razak, S. A. (2017). Influence of raw polysaccharide extract from mushroom stalk waste on growth and pH perturbation induced-stress in Nile tilapia, *Oreochromis niloticus*. *Aquaculture, 468*(17), 60-70.

Aly, S. M., Albutti, A. J. J. o. A. R., & Development. (2014). Antimicrobials use in aquaculture and their public health impact. *Aquaculture Reseach and Devielopment 5*(4), 2155-9546.

Amin, A., El Asely, A., Abd El-Naby, A. S., Samir, F., El-Ashram, A., Sudhakaran, R., & Dawood, M. A. O. (2019). Growth performance, intestinal histomorphology and growth-related gene expression in response to dietary Ziziphus mauritiana in Nile tilapia (Oreochromis niloticus). *Aquaculture*, 734301. Retrieved on 2019/07/10 from http:// www. sciencedirect.com/science/article/pii/ S0044848619308142. doi:https://doi.org/10.1016/j.aquaculture.2019.734301.

Amoah, A., Coyle, S. D., Webster, C. D., Durborow, R. M., Bright, L. A., & Tidwell, J. H. (2008). Effects of graded levels of carbohydrate on growth and survival of

largemouth bass, Micropterus salmoides. *Journal of the World Aquaculture Society, 39*(3), 397-405.

Anani, F., & Nunoo, F. (2016). Length-weight relationship and condition factor of Nile tilapia, Oreochromis niloticus fed farm-made and commercial tilapia diet. *International Journal of Fisheries and Aquatic Studies, 4*(5), 647-650.

Ayyat, M. S., Ayyat, A. M., Naiel, M. A., & Al-Sagheer, A. A. (2020). Reversal effects of some safe dietary supplements on lead contaminated diet induced impaired growth and associated parameters in Nile tilapia. *Aquaculture, 515*, 734-580.

Azevedo, P., Leeson, S., Cho, C., & Bureau, D. (2004). Growth, nitrogen and energy utilization of juveniles from four salmonid species: Diet, species and size effects. *Aquaculture, 234*(1-4), 393-414.

Baba, E., Uluköy, G., & Öntaş, C. (2015). Effects of feed supplemented with Lentinula edodes mushroom extract on the immune response of rainbow trout, Oncorhynchus mykiss, and disease resistance against Lactococcus garvieae. *Aquaculture, 448*, 476-482.

Bahnasawy, M. H. (2009). Effect of dietary protein levels on growth performance and body composition of monosex Nile tilapia, Oreochromis niloticus L. reared in fertilized tanks. *Pakistan Journal of Nutrition, 8*(5), 674-678.

Bharathi, S., Antony, C., Rajagopalasamy, C., Uma, A., Ahilan, B., & Aanand, S. (2019). Functional feed additives used in fish feeds. *International Journal of Fisheries and Aquaculture Studies, 7*(3), 44-52.

Bhuyain, M. A. B., Hossain, M. I., Haque, M. A., Jewel, M. A. S., Hasan, J., & Akter, S. (2019). Determination of the Proximate composition of cvailable fish feed ingredients in bangladesh. *Asian Journal of Agricultural Research, 13*, 13-19.

Bilen, S., Ünal, S., & Güvensoy, H. (2016). Effects of oyster mushroom (Pleurotus ostreatus) and nettle (Urtica dioica) methanolic extracts on immune responses and resistance to Aeromonas hydrophila in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 454*, 90-94.

Biró, J., Hancz, C., Szabó, A., & Molnár, T. (2009). Effect of sex on the fillet quality of Nile tilapia fed varying lipid sources. *Italian Journal of Animal Science, 8*(sup3), 225-227.

Bittencourt, N. d. L. R., Molinari, L. M., de Oliveira, D., de Abreu Filho, B. A., & Dias Filho, B. P. (2003). Haematological and biochemical values for Nile tilapia Oreochromis niloticus cultured in semi-intensive system. *Hemoglobin (g/dl), 10*(3.09), 6.58-15.98.

Breck, J. E. (2014). Body composition in fishes: Body size matters. *Aquaculture, 433*, 40-49.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*(1-4), 7-23.

Caipang, C. M. A., Mabuhay-Omar, J., & Gonzales-Plasus, M. M. (2019). Plant and fruit waste products as phytogenic feed additives in aquaculture. *AACL Bioflux, 12*(1), 261-268.

Chang, C.-S., Huang, S.-L., Chen, S., & Chen, S.-N. (2013). Innate immune responses and efficacy of using mushroom beta-glucan mixture (MBG) on orange-spotted grouper, Epinephelus coioides, aquaculture. *Fish & Shellfish Immunology, 35*(1), 115-125.

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., & Mylonas, C. C. (2010). Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture, 307*(1-2), 65-70.

Chelladurai, G., & Maran, B. A. V. (2019). Dietary supplementation of mushroom extract enhances growth and antioxidant levels of babylonia spirata (Mollusca: Gastropoda). *Aquaculture Reports, 15*, 100-218.

Chithra, E., Padmanaban, A., & Mohan, K. (2016). Potential use of Ganoderma lucidum polysaccharides as a feed supplement in diets on survival and growth performance of the grass carp, Ctenopharyngodon idella. *Internationalm Journal of Fisheries and Aquaculture Studies, 4*, 328-333.

Clauss, T. M., Dove, A. D., & Arnold, J. E. (2008). Hematologic disorders of fish. *Veterinary clinics of North America: Exotic Animal Practice, 11*(3), 445-462.

Craig, S., Helfrich, L. A., Kuhn, D., & Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding. *Virginia Cooperative Extension 4*(20), 420-256.

Datta, S. N., Kaur, V. I., Dhawan, A., & Jassal, G. (2013). Estimation of length-weight relationship and condition factor of spotted snakehead Channa punctata (Bloch) under different feeding regimes. *SpringerPlus, 2*(1), 436-450.

Dawood, M. A., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture, 10*(4), 950-974.

Dawood, M. A., Koshio, S., Ishikawa, M., & Yokoyama, S. (2015). Interaction effects of dietary supplementation of heat-killed Lactobacillus plantarum and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, Pagrus major. *Fish & Shellfish Immunology, 45*(1), 33-42.

Deepalakshmi, K., Mirunalini, S. J. I. J. o. P. S., & Research. (2011). Therapeutic properties and current medical usage of medicinal mushroom: Ganoderma lucidum. *International Journal of Pharmaceutical Science and Research, 2*(8), 1922-1929.

Din, A., Razak, S. A., & Sabaratnam, V. (2012). Effect of mushroom supplementation as a prebiotic compound in super worm based diet on growth performance of red tilapia fingerlings. *Sains Malaysiana, 41*(10), 1197-1203.

Dobšíková, R., Blahová, J., Mikulíková, I., Modrá, H., Prášková, E., Svobodová, Z., . . . Siwicki, A.-K. (2013). The effect of oyster mushroom β-1.3/1.6-D-glucan and oxytetracycline antibiotic on biometrical, haematological, biochemical, and immunological indices, and histopathological changes in common carp (Cyprinus carpio L.). *Fish & Shellfish Immunology, 35*(6), 1813-1823.

Docan, A., Grecu, I., & Dediu, L. (2018). Use of hematological parameters as assessment tools in fish health status. *Journal of Agroalimentary Processes and Technologies 24*(4), 317-324.

Du, J., Zhao, H., Wang, Y., Xie, H., Zhu, M., & Chen, J. (2019). Presence and environmental risk assessment of selected antibiotics in coastal water adjacent to mariculture areas in the Bohai Sea. *Ecotoxicology and Environmental Safety, 177*, 117-123.

Ebru, Y., & Cengiz, K. (2016). Feed additives in aquafeeds. *Lucrări Științifice-Universitatea de Științe Agricole și Medicină Veterinară, Seria Zootehnie, 66*, 155-160.

El-Naby, F. S. A., Naiel, M. A., Al-Sagheer, A. A., & Negm, S. S. (2019). Dietary chitosan nanoparticles enhance the growth, production performance, and immunity in Oreochromis niloticus. *Aquaculture, 501*, 82-89.

Encarnação, P. (2016). Functional feed additives in aquaculture feeds. In *Aquafeed Formulation* (pp. 217-237): Elsevier.

FAO. (2018). The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. In: FAO Rome, Italy. *Marine Policy, 100,* 332-333.

Fazio, F. (2018). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*. *500,* 237-242.

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture, 500*, 237-242.

Field, J. B., Elvehjem, C., & Juday, G. (1943). A study of the blood constituents of carp and trout. *Journal of Biological Chemistry, 148*, 261-269.

Fırat, Ö., Cogun, H. Y., Yüzereroğlu, T. A., Gök, G., Fırat, Ö., Kargin, F., & Kötemen, Y. (2011). A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus. *Fish Physiology and Biochemistry, 37*(3), 657-666.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology, 10*(1), 25-28.

Froese, R. (2006). Cube law, condition factor and weight–length relationships: History, meta-analysis and recommendations. *Journal of Applied Ichthyology, 22*(4), 241-253.

Furuya, V. R. B., Michelato, M., Salaro, A. L., Cruz, T. P. d., Urbich, A. V., Ribeiro, J. W. A. & Furuya, W. M. (2019). Mathematical models to predict growth, fillet traits, and composition of wild traíra, Hoplias malabaricus. *Revista Brasileira de Zootecnia, Aquaculture 48*, 1806-9290.

Gabriel, U. U., Akinrotimi, O. A., & Eseimokumo, F. (2011). Haematological responses of wild Nile tilapia Oreochromis niloticus after acclimation to captivity. *Jordan Journal of Biological Sciences, 147*(622), 1-6.

Goda, A. M. S. (2008). Effect of dietary Ginseng herb (Ginsana® G115) supplementation on growth, feed utilization, and hematological indices of Nile Tilapia, Oreochromis niloticus (L.), fingerlings. *Journal of the World Aquaculture Society, 39*(2), 205-214.

Godoy, A. C., Santos, O. O., Oxford, J. H., de Amorim Melo, I. W., Rodrigues, R. B., Neu, D., Ricardo V. N., & Boscolo, W. R. (2019). Soybean oil for Nile tilapia

(Oreochromis niloticus) in finishing diets: Economic, zootechnical and nutritional meat improvements. *Aquaculture, 512*, 734324.

Gomiero, L. M., Junior, G. A. V., & Naous, F. (2008). Relação peso-comprimento e fator de condição de Cichla kelberi (Perciformes, Cichlidae) introduzidos em um lago artificial no Sudeste brasileiro. *Acta Scientiarum. Biological Sciences, 30*(2), 173-178.

Grigorakis, K. (2017). Fillet proximate composition, lipid quality, yields, and organoleptic quality of Mediterranean-farmed marine fish: A review with emphasis on new species. *Critical Reviews in Food Science and Nutrition, 57*(14), 2956-2969.

Grisdale-Helland, B., Helland, S. J., & Gatlin III, D. M. (2008). The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar). *Aquaculture, 283*(1-4), 163-167.

Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2009). Effect of chemotherapy, vaccines and immunostimulants on innate immunity of goldfish infected with Aeromonas hydrophila. *Diseases of Aquatic Organisms, 88*(1), 45-54.

Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2010). Molecular studies, disease status and prophylactic measures in grouper aquaculture: Economic importance, diseases and immunology. *Aquaculture, 309*(1-4), 1-14.

Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture, 317*(1-4), 1-15.

Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2012a). Effect of Inonotus obliquus enriched diet on hematology, immune response, and disease protection in kelp grouper, Epinephelus bruneus against Vibrio harveyi. *Aquaculture, 344*, 48-53.

Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2012b). Inonotus obliquus containing diet enhances the innate immune mechanism and disease resistance in olive flounder Paralichythys olivaceus against Uronema marinum. *Fish & Shellfish Immunology, 32*(6), 1148-1154.

Harikrishnan, R., Kim, J.-S., Kim, M.-C., Balasundaram, C., & Heo, M.-S. (2011a). Hericium erinaceum enriched diets enhance the immune response in Paralichthys olivaceus and protect from Philasterides dicentrarchi infection. *Aquaculture, 318*(1-2), 48-53.

Harikrishnan, R., Kim, M.-C., Kim, J.-S., Balasundaram, C., & Heo, M.-S. (2011b). Protective effect of herbal and probiotics enriched diet on haematological and immunity status of Oplegnathus fasciatus (Temminck & Schlegel) against Edwardsiella tarda. *Fish & Shellfish Immunology, 30*(3), 886-893.

Hasan, M. R. (2010). On-farm feeding and feed management in aquaculture. *FAO Aquaculture Newsletter* (45), 45-48.

Hidalgo, M., Skalli, A., Abellán, E., Arizcun, M., & Cardenete, G. (2006). Dietary intake of probiotics and maslinic acid in juvenile dentex (Dentex dentex L.): Effects on growth performance, survival and liver proteolytic activities. *Aquaculture Nutrition, 12*(4), 256-266.

Hrubec, T. C., Cardinale, J. L., & Smith, S. A. (2000). Hematology and plasma chemistry reference intervals for cultured tilapia (Oreochromis hybrid). *Veterinary Clinical Pathology, 29*(1), 7-12.

Hua, K., Suwendi, E., & Bureau, D. P. (2019). Effect of body weight on lysine utilization efficiency in Nile Tilapia (Oreochromis niloticus). *Aquaculture, 505*, 47-53.

Ighwela, K. A., Ahmad, A. B., & Abol-Munafi, A. (2014). The selection of viscerosomatic and hepatosomatic indices for the measurement and analysis of Oreochromis niloticus condition fed with varying dietary maltose levels. *International Journal of Fauna and Biological Studies, 1*(3), 18-20.

Ighwela, K. A., Ahmed, A. B., & Abol-Munafi, A. (2011). Condition factor as an indicator of growth and feeding intensity of Nile tilapia fingerlings (Oreochromis niloticus) feed on different levels of maltose. *American-Eurasian Journal of Agricultural and Environmental Science, 11*(4), 559-563.

Ihie, I. S., Gabriel, S. S., & John, E.-I. (2018). Proximate and macro element composition of four fish species from lower river benue makurdi benue state Nigeria. *Journal of Nutrition Health Food Science, 6*(2), 1-6.

Jan, M., & Ahmed, I. (2016). Assessment of fecundity, gonadosomatic index and hepatosomatic index of snow trout, Schizothorax plagiostomus in river Lidder, from Kashmir Himalaya, India. *International Journal of Fishieries and Aquature Studies, 4*, 370-375.

Jansen, M. D., Dong, H. T., & Mohan, C. V. (2019). Tilapia lake virus: A threat to the global tilapia industry? *Reviews in Aquaculture, 11*(3), 725-739.

Jian, J., & Wu, Z. (2004). Influences of traditional Chinese medicine on non-specific immunity of Jian carp (Cyprinus carpio var. Jian). *Fish & Shellfish Immunology, 16*(2), 185-191.

Jimoh, W. A., Kamarudin, M. S., Sulaiman, M. A., & Dauda, A. B. (2019a). Assessment of prebiotic potentials in selected leaf meals of high dietary fibre on growth performance, body composition, nutrient utilization and amylase activities of a tropical commercial carp fingerlings. *Aquaculture Research, 50*(11), 3401-3411.

Jimoh, W. A., Kamarudin, M. S., Sulaiman, M. A., & Dauda, A. B. (2019b). Assessment of prebiotic potentials in selected leaf meals of high dietary fibre on growth performance, body composition, nutrient utilization and amylase activities of a tropical commercial carp fingerlings. *Aquaculture Research*, *50*(11), 3401-3411.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry, 113*(1), 9-16.

Kamilya, D., Ghosh, D., Bandyopadhyay, S., Mal, B., & Maiti, T. (2006). In vitro effects of bovine lactoferrin, mushroom glucan and Abrus agglutinin on Indian major carp, catla (Catla catla) head kidney leukocytes. *Aquaculture, 253*(1-4), 130-139.

Karimi, S., Kochinian, P., & Salati, A. (2013). The effect of sexuality on some haematological parameters of the yellowfin seabream, Acanthopagrus latus in Persian Gulf. *Iranian Journal of Veterinary Research, 14*(1), 65-68.

Katya, K., Yun, Y. h., Yun, H., Lee, J. Y., & Bai, S. C. (2016). Effects of dietary fermented by-product of mushroom, Pleurotus ostreatus, as an additive on growth, serological characteristics and nonspecific immune responses in juvenile Amur catfish, Silurus asotus. *Aquaculture Research, 47*(5), 1622-1630.

Kerry, J., NicGabhainn, S., & Smith, P. (1997). Changes in oxytetracycline resistance of intestinal microflora following oral administration of this agent to Atlantic salmon (Salmo salar L.) smolts in a marine environment. *Aquaculture, 157*(3-4), 187-195.

Kim, J.-S., Harikrishnan, R., Kim, M.-C., Jang, I.-S., Kim, D.-H., Hong, S.-H., . . . Heo, M.-S. (2011). Enhancement of Eriobotrya japonica extracts on non-specific immune response and disease resistance in kelp grouper Epinephelus bruneus against Vibrio carchariae. *Fish & Shellfish Immunology, 31*(6), 1193-1200.

Kim, M.-J., Kim, M.-C., Kim, T., Kim, K.-Y., Song, C.-B., Jeon, Y.-J., & Heo, M.-S. (2006). Effect of dietary supplementation of extracts of mushroom mycelium on survival and growth of juvenile flounder, Paralichthys olivacceus. *Journal of Aquaculture, 19*(4), 231-235.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6), 1332-1345.

Kumala, F. B., Wahjuningrum, D., & Setiawati, M. (2018). Effects of dietary algae, fungi and herb on the growth and innate immunity of Nile tilapia Oreochromis niloticus challenged with Streptococcus agalactiae. *Aquaculture, Aquarium, Conservation & Legislation, 11*(4), 1368-1377.

Kumar, P., Pal, A., Sahu, N., Jha, A. K., Kumar, N., Christina, L., & Priya, P. (2018). Dietary L-Tryptophan potentiates non-specific immunity in Labeo rohita fingerlings reared under elevated temperature. *Journal of Thermal Biology, 74*, 55-62.

Kumar, S., Raman, R., Pandey, P., Mohanty, S., Kumar, A., & Kumar, K. (2013). Effect of orally administered azadirachtin on non-specific immune parameters of goldfish Carassius auratus (Linn. 1758) and resistance against Aeromonas hydrophila. *Fish & Shellfish Immunology, 34*(2), 564-573.

Kümmerer, K. (2009). Antibiotics in the aquatic environment–a review–part II. *Chemosphere, 75*(4), 435-441.

Li, S., Li, Z., Sang, C., Zhang, J., & Chen, N. (2018). The variation of serum glucose, hepatic glycogen content and expression of glucose metabolism-related genes in hybrid grouper (female Epinephelus fuscoguttatus× male Epinephelus lanceolatus) in response to intraperitoneal insulin infusion. *Fisheries Science, 84*(4), 641-647.

Li, Z., Tran, N. T., Ji, P., Sun, Z., Wen, X., & Li, S. (2019). Effects of prebiotic mixtures on growth performance, intestinal microbiota and immune response in juvenile chu's croaker, Nibea coibor. *Fish & Shellfish Immunology, 89*, 564-573.

Liu, S., Wang, S., Cai, Y., Li, E., Ren, Z., Wu, Y., . . . Zhou, Y. (2020). Beneficial effects of a host gut-derived probiotic, Bacillus pumilus, on the growth, non-specific immune response and disease resistance of juvenile golden pompano, Trachinotus ovatus. *Aquaculture, 514*, 734446.

Liu, X., Steele, J. C., & Meng, X.-Z. J. E. p. (2017). Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Envrionmental Pollution, 223*, 161-169.

Lorenz, E. K., Barone, R. S. C., França, W. G., Sabioni, R. E., Koch, J. F. A., & Cyrino, J. E. P. (2018). Performance, hematology and immunology of Salminus brasiliensis fed diets containing swine liver hydrolysate. *Aquaculture, 483*, 46-52.

Magalhães, T., Mossolin, E. C., & Mantelatto, F. L. (2012). Gonadosomatic and hepatosomatic indexes of the freshwater shrimp Macrobrachium olfersii (Decapoda, Palaemonidae) from São Sebastião Island, Southeastern Brazil. *Pan-American Journal of Aquatic Sciences, 7*(1), 1-9.

Maina, J. N., Kavembe, G. D., Papah, M. B., Mashiteng, R., Wood, C. M., Bianchini, A., . . . Laurent, P. (2019). Sizes, condition factors and sex ratios of the scattered populations of the small cichlid fish, Alcolapia grahami, that inhabits the lagoons and sites of Lake Magadi (Kenya), one of the most extreme aquatic habitat on Earth. *Environmental Biology of Fishes, 102*(10), 1265-1280.

Martínez-Montemayor, M. M., Ling, T., Suárez-Arroyo, I. J., Ortiz-Soto, G., Santiago-Negrón, C. L., Lacourt-Ventura, Mercedes, Y. L., Anita, V., Walter, H. L., & Rivas, F. (2019). Identification of biologically active Ganoderma lucidum compounds and synthesis of improved derivatives that confer anticancer activities in vitro. *Frontiers in Pharmacology, 10*, 115-140.

Martínez, J. L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science, 321*(5887), 365-367.

Martins, M., Tavares-Dias, M., Fujimoto, R., Onaka, E., & Nomura, D. (2004). Haematological alterations of Leporinus macrocephalus (Osteichtyes: Anostomidae) naturally infected by Goezia leporini (Nematoda: Anisakidae) in fish pond. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 56*(5), 640-646.

Mazurkiewicz, J., Przybył, A., & Golski, J. (2008). Usability of Fermacto prebiotic in feeds for common carp (Cyprinus carpio L.) fry. *Nauka Przyroda Technologie, 2*(3), 15-20.

Mehana, E. E., Rahmani, A. H., Aly, S. M., Arshad H. R. (2015). Immunostimulants and fish culture. *Annual Research and Review in Biology*. 5(6), 477-489.

Michelato, M., de Oliveira Vidal, L. V., Xavier, T. O., de Moura, L. B., de Almeida, F. L. A., Pedrosa, V. B., Furuya, W. M., Wilson M. (2016). Dietary lysine requirement to enhance muscle development and fillet yield of finishing Nile tilapia. *Aquaculture, 457*, 124-130.

Mjoun, K., Rosentrater, K., & Brown, M. L. (2010). Tilapia: Environmental biology and nutritional requirements. *Environmental Biology and Nutritional Requirements. 10*(02), 164-174.

Mlay, P., Seth, M., Balthazary, S., Chiunda, R., Phiri, E., & Balemba, O. (2007). Total plasma proteins and hemoglobin levels as affected by worm burden in freshwater fish in Morogoro, Tanzania. *Livestock Research for Rural Development, 19*(2), 19019-19027.

Mohamed, F. A., Khogali, F. A., Mohamed, A. H., Deng, O. O., & Mohammed, A. A. (2016). Body weight characteristics and chemical composition of Nile tilapia (Oreochromis niloticus) collected from three different Sudanese dams. *International Journal of Fisheries and Aquatic Studies, 4*(5), 507-510.

Mohamed, H. E., Al-Maqbaly, R., & Mansour, H. M. (2010). Proximate composition, amino acid and mineral contents of five commercial Nile fishes in Sudan. *African Journal of Food Science, 4*(10), 640-654.

Mohan, K., Padmanaban, A., Uthayakumar, V., Chandirasekar, R., Muralisankar, T., & Santhanam, P. J. A. (2016). Effect of dietary Ganoderma lucidum polysaccharides on biological and physiological responses of the giant freshwater prawn Macrobrachium rosenbergii. *Aquaculture, 464*, 42-49.

Mohanta, K., Mohanty, S., Jena, J., & Sahu, N. (2008). Optimal dietary lipid level of silver barb, Puntius gonionotus fingerlings in relation to growth, nutrient retention and digestibility, muscle nucleic acid content and digestive enzyme activity. *Aquaculture Nutrition, 14*(4), 350-359.

Mohanty, B. P., Mahanty, A., Ganguly, S., Mitra, T., Karunakaran, D., & Anandan, R. (2017). Nutritional composition of food fishes and their importance in providing food and nutritional security. *Food Chemistry*, *293,* 561-570.

Morrison, A. (2017). Chapter no. 5 hepatosomatic index and gonadosomatic index. *docplayer*. Retrieved on 24/10/2019 from https://docplayer.net/33390250-Chapter-no-5-hepatosomatic-index-and-gonadosomatic-index.html.

Moslen, M., & Miebaka, C. (2017). Length-weight relationship and condition factor of Mugil cephalus and Oreochromis niloticus from a Tidal creek in the Niger Delta, Nigeria. *Archives of Agriculture and Environmental Science, 2*(4), 287-292.

Motlagh, S. P., Zarejabad, A. M., Nasrabadi, R. G., Ahmadifar, E., & Molaee, M. (2012). Haematology, morphology and blood cells characteristics of male and female Siamese fighting fish (Betta splendens). *Comparative Clinical Pathology, 21*(1), 15-21.

Mubarik, M. S., Asad, F., Zahoor, M. K., Abid, A., Ali, T., Yaqub, S., & Samina Q. (2019). Study on survival, growth, haematology and body composition of Cyprinus carpio under different acute and chronic salinity regimes. *Saudi Journal of Biological Sciences, 26*(5), 999-1002.

Murad, A., Everill, S., & Houston, A. (1993). Division of goldfish erythrocytes in circulation. *Canadian Journal of Zoology, 71*(11), 2190-2198.

Muttharasi, C., Muralisankar, T., Uthayakumar, V., Gayathri, V., Thangal, S., & Anandhan, K. (2019). Utilization of marine fisheries wastes for the production of the freshwater fish Cyprinus carpio. *Tropical Animal Health and Production*, *51*(19), 2305-2313.

Naeem, M., Ishtiaq, A., & Naz, S. (2017). Influence of dietary protein on proximate composition of mori, cirrhinusmrigala (hamilton). *Matrix Science Pharmaceutical, 1*(2), 1-5.

Nakajima, T., Suzuki, M., Harada, K., Inoue, M., & Mitsuhashi, S. (1983). Transmission of R plasmids in Vibrio anguillarum to Vibrio cholerae. *Microbiology and Immunology, 27*(2), 195-198.

Natarajan, M. (2006). Analysis of shrimp/fish feeds for proximate composition. *Training manual on shrimp and fish nutrition and feed management. CIBA Special Publication*, 104-109.

Navarrete, P., Mardones, P., Opazo, R., Espejo, R., & Romero, J. (2008). Oxytetracycline treatment reduces bacterial diversity of intestinal microbiota of Atlantic salmon. *Journal of Aquatic Animal Health, 20*(3), 177-183.

Navarrete, P., Toledo, I., Mardones, P., Opazo, R., Espejo, R., & Romero, J. (2010). Effect of Thymus vulgaris essential oil on intestinal bacterial microbiota of rainbow trout, Oncorhynchus mykiss (Walbaum) and bacterial isolates. *Aquaculture Research, 41*(10), e667-e678.

Ni, M., Wen, H., Li, J., Chi, M., Bu, Y., Ren, Y., & Ding, H. (2016). Effects of stocking density on mortality, growth and physiology of juvenile Amur sturgeon (Acipenser schrenckii). *Aquaculture Research, 47*(5), 1596-1604.

Norag, M. A. A., El-Shenawy, A. M., Fadl, S. E., Abdo, W. S., Gad, D. M., Rashed, M. A., & Prince, A. M. (2018). Effect of phytase enzyme on growth performance, serum biochemical alteration, immune response and gene expression in Nile tilapia. *Fish & Shellfish Immunology, 80*, 97-108.

Norousta, R., & Mousavi-Sabet, H. (2013). Comparative characterization of blood cells and hematological parameters between the mature and immature Caspian Vimba, Vimba vimba persa (Teleostei, Cyprinidae). *Aquaculture, Aquarium, Conservation & Legislation, 6*(3), 232-240.

Obasa, S., Akinyemi, A., Ogundijo, O., & Alade, O. (2011). Use of fish waste meal as a replacement for fish meal in the practical diets of African mud catfish Clarias gariepinus fingerlings. *Journal of Agricultural Science and Environment, 11*(1), 68-77.

Ogunji, J. O., & Wirth, M. (2000). Effect of dietary protein content and sources on growth, food conversion and body composition of tilapia Oreochromis niloticus fingerling fed fish meal diet. *Journal of Aquaculture in the Tropics, 15*(4), 381-389.

Öner, M., Atli, G., & Canli, M. (2008). Changes in serum biochemical parameters of freshwater fish Oreochromis niloticus following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. *An International Journal of Environmental Toxicology and Chemistry, 27*(2), 360-366.

Panserat, S. (2009). Nutritional regulation of gene expression for proteins involved in metabolism in cultured fish: focus on dietary carbohydrates. *Current Status of Molecular Research in Aquaculture*, *294*(1-2), 123-131.

Pauly, D., & Zeller, D. (2017). Comments on FAOs state of world fisheries and aquaculture (SOFIA 2016). *Marine Policy, 77*, 176-181.

Pula, H. J., Trenzado, C. E., García-Mesa, S., Fallola, C., & Sanz, A. (2018). Effects of different culture systems on growth, immune status, and other physiological parameters of tench (Tinca tinca). *Aquaculture, 485*, 101-110.

Rambhaskar, B., & Srinivasa Rao, K. (1987). Comparative haematology of ten species of marine fish from Visakhapatnam Coast. *Journal of Fish Biology, 30*(1), 59-66.

Rathore, S. S., & Yusufzai, S. I. (2018). Changes in haematological and serum biochemical indices of Nile tilapia (Oreochromis niloticus) fry fed dietary shrimp head meal. *Journal of Entomology and Zoology Studies, 6*(4), 663-667.

Ribas, J. L. C., Zampronio, A. R., & Silva de Assis, H. C. (2016). Effects of trophic exposure to diclofenac and dexamethasone on hematological parameters and

immune response in freshwater fish. *Environmental Toxicology and Chemistry, 35*(4), 975-982.

Ringø, E. (2020). Probiotics in shellfish aquaculture. *Aquaculture and Fisheries*, 5(1), 1-27.

Roberson, B. S. (1990). Bacterial agglutination. *Techniques in Fish Immunology*, 81-86.

Rodrigues, R. B., Hassemer, M. Z., Melo, I. W., Neu, D. H., Bittencourt, F., & Boscolo, W. R. (2019). Valine in diets for juvenile Nile tilapia (Oreochromis niloticus): growth performance, chemical composition, blood parameters and skeletal muscle development. *Spanish Journal of Agricultural Research, 17*(2), 602-611.

Rola, W. R., & Hasan, M. R. (2007). Economics of aquaculture feeding practices: a synthesis of case studies undertaken in six Asian countries. *FAO Fisheries Technical Paper, 505*, 1-31.

Romero, J., Feijoó, C. G., & Navarrete, P. (2012). Antibiotics in aquaculture–use, abuse and alternatives. *Health and Environment in Aquaculture, 159-199*.

Safari, O., & Sarkheil, M. (2018). Dietary administration of eryngii mushroom (Pleurotus eryngii) powder on haemato-immunological responses, bactericidal activity of skin mucus and growth performance of koi carp fingerlings (*Cyprinus carpio koi*). *Fish & Shellfish Immunology, 80*, 505-513.

Saha, N., Ullah, M. R., Islam, M. S., & Hossain, M. B. (2019). Morphometric relationships between length-weight and length-length and condition factor of four small indigenous fishes from the Payra River, southern Bangladesh. *Archives of Agriculture and Environmental Science, 4*(2), 230-234.

Sahu, B. B., Barik, N. K., Routray, P., Agnibesh, A., Paikaray, A., Mohapatra, S., & Sundaray, J. K. (2017). Comparative studies on carcass characteristics of marketable size farmed tilapia (Oreochromis niloticus) and silver barb (Puntius gonionotus). *International Journal of Fisheries and Aquatic Studies, 5*, 272-277.

Sebastião, F., Nomura, D., Sakabe, R., & Pilarski, F. (2011). Hematology and productive performance of nile tilapia (Oreochromis niloticus) naturally infected with Flavobacterium columnare. *Brazilian Journal of Microbiology, 42*(1), 282-289.

Selvaraj, V., Sampath, K., & Sekar, V. (2005). Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (Cyprinus carpio) infected with Aeromonas hydrophila. *Fish & Shellfish Immunology, 19*(4), 293-306.

Shahkar, E., Hamidoghli, A., Yun, H., Kim, D.-J., & Bai, S. C. (2018). Effects of dietary vitamin E on hematology, tissue α-tocopherol concentration and non-specific immune responses of Japanese eel, Anguilla japonica. *Aquaculture, 484*, 51-57.

Shamaki, B. U., Sandabe, U. K., Fanna, I. A., Adamu, O. O., Geidam, Y. A., Umar, I. I., & Adamu, M. S. (2012). Proximate composition, phytochemical and elemental analysis of some organic solvent extract of the wild mushroom Ganoderma lucidum. *Journal of Natural Sciences Research, 2*(4), 24-35.

Siddiqui, A. Q., Howlader, M. S., & Adam, A. A. (1988). Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, Oreochromis niloticus. *Aquaculture, 70*(1-2), 63-73.

Singdevsachan, S. K., Auroshree, P., Mishra, J., Baliyarsingh, B., Tayung, K., & Thatoi, H. (2016). Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: A review. *Bioactive Carbohydrates and Dietary Fibre, 7*(1), 1-14.

Singh, S., & Srivastava, A. (2017). Variations in hepatosomatic index (HSI) and gonadosomatic index (GSI) in fish heteropneustes fossilis exposed to higher sub-lethal concentration to arsenic and copper. *Journal of Ecophysiology and Occupational Health, 15*(3-4), 89-93.

Sogbesan, O., Ahmed, Y., & Ajijola, K. (2017). Growth performance, nutrient utilization, Somatic indices and cost benefit analyses of african basil leaf additive diets on clarias gariepinus (Burchell, 1822) fingerlings. *Journal of Animal Resources Nutrtion 34*(2), 167-173.

Sørensen, M., Nguyen, G., Storebakken, T., & Øverland, M. (2010). Starch source, screw configuration and injection of steam into the barrel affect the physical quality of extruded fish feed. *Aquaculture Research, 41*(3), 419-432.

Sotolu, A. (2009). Comparative utilizations of fish waste meal with imported fishmeal by African catfish (Clarias gariepinus). *American-Eurasian Journal of Scientific Research, 4*(4), 285-289.

Supramani, S., Ahmad, R., Ilham, Z., Annuar, M. S. M., Klaus, A., & Wan-Mohtar, W. A. A. Q. (2019). Optimisation of biomass, exopolysaccharide and intracellular polysaccharide production from the mycelium of an identified Ganoderma lucidum strain QRS 5120 using response surface methodology. *AIMS Microbiology, 5*(1), 19-38.

Taufek. (2016). The effect of dietary cricket meal (Gryllus bimaculatus) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (Clarias gariepinus). *42*(4), 1143-1155.

Taufek, Muin, H., Raji, A. A., Razak, S. A., Yusof, H. M., & Alias, Z. J. J. o. t. W. A. S. (2016). Apparent digestibility coefficients and amino acid availability of cricket meal, Gryllus bimaculatus, and fishmeal in African catfish, Clarias gariepinus, Diet. *Journal of the World Aquaculure Society, 47*(6), 798-805.

Taufek, N. M., Muin, H., Raji, A. A., Razak, S. A., Yusof, H. M., & Alias, Z. (2016). Apparent digestibility coefficients and amino acid availability of cricket meal, Gryllus bimaculatus, and fishmeal in African catfish, Clarias gariepinus, diet. *Journal of the World Aquaculture Society, 47*(6), 798-805.

Teame, T., Natarajan, P., & Tesfay, Z. (2016). Proximate and mineral composition of some commercially important fish species of tekeze reservoir and lake Hashenge, Ethiopia. *International Journal of Fisheris Aquaculture Studies, 4*, 160-164.

Thatoi, H., & Singdevsachan, S. K. (2014). Diversity, nutritional composition and medicinal potential of Indian mushrooms: A review. *African Journal of Biotechnology, 13*(4), 523-545.

Tiengtam, N., Khempaka, S., Paengkoum, P., & Boonanuntanasarn, S. (2015). Effects of inulin and Jerusalem artichoke (Helianthus tuberosus) as prebiotic ingredients in the diet of juvenile Nile tilapia (Oreochromis niloticus). *Animal Feed Science and Technology, 207*, 120-129.

Ulziijargal, E., & Mau, J.-L. J. I. j. o. m. m. (2011). Nutrient compositions of culinary-medicinal mushroom fruiting bodies and mycelia. *International Journal of Medicine 13*(4), 343-349.

Valladão, G. M. R., Gallani, S. U., Kotzent, S., Assane, I. M., & Pilarski, F. (2019). Effects of dietary thyme essential oil on hemato-immunological indices, intestinal morphology, and microbiota of Nile tilapia. *Aquaculture International, 27*(2), 399-411.

Van Doan, H., Doolgindachbaporn, S., & Suksri, A. (2016). Effects of Eryngii mushroom (Pleurotus eryngii) and Lactobacillus plantarum on growth performance, immunity and disease resistance of Pangasius catfish (Pangasius bocourti, Sauvage 1880). *Fish Physiology and Biochemistry, 42*(5), 1427-1440.

Van Doan, H., Hoseinifar, S. H., Chitmanat, C., Jaturasitha, S., Paolucci, M., Ashouri, G., . . . Esteban, M. Á. (2019). The effects of Thai ginseng, Boesenbergia rotunda powder on mucosal and serum immunity, disease resistance, and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Aquaculture, 513*, 734-388.

Van Doan, H., Hoseinifar, S. H., Esteban, M. Á., Dadar, M., & Thu, T. T. N. (2019). Mushrooms, Seaweed, and Their Derivatives as Functional Feed Additives for Aquaculture: An Updated View. In *Studies in Natural Products Chemistry* (Vol. 62, pp. 41-90): Elsevier.

Van Norren, K., Kegler, D., Argiles, J., Luiking, Y., Gorselink, M., Laviano, A., . . . Van Der Beek, E. (2009). Dietary supplementation with a specific combination of high protein, leucine, and fish oil improves muscle function and daily activity in tumour-bearing cachectic mice. *British Journal of Cancer, 100*(5), 713.

Wan, W. A. A. Q. I., Latif, N. A., Harvey, L. M., McNeil, B. J. B., & biotechnology, a. (2016). Production of exopolysaccharide by Ganoderma lucidum in a repeated-batch fermentation. *Biocatalysis and Agricultural Biotechnology 6*, 91-101.

Weinert, N. C., Volpato, J., Costa, Á., Antunes, R. R., Camargo de Oliveira, A., Scabelo Mattoso, C. R., & Saito, M. E. (2015). Hematology of Nile tilapia (Oreochromis niloticus) subjected to anesthesia and anticoagulation protocols. *Semina: Ciências Agrárias, 36*(2) 19829

Winfree, R. A., & Stickney, R. R. (1981). Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. *The Journal of Nutrition, 111*(6), 1001-1012.

Wong, W. H., Cheung, S. G., & Shin, P. K. (2012). Lipid content and fatty acid composition of the clam rangia cuneata (GB Sowerby, 1831) in upper barataria estuarine, louisiana. *Journal of Life Science, 6*, 411-417.

Xiao, W., Zou, Z., Li, D., Zhu, J., Yue, Y., & Yang, H. (2019). Effect of dietary phenylalanine level on growth performance, body composition, and biochemical parameters in plasma of juvenile hybrid tilapia, Oreochromis niloticus× Oreochromis aureus. *Journal of the World Aquaculture Society 51*(2), 437-451.

Yıldız, M. (2004). The study of fillet quality and the growth performance of rainbow trout (Oncorhynchus mykiss) fed with diets containing different amounts of vitamin E. *Turkish Journal of Fisheries and Aquatic Sciences, 4*(2), 81-86.

Yin, G., Ardo, L., Thompson, K., Adams, A., Jeney, Z., Jeney, G. J. F., & Immunology, S. (2009). Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. *Fish & Shellfish Immunology, 26*(1), 140-145.

Yonar, S. M., Yonar, M. E., Pala, A., Sağlam, N., & Sakin, F. (2020). Effect of trichlorfon on some haematological and biochemical changes in Cyprinus carpio: The ameliorative effect of lycopene. *Aquaculture Reports, 16*, 100-246.

Yousefi, S., Hoseinifar, S. H., Paknejad, H., & Hajimoradloo, A. (2018). The effects of dietary supplement of galactooligosaccharide on innate immunity, immune related genes expression and growth performance in zebrafish (Danio rerio). *Fish & shellfish Immunology, 73*, 192-196.

Yuji-Sado, R., Raulino-Domanski, F., de Freitas, P. F., & Baioco-Sales, F. (2015). Growth, immune status and intestinal morphology of Nile tilapia fed dietary prebiotics (mannan oligosaccharides-MOS). *Latin American Journal of Aquatic Research, 43*(5), 944-952.

Zhou, X., Li, M., Abbas, K., & Wang, W. (2009). Comparison of haematology and serum biochemistry of cultured and wild Dojo loach Misgurnus anguillicaudatus. *Fish Physiology and Biochemistry, 35*(3), 435-441.

Zhou, X., Tian, Z., Wang, Y., & Li, W. (2010). Effect of treatment with probiotics as water additives on tilapia (Oreochromis niloticus) growth performance and immune response. *Fish Physiology and Biochemistry, 36*(3), 501-509.

Zhu, L., Yang, Q., Huang, L., Wang, K., Wang, X., Chen, D., Yi, G,. Xiaoli, H., Ping, O., & Lai, W. (2017). Effectivity of oral recombinant DNA vaccine against Streptococcus agalactiae in Nile tilapia. *Developmental & Comparative Immunology, 77*, 77-87.

Zou, H. K., Hoseinifar, S. H., Miandare, H. K., & Hajimoradloo, A. (2016). Agaricus bisporus powder improved cutaneous mucosal and serum immune parameters and up-regulated intestinal cytokines gene expression in common carp (Cyprinus carpio) fingerlings. *Fish & Shellfish Immunology, 58*, 380-386.