UNIVERSITY OF CAPE COAST

EFFECTS OF GINGER AND GARLIC SUPPLEMENTS ON CULTURE PERFORMANCE OF BLACKCHIN TILAPIA (*SAROTHERODON MELANOTHERON*)

BY

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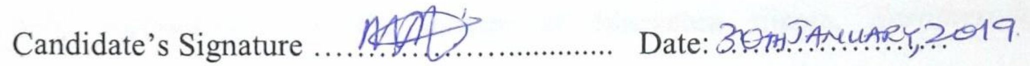
AUGUST 2018

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.



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Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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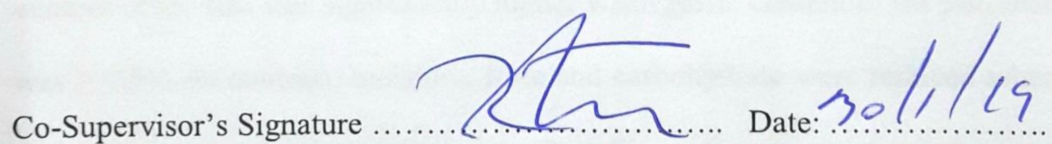

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ABSTRACT

Previous studies suggest that plant products can be used in place of synthetic therapeutic chemical agents to improve the growth, health, and meat quality of cultured fish. These effects of plant supplements are specific on different fish species. Therefore, this study investigated the potential of ginger and garlic as feed supplements for the culture of blackchin tilapia, *Sarotherodon melanotheron.* This fish is an important source of protein to many coastal communities. Powdered forms of these supplements were fed to the fish at four levels (0.5%, 1%, 1.5% and 2%) as part of a commercial feed. Ten fingerlings per m^2 (average weight = 2.74 ± 0.30 g each) were cultured for 24 weeks in hapas suspended in concrete tanks. The performance of these fingerlings was compared with those that were not fed with the supplements. Results showed that both supplements did not affect growth of the fish, its feed utilisation, blood constituents, serum biochemical composition and lysozyme activity. However, the condition index, protein, fat and mineral content of the fish were significantly higher when garlic content of the fish feed was $\geq$ 1.5%. In contrast, moisture, fibre and carbohydrate were reduced when the garlic content of the feed was $\geq$ 1.5%. Ginger, particularly at a concentration of 1.5% induced significantly higher protein and moisture whereas it decreased fat, fibre, carbohydrate and ash content of the fish. These results suggest that ginger and garlic can be used to enhance the physiological condition and nutritional quality of *S. melanotheron* under culture.

KEY WORDS

Aquaculture

Plant supplements

Sarotherodon melanotheron

Physiological condition

Growth

Serum lysozyme activity

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DEDICATION

This work is dedicated to my dear family, especially my husband–Francis, kids–Anna and Janice Odei Antwi, and my mother Agnes Lydia Acquaye.

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LIST OF ACRONYMS/ ABBREVIATIONS

AOAC: Association of Official Analytical Chemists

AGR: Absolute Growth Rate

DO: Dissolved Oxygen

EDTA: Ethylene Diamine Tetra-Acetate

ELISA: Enzyme Linked Immuno Sorbent Assay

FAO: Food and Agriculture Organization of the United Nations

FCR: Feed Conversion Ratio

FER: Feed Efficiency Ratio

FMW: Final Mean Weight

HCT: Haematocrit

HGB: Haemoglobin

ICLARM: International Centre for Living Aquatic Resources Management

MCH: Mean Corpuscular Haemoglobin

MCHC: Mean Corpuscular Haemoglobin Concentration

MCV- Mean Corpuscular Volume

MoFAD: Ministry of Fisheries and Aquaculture Development

MWG: Mean Weight Gain

NFE: Nitrogen Free Extract

PCT: Platelet Count

PWG: Percentage Weight Gain

RBC: Erythrocyte Count

SGR: Specific Growth Rate

WBC: Leucocyte Count

WHO: World Health Organization

CHAPTER ONE

INTRODUCTION

This chapter explains the background to the study, presents the research problem and its significance as well as highlights the main and specific objectives of this study.

Background to the Study

The Relevance of Aquaculture

Over the years, Aquaculture has substantially increased fish availability to humanity and contributes to satisfy the growth in fish demand, thereby relieving the pressure on wild fish stocks (World Bank, 2014; FAO, 2016). This is evident in the fish production data in which aquaculture fish production contributed 44.1% of the total production from both capture fisheries and aquaculture in 2014, which was an increment from 42.1% recorded in 2012 and 31.1% recorded in 2004 according to FAO (2016).

In Africa, aquaculture production in 2014 amounted to 1710.9 thousand tonnes representing only 2.32% of the Global aquaculture production. Nonetheless this was an improvement from 0.45% in 1995 gradually to 1.46% in 2005 and 2.23% in 2012. Egypt has consistently been the leading producer in Africa contributing 66.46% followed by Nigeria with 18.31% and the rest Sub-Saharan Africa contributing 14.24% whiles the remaining north African countries together contributed 0.99% (FAO, 2016). When the needed effort and attention is given to the industry, aquaculture in Africa is likely to grow to meet the needs of both local as well as international markets.

One of the challenges that the ever growing world faces and needs to be addressed, especially in developing countries like Ghana, is to ensure that there is both adequate food and nutrition security for all (Haraksingh et al., 2016). In Ghana, presently, there is a shortfall of about 380,000 metric tons in the domestic fisheries production needed to meet our food security requirements (MOFAD, 2016). The steady growth in aquaculture production therefore presents a reliable opportunity to contribute to food security and good nutrition for all.

However, aquaculture faces a number of challenges and disease occurrence is a major threat causing significant losses and limiting production in the sector (Subasinghe, Rohana & McGladdery 2001). When the necessary support is given for its sustainable development, aquaculture can limit its challenges and produce sufficient fish to contribute towards eradication of hunger, food insecurity and malnutrition (Haraksingh et al., 2016). In the Sub-saharan Africa, Hecht and Endemann (1998) revealed that bacterial and parasitic infections are a major setback in aquaculture production. For the sustained growth and development of aquaculture in this region, diversifying the species cultured can enable the use of various water resources yet unused for the production of food fish.

Presently, aquaculture in Ghana is mainly focused in freshwater (on the Volta lake) with little or no development in coastal aquaculture (MOFAD, 2016). This situation is primarily due to the non-availability of a developed suitable species for culture in this environment and needs to be addressed. One coastal species that can be considered is the black chin tilapia.

The Black Chin Tilapia (*Sarotherodon melanotheron*)

Tilapia are valuable species in Ghana and West Africa and the Black-chinned tilapia, *Sarotherodon melanotheron* is an important fish resource in the coastal waters of Ghana; serving as a source of protein and livelihood for some coastal dwellers (Abban, Asante & Falk, 2000). Over the years, many studies have contributed information related to its culture performance including Igonifagha, Deekae and Marioghae (1996); Legendre and Trebaol (1996); Apenuvor (2015) and Tseku (2016). The species is amenable to culture conditions in ponds, tanks, enclosures and cages (Campbell, 1987) making it a suitable candidate for coastal aquaculture. Its successful culture can therefore serve as supplementary livelihood for coastal dwellers in the face of dwindling catches from marine and coastal waters. However, culturing of the species will also come at the expense of exposing it to conditions that can make it vulnerable to diseases.

Vulnerability of Fish to Diseases in Aquaculture

As a result of the environment in which they live, fishes are exposed to a variety of infectious agents namely parasites, bacteria, fungi, and viruses that can cause disease when not controlled. Generally, fish culturists try to maintain an environment that is conducive to good fish health but culture conditions that pertain in the aquaculture set-up such as high stocking density, handling, accumulation of wastes, natural flora and fauna, sub-optimal water quality levels, etc., can pose additional stress on the culture species. Moreover, stress is known to increase fish disease susceptibility which compromises fish health and growth and thus promote disease outbreaks (Ashley, 2007; Turnbull, 2012; Nya & Austin, 2009a). Turnbull (2012) therefore concludes

that disease is not just the presence of pathogens but results from a combination of factors. Furthermore, as aquaculture keeps expanding and new species are farmed, new diseases will emerge that can adversely affect both wild and cultured fish (Murray & Peeler, 2005).

Impact of Diseases on Aquaculture

Diseases have been identified as a cause of significant economic losses to the aquaculture industry (Citarasu, 2012; Newaj-Fyzul & Austin, 2014). Moreover, when outbreaks occur, it is difficult to control and/or administer protocols for effective remedy, not to mention the high cost involved including the threat to the environment. To the farmer, diseases cause losses directly through mass mortalities and reduced growth (Ashley, 2007). This results in reduced profit or heavy losses in terms of productivity, expenditure and income. To a larger extent it affects employment, market access or shares, investment and consumer confidence, and can consequently lead to food shortages, industry failure or closure of business (Bondad-reantaso et al., 2005; Brun, Rodgers, Georgiadis & Bjondal, 2009). Fish diseases are therefore a threat to aquaculture and food security in general and efforts that can mitigate its occurrence are important.

Overview of Fish Diseases

Diseases that affect fish under culture are categorized into:

(a) Infectious diseases, which are caused by pathogenic organisms present in the environment. They are mostly contagious and treatment may be necessary to control the disease outbreak.

(b) Non-infectious diseases, which are caused by environmental problems, nutritional deficiencies, or genetic anomalies. These are not contagious and usually cannot be cured by medications. They are best prevented and controlled by provision of good water quality, adequate nutrition and good management practices.

Infectious diseases are more prevalent and grouped as parasitic, bacterial, fungal, or viral diseases.

Parasitic diseases

Parasites are responsible for a number of infectious diseases in fish. There are two forms, the opportunistic and obligate parasites. Under normal circumstances, the obligate parasite does not kill the host since its life is dependent on that of the host, thus there are many parasites in wild fish which do not pose any problems (Mesalhy Aly, 2013). However, under an intensive culture situation, the fish are abnormally stressed at the same time crowded. The closeness of one fish to another also increases the possibility of infection and mortality.

Parasitic diseases of fish are categorized into protozoan, crustacean and helminthic diseases. Crustaceans are mostly external parasites which can cause severe diseases. The protozoans on the other hand cause either external or internal diseases according to where they affect the fish. With the helminths, most monogeneans and annelids cause external diseases, while majority of digeneans cause internal diseases. Nematodes, acanthocephalans and cestodes are all associated with parasitic infestations that are internal (Mesalhy Aly, 2013).

Bacterial diseases

Bacteria are the most significant pathogens in cultured and wild finfish and shellfish worldwide (Citarasu, 2012). Most of the causative micro-organisms are naturally occurring saprophytes, utilizing the organic and mineral matter in the aquatic environment to grow and multiply. It has been shown that the normal bacterial flora of fish reflects the bacterial population of the water in which they swim (Mesalhy Aly, 2013). The majority of fish pathogenic bacteria are short, gram-negative rods (families: Enterobacteriaceae,, Pseudomonadaceae and Vibrionaceae). The long gram-negative myxobacteria (family: Cytophagaceae) which are not recognized as pathogens of warm-blooded animals, have also been found to cause heavy mortality in fish stocks (Mesalhy Aly, 2013). The general symptoms of bacterial infections are septicaemia and ulcers. Gram-positive bacteria are not common, but can cause severe losses in certain species of fish under particular conditions (Mesalhy Aly, 2013).

Fungal diseases

Fungi are also responsible for a number of economically important diseases in farmed fish. They cannot use photosynthetic pathways for energy production as they have no chloroplasts and therefore must live a saprophytic or parasitic existence. The Oomycetes group (namely *Saprolegnia, Branchomyces and Achyla*) is the most important of the fungal pathogens and are commonly seen during cold seasons and is linked with stress factors. They are widely distributed in aquatic habitat and very few are parasitic. They have a common characteristic feature of producing motile biflagellate spores that can cause infection to occur at any time. According to Verma (2008),

Saprolegniasis is a common and highly prevalent fungal disease that affects all species and ages of freshwater and estuarine fish. Many factors contribute to the development of fungal infections in fish, either favouring the proliferation of the fungus or weakening of the fish. It is when these factors combine to overwhelm the fish that leads to infection (Verma, 2008).

Viral diseases

Viruses are small infectious agents in the aquatic environment which infect wild and farmed fin and shellfish species, often causing high levels of mortality (Citarasu, 2012) with negative impacts on the economy of fish production. Members of twelve virus families have been identified in wild and cultured fish worldwide (Mesalhy Aly, 2013). Out of nine fish diseases reported by the World Organization for Animal Health in the Aquatic Animal Health Code (2009), seven were viral in origin. Viral diseases are responsible for the highest losses in aquaculture production. They mostly affect fish at the early stages of development and in the adult fish (which are more economically valuable) causing a high percentage of mortality (Falco, Martinez-Lopez, Coll & Estepa, 2012). The presence of these pathogenic agents is a threat to the culture of aquatic animals and measures to prevent, curb them or make the organism robust against them are important.

Overview of Fish Immunity

Fishes, like other vertebrates possess both innate (non-specific) and adaptive (specific) immune defence systems. The innate immune system is the fundamental defence mechanism of fish and a crucial factor in disease resistance (Magnadottir, 2006). The adaptive response of fish is delayed but is

essential for long-lasting immunity and is a key factor in successful vaccination (Secombes & Wang, 2012). The innate immune system also plays an instructive role in the adaptive immune response and co-operates in maintaining homeostasis (Magnadottir, 2006). The present study will focus on the innate immune defence system.

Innate (Non-specific) immune system

The innate immune system is the first line of defense against invading pathogens. According to Harikrishnan, Balasundaram, Kim, Kim, Han & Heo (2009), the major components of the innate immunity are macrophages, monocytes, granulocytes and humoral elements, including lysozyme or complement system. These components consist of proteins involved in the recognition of pathogen associated molecular patterns like bacterial and fungal glycoproteins and intracellular components released through injury or infection (Magnadottir 2006). Several external and internal factors such as temperature changes, handling and crowding stress can have suppressive effects on innate parameters, whereas several food additives and immunostimulants can enhance different innate factors (Magnadottir, 2006). Furthermore, Magnadottir (2006) illustrated that the innate immune system in fish is divided into physical barriers (composed of scales, mucous surfaces of skin and gills and the epidermis), cellular components (thus phagocytic, cytotoxic, epithelial and dendritic cells) and humoral components (such as pathogenic growth inhibitors, lytic enzymes, agglutinins, precipitins, natural antibodies, cytokines, chemokines and antibacterial peptides).

Susceptibility of fish to infectious disease

Infectious disease occurs when a virulent pathogen, whether obligate or facultative, is able to overwhelm the defence mechanisms of a susceptible host (fish) under environmental conditions that are conducive to the disease process. Thus the defence mechanism of the fish plays a key role in the success of an infectious disease. For this reason, approaches to improve the defense mechanism of the fish under proper management conditions can reduce susceptibility of fish to disease and minimise stress impacts (Ashley, 2007). Limiting disease occurrence in the rearing period will contribute to enhance fish production and minimize losses in the aquaculture sector. In this direction, development of methods for improving fish health and sustainable control of fish diseases are a motivation for aquaculture research.

Conventional ways of managing fish disease in aquaculture

Generally synthetic agents such as antibiotics, disinfectants and therapeutants are commonly used for management and prevention of fish diseases in intensive aquaculture (Choudhury, Pal, Sahu, Kumar, Das & Mukherjee, 2005). However, these methods have been criticised for having some negative impacts including bioaccumulation of drugs in tissues of the fish, development of antibiotic-resistant bacteria strains and immunosuppression (Esiobu, Armenta & Ike, 2002). In addition, chemical and antibiotic residues in aquaculture environments and subsequently in fish products could cause unfavourable ecological and public health hazards (Jones, Voulvoulis & Lester, 2004). As prevention is better than cure, management of fish disease should focus on preventive and ecologically friendly remedies for the health of fish, the environment and consumers.

Contemporary ways of limiting disease outbreak in aquaculture

In recent times, there has been a keen interest in the use of natural products for disease control in aquaculture due to their multiple bioactivities as alternatives to antibiotics and disinfectants (Reverter, Bontemps, Lecchini, Banaigs & Sasal, 2014). Attention has focused on immunostimulants and plant products that could have a beneficial effect in disease control (Karata, Arda & Candan, 2003).

An immunostimulant is a substance that enhances the defense mechanisms or immune response of an organism thus rendering the animal more resistant to diseases and external agressions (Anderson, 1992 cited in Reverter et al., 2014). The application of immune-stimulants has been considered a more effective approach to health management in aquaculture because it reduces stress response, increases the activity of innate immune parameters and improves disease resistance as well as promotes growth and performance under culture (Harikrishnan, Balasundaram & Heo, 2011; Kum & Sekkin, 2011; Talpur & Ikhwanuddin, 2012).

Immuno-stimulating substances include synthetic chemicals, bacterial derivatives (probiotics, prebiotics, synbiotics), polysaccharides or animal and plant extracts, vitamins, carotenoids, medicinal plants, etc. (Harikrishnan, Balasundaram, & Heo, 2011). Of these, medicinal plants can be said to be the most significant because of their versatile nature. Thus they are effective, environmentally-friendly, easily available, economically attractive and non-biomagnifiable, (Citarasu, 2010). They therefore represent an alternative to the use of synthetic drugs and/or antibiotics.

Natural plant products in aquaculture

Plants contain natural products such as alkaloids, terpenoids, glycosides, flavonoids, phenolics, tannins, saponins, steroids and essential oils (Citarasu, 2010; Chakraborty & Hancz, 2011). These plant derivatives are described to improve fish health and also promote fish growth by stimulating the appetite, and defence response of the fish (Reverter et al., 2014). They are said to augment the tonicity and also maturation of cultured species and can act as, aphrodisiac and anti-pathogens in cultured fish (Citarasu, 2010; Chakraborty & Hancz, 2011). The application of plant products could lessen the cost of treatment and be more sustainable as they are likely to be more biodegradable than other synthetic molecules and less prone to cause drug resistance in pathogens because of “the high diversity of plant extract molecules” (Logambal, Venkatalakshmi & Michael 2000; Olusola, Emikpe & Olaifa, 2013).

Maqsood, Singh, Samoon and Munir, (2011) stated that “the most important advantage of using immunostimulant plants in aquaculture is that they contain natural organic materials that do not cause threat to fish health or to the environment or to human health”. Additionally, with the increasing preference for organic foods, the use of plant products in aquaculture would make aquaculture produce more appealing to consumers.

In this regard, a variety of plant products (with known medicinal properties and beneficial effects on humans) have been applied in aquaculture to explore their potential effect in enhancing health status, improving disease resistance and promoting growth in various culture species. In a review of the use of plants in aquaculture, Reverter et al. (2014) enlisted a number of plant

products that have been tried on fish including mistletoe (*Viscum album*), nettle (*Urtica dioica*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), onion (*Allium cepa*), motherwoot herb, bermuda grass (*Cynodon dactylon*), long pepper (*Piper longum*), coat buttons (*Tridax procumbens*), monkey head mushroom (*Hericium erinaceum*), kelp (*Ecklonia cava*) indian ginseng (*Achyrantes aspera*), purple fruited pea eggplant (*Solanum trilobatum*), chinese cedar (*Toona sinensis*), chaga mushroom (*Inonotus obliquus*), etc. In Ghana, ginger and garlic are readily available and widely accepted plant products used as spices in daily cuisine and as a component in herbal preparation for various health benefits. The properties and benefits of ginger and garlic are elucidated in the following session.

Properties and benefits of Ginger (Zingiber officinalis)

Ginger is a well-known herbal medicine containing alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, vitamins, carotenoids and minerals (Talpur, Ikhwanuddin & Ambok, 2013). Alongside, it is endowed with natural antioxidants such as gingerols, shogaols and zingerone (Hori et al., 2003) and essential oils which have potent anti-inflammatory effects (Zarate & Yeoman, 1996). Reported benefits from ginger include anti-platelet, anti-bacterial, anti-fungal, anti-viral, anti-worm and anti-oxidative activities, with effects on gastrointestinal and cardiovascular systems. Additionally, it possesses anti-lipidemic, anti-hyperglycemic, and anti-tumour properties (Talpur et al., 2013). It is also known to be effective as an immune-modulator in human and other animals including fish (Nya & Austin, 2009b; Apines-Amar, Amar, Faisan, Rolando, Pakingking, & Satoh, 2012 and Talpur et al., 2013). Ginger application in aquaculture is gaining grounds as a digestive

stimulant and immune booster.

Properties and benefits of Garlic (Allium sativum)

Garlic is also a widely accepted spice. The bioactive components of garlic include several sulfur-containing compounds such as alliin, diallylsulfides and allicin that partly account for some of its effects (Amagase, Petesch, Matsuura, Kasuga & Itakura, 2001; Butt, Sultan & Iqbal, 2009). These components are known to possess anti-bacterial, anti-fungal, anti-parasitic, anti-viral, anti-oxidant (Santhosha, Jamuna & Prabhavathi, 2013), as well as anti-thrombotic, vasodilatory and anti-cancer activities (Butt et al., 2009; Agarwal, 1996). Ankri and Mirelman (1999) have pointed out that garlic can hamper the growth of those bacteria that are resistant to certain antibiotics. Application of garlic in fish farming has become popular for enhancing the activity of defence systems, conferring protection against diseases and acting as a growth promoter (Metwally, 2009; Nya & Austin, 2009; Talpur & Ikhwanuddin, 2012).

The Research Problem

In aquaculture, fish diseases constitute one of the major problems and efforts to minimize the occurrence of diseases are crucial to prevent mortality and morbidity. This can in turn promote rapid growth and optimal feed conversion of cultured fish in fresh, estuarine and marine waters. According to Oliva-Teles (2012), the nutritional status of the cultured fish plays a crucial role in its health, hence feed supplementation is practiced with the aim of improving fish health as well as optimizing feed utilization, quality of flesh and consequently fish growth and performance (Saleh, Michael & Toutou,

2015). In view of this, the search for new feed additives has become an important area for aquaculture researchers as noted by Cho and Lee (2012).

S. melanotheron is a suitable aquaculture species in the coastal waters of Ghana. However, earlier studies on its aquaculture performance reported rather low growth rates, and measures such as monosex male culture, lower density, and altering quantity and distribution of the feed did not help to improve growth (Campbell, 1987). Additionally, the aquaculture performance of *S. melanotheron* studied in brackish water by Gilles, Amon-Kothias & Agnese (1998), Legendre, Hem & Cisse (1989) and Ouattara, Teugels, N'Douba & Philippart (2003) also recorded growth rates that were not impressive for the commercial culture of this species. They also recorded high feed conversion ratio indicating that the fish poorly utilized artificial diet. In all these studies however, there was no mention of supplementing its diet to aid in feed utilization and improving culture performance. Furthermore, the effect of plant based feed supplement on growth parameters is under research.

In order to improve its culture performance, application of natural plant products which in recent aquaculture have been used as potential growth promoters, anti-stress and immune-stimulants on various species (Choudhury et al., 2005), can be tested on the blackchin tilapia. Supplementing ginger and garlic in artificial feed for *S. melanotheron* may help to reduce stress and facilitate growth performance whiles keeping fish in good health, limiting incidence of diseases and saving the environment from unnecessary load of antibiotics, therapeutants and other chemicals during the rearing period.

The Study Objectives

The main goal of this study is to determine the effect of dietary ginger and garlic supplementation on growth and health status of *Sarotherodon melanotheron*.

The specific objectives are to:

a. determine the appropriate dosage of ginger and garlic powder supplements for maintaining health and improving growth of *S. melanotheron* under culture.

b. assess the effect of ginger and garlic supplementation on

i. growth, feed utilization and physiological condition.

ii. proximate biochemical composition

iii. blood composition

iv. blood chemistry and

v. immunity of *S. melanotheron*

The Research Hypotheses

The null and alternate hypotheses for this study are as follows;

H_0: Ginger feed supplement does not affect growth performance of reared *Sarotherodon melanotheron*.

H_1: Ginger feed supplement affects growth performance of reared *Sarotherodon melanotheron*.

H_0: Garlic feed supplement does not affect growth performance of reared *Sarotherodon melanotheron*.

H_1: Garlic feed supplement affects growth performance of reared *Sarotherodon melanotheron*.

H_0: Ginger feed supplement does not influence the proximate biochemical composition of the reared *Sarotherodon melanotheron*.

H_0: Ginger feed supplement influences the proximate biochemical composition of the reared *Sarotherodon melanotheron*.

H_0: Garlic feed supplement does not influence the proximate biochemical composition of the reared *Sarotherodon melanotheron*.

H_0: Garlic feed supplement influences the proximate biochemical composition of the reared *Sarotherodon melanotheron*.

H_0: Ginger feed supplement does not influence the physiological defence mechanisms of the reared *Sarotherodon melanotheron*.

H_0: Ginger feed supplement influences the physiological defence mechanisms of the reared *Sarotherodon melanotheron*.

H_0: Garlic feed supplement does not influence the physiological defence mechanisms of the reared *Sarotherodon melanotheron*.

H_0: Garlic feed supplement influences the physiological defence mechanisms of the reared *Sarotherodon melanotheron*.

The Significance of the Study

S. melanotheron is an important coastal fish resource whose culture can serve as a source of livelihood for coastal dwellers. To efficiently culture this species, it is important to study some factors that may affect its growth under culture. *S. melanotheron* has an existing market with preference for smaller sizes suitable for fried fish recipe locally called “kyenam” which is popularly used in local dishes across Ghana. Its culture in some of the numerous coastal water bodies can help to supplement the declining small

pelagic fish catch in our coastal waters and serve as a source of supplementary livelihood for coastal dwellers.

Incorporation of plant supplements in the diet of *S. melanotheron* can provide information on the possibility of using an environmentally friendly product to aid in fish growth, better health condition and disease resistance to facilitate the sustainable culture of the species without negatively affecting the environment and the quality of fish produced (Saleh et al., 2015).

Results of this study will be useful to aquaculturists, researchers and policy makers, by informing ways to improve feed utilization and efficiency and management practices for the culture of *S. melanotheron*. Also, this study will provide a means of managing stress impacts to minimize losses due to mortalities. Additionally, it will provide an alternative to limit the use of chemicals and synthetic products to either treat or prevent infectious diseases and help to achieve environmentally friendly and sustainable aquaculture.

Limitations of the study

The study was conducted with fingerlings of *S. melanotheron* produced from broodstock that were collected from a single reservoir (Bremsu Reservior at Effutu-Abasa in the Central Region, Ghana). This was chosen because the population from this environment compared to those of surrounding water bodies are larger and therefore seem to have the growth potential that is favourable for culture. Considering the fact that different populations may have some inherent genetic potential differences (Abban, Asante & Falk 2000), this approach can be considered a limitation of the study. Additionally, the source of ginger and garlic used for preparing the supplements, the number of subjects (10 fish per replicate) used, the method of incorporation of

supplement into the basal diets to constitute the experimental treatments, the culture facility (hapa-in-tank system) and the source of water (de-chlorinated tap water) for the culture could be considered as limitations to the study.

Definition of Terms

Adaptive immune system: A part of the immune system composed of specialised systemic cells and processes that eliminate pathogens or prevent their growth.

Aquaculture: The farming of aquatic animals and plants.

Disease: A disorder in an organism that affects a specific location and impairs it's normal functioning

Immunity: A balanced state of multicellular organisms that have adequate biological defences to fight infection and tolerance to autoimmune diseases.

Immuno-stimulant: Substances that stimulate the immune system by inducing activation or increasing activity of any of its components.

Innate immune system: A non-specific defense that comes into play upon attack by an antigen.

Intensive culture: A situation in which a large number of fish are confined to a relatively small space and the all factors of production, thus fish, feed and environment are controlled.

Macrophages: A type of white blood cell derived from monocytes that are capable of engulfing pathogens

Parasite: An organism that live on/in another organism and benefits by deriving nutrients at the host's expense.

Pathogen: A microorganism that can cause a disease

Phagocytes: A type of immune cell within the body capable of engulfing and absorbing bacteria and other small cells and particles

Stress: Any environmental situation that can obstruct the physiology of fish

Organization of the Study

The study is structured into five chapters as follows: Chapter one introduces the study and covers areas such as, background of the study, research problem and significance of the study, the study objectives as well as limitations and the hypotheses to which the study aim to verify. Chapter two reviews some literature pertaining to the species of interest and earlier works that have explored the hypotheses of this study. Chapter three focuses on the methodology that was used for the study, explains the procedures used to gather the relevant data for the study and the analyses of the data. In Chapter four, the results obtained is presented while Chapter five interprets the findings of this study with reference to relevant literature and previous findings from similar works on the same or related species. It then summarises the research in the conclusion and recommendations.

Chapter Summary

This chapter described the importance of aquaculture to global food and nutrition security and how diseases are a constraint to production of fish, detailing the impacts of diseases on this sector. It also looked at an overview of diseases in fish and their immune system. Contemporary ways of managing fish diseases were revised and conventional ways of dealing with them were also looked at bringing us to the point of applying plant products that can boost immunity and performance of fish under culture. The research problem

was stated and the objectives set to guide this work were outlined. The hypotheses and significance of the study were explained. It ended by detailing the limitations, definition of terms and organization of the study.

CHAPTER TWO

LITERATURE REVIEW

This chapter first examines information about the biology and physiology of *S. melanotheron* as a native species as well as its culture performance under experimentation. It then reviews relevant literature on the use of ginger and garlic supplements in aquaculture, focusing on the effects on growth and feed utilization; health status and disease resistance of cultured fish.

Characteristics and importance of the Black chin tilapia

The species is characterized by relatively lower numbers of vertebrae (26–29, usually 27–28), 12–19 lower gill rakers compared with other tilapia species. It has 14–16 dorsal spines, and a deep preorbital bone. It is a paternal brooder, and has a strong preference for brackish water (Trewavas, 1983). Its colouration varies with location, sexual activity, and environmental background producing a protective chameleon effect. The throat and ventral surfaces are usually white. The black spots on the chin and throat vary considerably both within and among populations. The matured males often have a proportionately larger head caused by month brooding and loss of weight during incubation (Trewavas, 1983).

S. melanotheron is native to west Africa and has a high value in coastal fishery being the dominant fish species (65- 98 %) in about 60 lagoons and estuaries along the coast of Ghana (Abban et al., 2000). The juvenile size of this fish locally called "mpatua" along the central coastal belt in Ghana is an important source of protein for many coastal dwellers. Culturing of this species will increase its availability to a wider portion of the country's

population and can contribute to food and nutrition security. Information about its biology and physiology as a native species is very important and is useful for propagation and culturing of the species.

Biology of *Sarotherodon melanotheron*

There are many reports on the blackchin tilapia in Ghana and beyond. For its feeding habit, *S. melanotheron* is reported to be omnivorous, thus subsisting on a variety of food items of both animal and plant origins (Fagade 1971; Blay 1998) and can change its diet in function to the environment (Kone &Teugels, 2003).

A sex ratio of 1:1 has been reported by a number of authors such as Faunce (2000) and Oribhabor and Adisa-Bolanta (2009) indicating that they occur in equal numbers in nature. However, a sex ratio of 1:1.5 in favour of the females was observed by Arizi, Obodai and Aggrey-Fynn (2014). For the size at first maturity, 5.5 cm SL and 4.6 cm SL have been reported for *S. melanotheron* populations in the Benya lagoon and the Kakum river estuary of Ghana respectively (Blay 1998). These sizes were smaller compared to 12.8 cm SL and 14.5 SL reported by Faunce (2000) for males and females respectively in an impounded mangrove ecosystem in Florida. Also, Arizi et al. (2014) recorded 13.4 cm SL as the size for first sexual maturity in the Dominli lagoon in Ghana, indicating that populations in different environments have varying maturity sizes.

For *S. melanotheron,* as with the tilapias in general, the presence of mature gonads encountered throughout the year suggested that there is continuous breeding with peak seasons during April and May (Faunce 2000; Koné & Teugels, 2003; Arizi et al., 2014). But Panfili et al. (2004) observed

that there was a clear seasonal cycle of reproduction in estuaries with a peak at the beginning of the wet season (May-July) in the Senegalese Saloum Estuary and the Gambia River estuary. Under laboratory conditions, Eyeson (1979) found spawning occurred on an average once in 22 days, although there were periods of inactivity for 2 months or more. Females produce between 200 and 900 eggs per brood (Trewavas, 1983). The number of eggs that can be incubated by the male ranges from less than 20 (male of 20 g) to over 700 (male of 310 g) (Campbell, 1987). The fecundity recorded for *S. melanotheron* by Arizi et al. (2014) ranged from 97 to 379 eggs with a mean of 206 eggs.

The eggs are orange and have a length range from 2.0 to 3.5 mm weighing 10 to 20 mg (Trewavas, 1983). Eyeson (1979) found a range of 1.9 × 2.1 to 2.6 × 3.0 mm and Trewavas, (1983) reported that the number of eggs decreased with increasing female size but the egg size increased. Hatching of eggs occurs within 4–6 days at 27°C. The new fry are 4.1 mm in length and weigh about 20 mg. The fry are released 10 to 11 days after hatching measuring 8.6 to 11.5 mm. The total buccal incubation time is about 14 days (mode), and may continue for as long as 19 days (Eyeson, 1979; Trewavas, 1983) being at least 3 days longer than other mouth brooding tilapias (Philippart & Ruwet, 1982).

Physiology of *S. melanotheron*

Naturally, *S. melanotheron* exhibits an isometeric growth pattern, thus an increase in its length is directly proportional to an increase in its weight (Ayaode & Ikulala 2007). By virtue of its habitat (brackish water), this species is exposed to regular or random changes in environmental conditions especially salinity, which plays an important role in its physiology. Panfili et

al. (2004) observed in the Senegalese Saloum Estuary and the Gambia River estuary that *S. melanotheron* is able to withstand saltier environments by limiting its growth, reducing the size-at-maturity, and changing its fecundity, with the most profound changes being visible in hypersaline conditions. With this adaptation, it is reported that it can tolerate euryhalinity up to 90 ppt in the Casamance Estuary and even above 120 ppt (Albaret 1987; Lévêque 1999).

In terms of growth rates for *S. melanotheron,* it can tolerate from fresh to hypersaline waters, but the optimal conditions may be intermediate salinities that is close to that of the seawater (Panfili et al., 2004). This makes *S. melanotheron* suitable for culture in a wider range of environments depending on what is available for use.

Additionally, *S. melanotheron* has been shown to exhibit high tolerance to low levels of dissolved oxygen (Dussart, 1963) and can withstand high levels of turbidity. It can also be quite resistant to pollution and tolerate a high range of temperatures from 17 ^{o}C to more than 32°C (Welcornrne, 1972).

Haematological analysis on *S. melanotheron* by Obemeata, Aduabobo and Wokoma (2012) revealed that the red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), thrombocytes, and lymphocytes of the control group were significantly higher ($p \leq 0.05$) than the crude oil treated groups while the white blood cells (WBC), neutrophils, leucocrit (Lct) and monocytes of the crude oil treated groups were significantly higher than the control group, indicating an immune response to the toxicant.

Tine, Bonhomme, McKenzie1 and Durand (2010) showed through their study that, although *S. melanotheron* can colonise extremely saline

environments, the overexpression of the gene Hsp70 combined with the higher Naka mRNA expression revealed the presence of a chronic stress on the fish which may explain the impaired growth performance and precocious reproduction that have been demonstrated in some populations of this species. Lorin-Nebel, Avarre, Faivre, Wallon and Durand (2012) demonstrated in their study that there exist a severe osmotic imbalance in *S. melanotheron* living in hypersaline environments. Growing the species in a suitable environment may therefore encourage better growth and performance and thus needs further studies.

In studying the cold tolerance and behavioural responses of *S. melanotheron*, to declining temperatures at salinities of 5, 15, and 35 parts per thousand (ppt), Jennings (1991) found that behavioural activity declined with decreasing temperature and ceased between 10-12°C. Also certain behavioural actions were significantly more frequent at salinity of 15 or 35 ppt than at 5 ppt. In another study, Chinda, Braide and Oranye (2008) demonstrated how pH affects the blood glucose levels of *S. melanotheron*. They found significant increase in blood glucose levels with a decrease in pH (pH 4 and 3.8) of the environment whereas blood glucose level of the control group (pH 7) remained constant throughout the study period. The acute change in level of blood glucose with pH implies a stress response by the fish which can alter normal growth and development.

Culture studies on *S. melanotheron*

In aquaculture, the growth of an organism depends on the environmental factors, specific potential of the species and the stocking density (Weatherley, 1976; Delince, 1992). For commercial scale production,

large numbers of fish are confined to the smallest possible space. The optimal stocking of fish is that amount of fish at the start of the production period that guarantees the highest possible yield of fish on harvesting.

The aquaculture potential of *S. melanotheron* has been studied in brackish water by Eyeson (1983), Legendre (1986), Gilles, Amon-Kothias & Agnese (1998) and Legendre, Hem & Cisse (1989). The growth rates obtained in their study were not good enough to favour its commercial culture. In Ebrie Lagoon, Legendre et al. (1989) noted feed conversion ratios (FCR) varying from 8.7 to 9.1 for this species fed a 31% crude protein (CP) pelleted feed. Ouattara et al., (2003) also recorded FCRs higher than 11 whereas lower FCRs of 1.5 to 3 have been obtained in intensive tilapia culture (Coche, 1982). The high FCRs recorded indicated that *S. melanotheron* utilized artificial feed very poorly. In a growth performance study of *S. melanotheron* conducted by Anani, Ofori-Danson and Abban (2010) in the Aglor Lagoon in Ghana, the characteristic of poor feed utilization was highlighted in that there was no significant difference between *S. melanotheron* fed on 29.80% CP feed and those that fed on bamboo generated periphyton but the two fed groups showed a significant improvement compared to those that were not fed (control). These demonstrations suggest that perhaps improving the protein quality of feed given this species may help boost its growth and performance under culture.

Regarding its growth and feed utilization, application of methods that can enhance nutrients digestion and assimilation in this species may help to improve its aquaculture performance. Currently, there are no studies on effects of plant dietary supplements on this species. Studies on the properties and

benefits of ginger and garlic present a good opportunity to experiment their potential on the culture performance of *S. melanotheron*.

Ginger and Garlic Supplements on Growth and Feed Utilization in cultured fish.

In aquaculture set-ups, about 40 to 50 percent of production costs is for feed (Abowei & Ekubo, 2011), making nutrition critical to improve production and economic efficiency. Since the use of some synthetic and antibiotics supplements can cause side effects and bacterial resistance, the use of natural food additives, such as herbal products and extracts is recommended by WHO (Levic, Sinisa, Djuragic & Slavica 2008). Herbal supplements have been shown to lead to greater digestive enzymes secretion and improved appetite, feeding rate and food efficiency (Jiang, Li, Ferguson, Wang, Liu & Li, 2007) thereby yielding greater fish growth and biomass production.

Several authors have reported significant positive effects of administering garlic in diets on growth and feed utilization of many species such as the African catfish, *Clarias gariepinus* (Agbebi, Ogunmuyiwa & Herbert, 2013); rainbow trout, *Oncorhynchus mykiss* (Gabor, Sara, Bentea, Creta & Baciu, 2012; Nya & Austin, 2009a); swordtail, *Xiphophorus helleri* (Kalyankar, Gupta, Bansal, Sabhlok & Singh, 2013); Nile tilapia, *Oreochromis niloticus* (Shalaby, Khattab & Abdel Rahman, 2006; Mesalhy, Abdelatti & Mohamed, 2008; Metwally, 2009; Aly & Mohamed, 2010), Asian sea bass, *Lates calcarifer* fingerlings (Talpur & Ikhwanuddin, 2012); sea bass, *Dicentrarcus labrax* fry (Saleh et al., 2015); orange-spotted grouper (Guo, Kuo, Chuang, Hong, Chou & Chen, 2012). In all these studies, there was a significant increase in growth rate, weight-gain and feed conversion in garlic-

fed groups, as well as enhanced feed efficiency and fish growth. However, studies by other authors including Gholipour Kanani, Nobahar, Kakoolaki and Jafarian (2014) on sturgeons, *Huso huso* did not record significant positive effects of garlic administration on growth and feed utilization even though it was not detrimental.

Ginger has been used as digestive stimulant since ancient times and it has been shown that it stimulates growth of aquatic organisms (Butt & Sultan, 2011; Nya & Austin 2009b). Venkatramalingam, Godwin and Citarasu, (2007); Nya and Austin (2009b) reported increment of gastric enzyme activity after consumption of ginger as a food additive in *Penaeus monodon* and *Oncorhynchus mykiss* respectively. Rahimi, Zanguee, Mousavi and Zakeri, (2015) also demonstrated that addition of ginger extract to feed increases activity of some digestive enzymes (amylase and alkaline phosphatase) and stored energy compounds (lipid and glycogen) in the liver of *Mesopotamichthys sharpeyi* fingerlings, thereby increasing metabolism which plays a crucial role in the growth of fish. Similarly, Hassanin, Hakim and Badawi (2014) in their study revealed that *Oreochromis niloticus* (initial weight of 30.00 ± 1.00g) fed diets containing ginger for ten weeks had a significant ($p < 0.05$) increase in total final body weight, body gain, body gain percent, specific growth rate, and also utilized their feed more efficiently (lower values of FCR) than those fed the control diets even though the average daily feed intake was not significantly ($P > 0.05$) different with all groups. Gholipour Kanani, et. al., (2014) who studied dietary treatments of garlic and ginger at 1.0 g per 100 g of feed for 60 days in *Huso huso* found that specific growth rate, body weight gain and condition factor were significantly

increased in ginger treatment group but not in the garlic and control groups at the end of the trial.

The outcomes of testing ginger and garlic on growth and feed utilization of various culture species draws attention to the fact that application of these plant products to *S. melanotheron* may contribute to improve its use of artificial diet and to enhance the possibility of culturing this species for commercial purposes.

Ginger and Garlic Supplements on Blood Composition and Biochemical Parameters of cultured fish

One of the important ways to distinguish appropriate and inappropriate prescriptions of medicinal plants is the assessment of their effects on haematological and biochemical parameters in experimental animals (Kolawole, Kolawole & Akanji, 2011). Therefore, studies on the use of plant supplements in fish perform this assessment to ensure whether they are beneficial or detrimental to fish health.

Talpur and Ikhwanuddin (2012) demonstrated significantly increased levels of erythrocytes, leucocytes, haematocrit, and haemoglobin in Asian sea bass *Lates calcarifer* fingerling when fed garlic supplements. In the same study, there was significantly higher serum protein, albumin and globulin levels in the garlic treated groups. There was also a reduction in serum glucose, lipids, triglycerides and cholesterol levels in treated groups compared to the control indicating that garlic led to enhanced health status of the fish. In a study by Gholipour Kanani et al. (2014) of dietary treatments of garlic and ginger at 1.0% each, they observed the highest levels of erythrocyte and hematocrit in fingerlings of *H. huso* in the ginger group after 60 days.

Globulin increased significantly in the ginger group, and the highest level of albumin was observed in garlic group. This implies that the dosage of plant products applied to fish is important on various fish species. Considering that there is a vast number of fish species being cultured, it is important to establish appropriate plant product doses that may be beneficial to any particular species.

Ginger and Garlic Supplements on Immunity and Disease Resistance of cultured fish.

According to Reverter et al. (2014), many researchers have observed some immunological parameters after intraperitoneal injection or oral administration of some plant extracts on different fish species and found that treated fish showed improvement in some immunological parameters. These include increased lysozyme activity, phagocytic activity, complement activity, increased respiratory burst activity and increased plasma protein (albumin and globulin).

Lysozyme plays an important role in the defence of fish by inducing antibacterial activity in the presence of complement (Harikrishnan et al., 2011). Phagocytosis is one of the main mediators of innate immunity to pathogens, while respiratory burst is also a crucial effector mechanism for limiting the growth of fish pathogens (Divyagnaneswari, Christybapita & Michael, 2007). Increase in plasma protein: albumin and globulin, is considered as a strong innate response in fishes (Reverter et al., 2014). Talpur and Ikhwanuddin (2012) observed that the phagocytic activity, respiratory burst, lysozyme, anti-protease and bactericidal activities were enhanced following feeding with garlic, and this improved immunity by making *Lates*

calcarifer more resistant to infection by *Vibrio Harveyi* after a challenge test. Also, Hassanin et al. (2014) demonstrated significantly improved lysozyme activity and immunoglobulin M (IgM) in Nile tilapia due to supplementing their diets with ginger, and consequently, leading to protection of fish against pathogenic strain of *Aeromonas hydrophila*. These studies have demonstrated that ginger and garlic supplements in diets can confer some resistance against infectious diseases in some fish species and hence their efficacy in other fishes need to be investigated.

Results by Guo et al. (2012) demonstrated that the cumulative mortality of the orange-spotted grouper (*Epinephelus coioides*) fed with 1.3% garlic diet was significantly lower ($p < 0.01$) than that of those fed with 4% garlic diet and control diet following a challenge with *Streptococcus iniae*, indicating that the inclusion of lower dosage of garlic in the diet of orange-spotted grouper enhanced resistance to infection by *S. iniae.* Karata et al. (2003) reported that aqueous extracts of mistletoe, nettle and ginger in diet of rainbow trout caused an enhanced extracellular respiratory burst activity ($P< 0.001$) compared to the control group. There was also an increased total plasma protein level (except 0.1% ginger), and increased rate of phagocytosis and extracellular burst activity of blood leukocytes, conferring an improved health status in the rainbow trout. These findings indicate that supplementing fish diets with plant products have beneficial effects on the fish's health and enhance the immune system, and therefore can play a crucial role in avoiding disease outbreaks in aquaculture systems. However, they also signify the importance of suitable dosing to obtain the desired effects. More importantly, some studies have shown that plant extracts can be toxic to fish if applied at

unsuitable doses (Ekanem & Brisibe 2010; Kavitha, Ramesh, Kumaran & Lakshmi, 2012). It is therefore important to investigate the appropriate doses of any particular plant product on different fish species.

A study by Militz, Southgate, Carton and Hutson (2013), demonstrated that garlic extract administered as a dietary supplement is one of the most practical methods to prevent *Neobenedenia sp*. (monogenean parasite) infection in *Lates calcarifer*. In their study, dietary supplementation of garlic extract in the diet during long-term supplementation (for 30 days) at either 50 or 150 mL kg^{-1} significantly reduced *Neobenedenia sp*. oncomiracidia infection success of *L. calcarifer* ($p < 0.05$) to less than 10% compared to the control diet (without garlic extract) and short-term supplementation (for 10 days) having nearly three times more success rate. Thus a longer period of supplementation with the garlic extract conditioning was necessary to reduce infection success of *Neobenedenia sp.* to juvenile *L. calcarifer.* Additionally, Sahu (2007) showed in a study that when supplementing garlic in the diet of juvenile carp, *Labeo rohita*, for 20 and 40 days, several immunological indices namely leucocyte density, superoxide anion production and antimicrobial activity of the serum, continually increased with longer periods of conditioning. Nya and Austin (2009) also reported that garlic is beneficial for the control of *Aeromonas hydrophila* infection in rainbow trout, and furthur extended the study in Nya and Austin (2011) where they demonstrated that there exists a significant long-term memory effect (about two weeks) after the cessation of the feeding regime with garlic. Together, these studies support the view by Nya and Austin (2011) that the “persistence of an immune activator may be critical in maintaining long-term protection against disease causing

situations''. In this view, supplementing the diet of *S. melanotheron* for the culture period (24 weeks) may give lasting protection to the fish. This will enable it withstand the impact of stress and possible pathogenic invasion throughout the culture period. It turns out however, that a study by Gholipour Kanani et al. (2014) indicates that lysozyme activity of *Huso huso* fed with ginger and garlic was suppressed. On the contrary, Nya and Austin (2011) found that there was an increase in the serum lysozyme activity in rainbow trout treated with garlic.

Harikrishnan, Balasundaram and Heo (2011), have raised the fact that the effects of immuno-stimulants on fish are species specific and this review can attest to that fact. The similarity and contradicting results may all have to do with the inherent genetic make-up and differences among the many cultured species. Then, one cannot conclude that what has worked for one species will work for the other unless investigations are carried out. The present study will therefore evaluate the effect of supplementing ginger and garlic powder in the diet of *S. melanotheron* on their growth, feed utilization, proximate composition, blood composition and immune status of the fish.

Chapter Summary

This chapter revised literature on the biology, physiology and culture of *S. melanotheron*, it provided information needed to set-up and culture the species considering what it can adapt to and the possibility of applying a plant supplement in its diet to aid culture performance was looked at. Application of ginger and garlic in aquaculture was assessed to bring out the effects achieved in various culture species.

CHAPTER THREE

MATERIALS AND METHODS

This chapter describes the activities undertaken and the procedures followed to achieve the objectives of this study.

Study Site

The study was conducted in 3 concrete tanks located behind the School of Biological Sciences of the University of Cape coast in Cape coast, Central region, Ghana. The tanks belong to the Department of Fisheries and Aquatic Sciences of the School. The size, i.e. length x breath x height, of each tank measured 3.71 x 3. 70 x 1.20 m.

Preparation of tanks for holding fish

Before using the tanks for the study, they were disinfected by exposing them to sunlight for two weeks. To do this, all water in the three tanks was drained and the bottom allowed drying for two weeks. The dried sludge was scraped, swept and removed. The tanks were then scrubbed thoroughly under running tap water to remove all residue and dirt. They were allowed to dry again for another two weeks to ensure that all living organisms were eliminated. After drying, the tanks were filled with tap water. This water was left to stand for a month for its chlorine content to evaporate and for some natural food (phytoplankton) to develop. Hapas (size = $1x1x1m^3$) constructed from nylon nets (¼ inch mesh size) were used for this experiment. Nine hapas were installed in each tank using bamboo poles as support. In total, twenty-seven hapas were used for this study. These hapas were arranged equidistantly in the tanks. The Set-up for the study is shown in Figure 1.

Fingerlings Production

The production of fingerlings for the study followed the approach used by previous studies (Ayoola, Oluwatoyin, & Akapo, 2012; Tseku 2016). Twenty brood fish (10 males and 10 females) were used. These were obtained from the Brimsu Reservoir located on the Kakum River in November 2016 by cast netting with the help of a fisherman. The fingerlings production and experimental treatments were carried out in a freshwater system since the source of the fish was from a freshwater body. The broodfish were held in hapas (size = 1x1x1m) constructed from nylon mosquito net (size) mounted in a prepared concrete tank. The broodstock were allowed to stay for 7 days to acclimatize to the new environment. Afterwards they were stocked at a density of 4 fish $m^{-2}$and a sex ratio of 1:1 (male to female) in line with Tseku (2016). They were also fed a 40% crude protein (CP) diet twice daily (9:00 am and 3:00 pm) at 3% body weight according to Ng & Romano (2013).

After 5 weeks of breeding, an estimated number of 780 fry were harvested from two females and two males. The fry were then stocked in mosquito nylon hapas at a density of 360 fry m^{-2}. They were fed *ad libitum*, 5 times daily, with a 17α-methyl testosterone hormone incorporated feed (60 mg hormone per kilo of feed) for 28 days. This was done to achieve an all-male population which is favourable for growth. The CP content of feed was 47%, which was higher than that of the feed used for the broodstock. The justification for this is that younger fish have a higher protein requirement for proper growth and development (Halver & Hardy, 2002; Pillay & Kutty, 2005). After the hormone treatment period, the post-fry stage of the fish (average weight = 0.74 ± 0.32 g) were further reared for two weeks.

The resulting fingerlings were hand sorted and those weighing above 1.5 g (average weight 1.53 ± 0.05g), sample of fingerlings shown in Figure 2, were transferred to the mounted experimental hapas to be conditioned for 7 days. They were fed with a normal diet (without hormone) with CP at 47%. This diet is referred to as basal diet 1 in this report. The feed ration given was 10% of the biomass of fish in each hapa. The ration was given thrice a day in three equal parts at 09.00 am 12: 00 and 4:00 p.m. After the conditioning, they were starved for a day and weighed for the experimental treatment to begin.

Basal Diet Composition

In order to meet the nutritional requirement of the fish at the various developmental stages, commercially available fish feed containing the required nutrients for tilapia were used as the basal diets as applied in commercial tilapia production.

For the first 8 weeks, fish were given the basal diet 1 (47% CP; size = 0.2 - 0.3mm) as feed. From the 9th to 16th week, basal diet two (40% CP; pellet size =2.0 mm) was given, and for the 17th to 24th week, fish were given basal diet 3 (33% CP; pellet size = 2.5 mm). These feeding regimes are consistent with the nutrient requirement of the fish as found by the FAO (2017). The nutritional composition of the basal diets used is shown in Table 1. This composition is the same as those indicated on their feed containers. The costs per kg of the basal diets were Gh₵14.00, Gh₵5.15 and Gh₵ 4.00 for basal diets 1, 2 and 3 respectively. In order to ensure that the nutrients levels

Figure 1: Set-up of hapa-in-tank system used for culturing *S. melanotheron* for 24-weeks

Figure 2: Sample of fingerlings produced for the experimental study

indicated on the feed purchased from the market were available to the fish, a proximate analysis of the basal diets and the supplements was also carried out as described in Appendix A. The results are presented in Tables 2 and 3.

Preparation of Feed Supplements: Ginger and Garlic Powder

Ginger and garlic were procured from a market in cape coast shown in Figures 3 and 4. The use of these ingredients as feed supplement was done by following the approach described by Hassanin et al. (2014). They were washed, peeled, sliced, sun-dried, and milled into powdery form using a household grinder. After milling, the powder was sieved using a fine mesh, and the residual fibre discarded while the fine powder was stored in sealed plastic containers at 4^oC (in a refrigerator) until use as done in previous studies (Guo et al., 2012). The cost per Kg of the ginger powder was Gh₵ 117.51 while that of the garlic powder was Gh₵ 95.97.

Preparation of Treatment Diets

The powdered ginger and garlic were incorporated into the three basal diets at four different levels of 5, 10, 15 and 20 g/kg feed consistent with the work of Talpur and Ikhwanuddin (2012) to constitute four doses of supplementation. The control diet was the basal feed without additional supplements. For younger fish (during the first 8 weeks), ginger and garlic powder was incorporated by mixing directly into the basal diet 1 using a household dry blender at low speed to ensure a homogeneous mixture (Nya & Austin, 2011) to achieve the four levels of experimental diets shown in Table 4, illustrated in Figures 5 and 6. The modified feed was stored in air-tight containers at room temperature until use.

Table 1: Nutritional composition of the various sizes of feeds used as basal diet (control) for the study as indicated on feed labels

	Basal Feed Type (size)		
Nutritional composition	Diet 1 (0.2-0.3mm)	Diet 2 (2.0 mm)	Diet 3 (2.5 mm)
% Crude protein	47.0	40.0	33.0
% Lipid	9.0	7.0	6.0
% Crude fibre	1.0	2.5	3.5
% Ash	-	11.0	8.0
% Moisture	-	9.0	9.0
% Total Phosphorus	-	1.3	1.2
vitamin A (mg/kg)	4.2	3.45	3.45
vitamin D (mg/kg)	0.0535	-	-
vitamin E (mg/kg)	280	-	-
vitamin C (mg/kg)	490	150	150
% Calcium	2.4	-	-
Gross energy (MJ/ kg)	19.0	-	-
Digestible energy (MJ/kg)	17.4	-	-

Table 2: Proximate nutritional composition of the basal diets determined using standard procedures adopted from AOAC (2005) to confirm nutrients available to the fish

Nutritional composition	Basal Feed Type (size)		
	Basal diet 1 (0.2-0.3mm)	Basal diet 2 (2.0 mm)	Basal diet 3 (2.5 mm)
% Crude protein	45.31	38.78	32.27
% Lipid	7.25	3.27	3.58
% Crude fibre	2.53	6.75	6.49
% Ash	10.12	8.16	8.35
% Moisture	11.03	10.63	9.14
% Nitrogen Free Extract	23.76	32.41	40.17

Table 3: Proximate nutritional composition of supplements used for the study

Feed composition	Ginger powder	Garlic powder
% Crude protein	8.80	12.66
% Lipid	3.47	3.02
% Crude fibre	3.59	2.79
% Ash	4.46	4.50
% Moisture	15.42	15.19
% Nitrogen Free Extract	64.26	61.84

Figure 3: Fresh ginger used to prepare ginger supplement

Figure 4: Fresh garlic used to prepare garlic supplement

Table 4: Preparation of supplemented diets: quantities of basal diet and supplements put together to constitute the 9 treatment diets used for the study during weeks 1 to 8

Supplement	Dose (%)	Quantity of Basal diet (g)	Quantity of supplement added (g)	Total quantity of supplemented feed (g)
No supplement	0	100.0	0.0	100
Ginger powder	0.5	99.5	0.5	100
	1.0	99.0	1.0	100
	1.5	98.5	1.5	100
	2.0	98.0	2.0	100
Garlic powder	0.5	99.5	0.5	100
	1.0	99.0	1.0	100
	1.5	98.5	1.5	100
	2.0	98.0	2.0	100

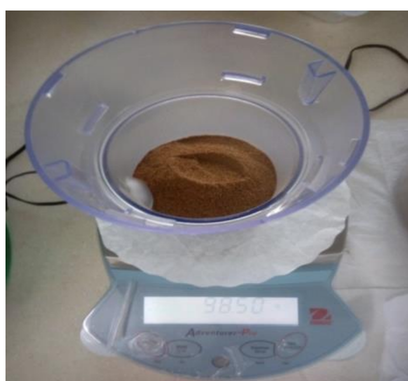

Figure 5: Basal diet-1 being weighed to prepare supplemented diet

Figure 6: Ginger powder added to basal diet -1, ready to be mixed into supplemented diet

For older fish (age from 9th to 24th week), ginger and garlic powder was incorporated into basal diets 2 and 3. First the ginger or garlic powder was soaked in chilled distilled water (in order to offset the heat during drying) for 5 minutes. The mixture was then vortexed at moderate speed (speed level = 6) using a vortex mixer (model: Vortex-Genie 2 G560 E) for three minutes to ensure uniform mixing (Guo et al., 2012).

The resulting ginger or garlic- powder in water solution was poured slowly on the basal diets from the vortexing container and immediately stirred to achieve a uniformly soaked mixture (method modified from Guo et al., 2012). The ratio of water to feed used was 19.28% (w/w). The ginger and garlic powder solution was added to the basal diets in order to prepare the four doses of supplemented feed shown in Table 5. The soaked feed was then air dried until the feed was hardened again, illustrated in Figure 7. The basal diets without supplementation served as the control. The supplemented feed pellets were prepared as needed and stored in air tight containers at room temperature until use. The final costs after formation of the treatment feed types are presented in Table 6.

Culture of Fingerlings on treatment diets

Experimental Set-up

Two hundred and seventy fishes of average weight (2.74 ± 0.18 g) were distributed randomly into the 27 individual nylon hapas at a density of 10 fish m^{-3}(Cisse,1985). Based on a completely randomized design (Bhujel, 2008). The 27 hapas were assigned into nine groups for the nine treatments with each treatment represented in each tank as shown in Table 7.

Table 5: Preparation of supplemented diets: quantities of pelletized basal diet and supplements added to constitute the 9 treatment diets used in the study from weeks 9 to 24

Supplement	Dose (%)	Quantity of Basal diet (g)	Quantity of supplement (g) vortexed in 100 ml distilled water	Total quantity of supplemented diet (g)
No supplement	0	500.0	0.0	500
Ginger powder	0.5	497.5	2.5	500
	1.0	495.0	5.0	500
	1.5	492.5	7.5	500
	2.0	490.0	10.0	500
Garlic powder	0.5	497.5	2.5	500
	1.0	495.0	5.0	500
	1.5	492.5	7.5	500
	2.0	490.0	10.0	500

Figure 7: Drying of eight supplemented diets prepared using basal diet-3

Table 6: Cost per kg (GhȻ) of basal and supplemented diets administered to *S. melanotheron*

Basal diet Used	Feed Treatments								
	Control	Ginger supplemented diets				Garlic supplemented diets			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Basal diet 1 (GhȻ)	14.00	14.52	15.04	15.55	16.07	14.41	14.84	15.23	15.64
Basal diet 2 (GhȻ)	5.15	5.71	6.27	6.84	7.40	5.60	6.06	6.51	6.97
Basal diet 3 (GhȻ)	4.00	4.57	5.14	5.70	6.27	4.46	4.92	5.38	5.84

Table 7: The arrangement of experimental units for administering nine treatments to *S. melanotheron* fingerlings

Treatments	Arrangement of replicates (Labels assigned to hapas in each tank)		
	Tank 1	Tank 2	Tank 3
Control feed	1	14	27
1.0% Garlic feed	2	18	22
0.5% Ginger feed	3	11	19
2.0% Garlic feed	4	12	26
1.5% Ginger feed	5	17	25
1.5% Garlic feed	6	10	20
2.0% Ginger feed	7	15	23
0.5% Garlic feed	8	16	24
1.0% Ginger feed	9	13	21

Administration of feed treatments

Fish were fed according to their body weight at ambient temperature as recommended by Ng & Romano (2013). The feed ration was adjusted monthly after sampling as 10%, 8%, 7%, 6%, 4.5% and 3% for the first, second, third, fourth, fifth and sixth months respectively. Feed was administered three times daily between 9:00 – 9:30 am, 12: 30 – 1:00 pm and 4:30 – 5:00pm for 6 days a week. On many occasions, (especially in the afternoons) a session was not given if previous feed was found to be floating in the hapa. Feed given was recorded daily. Figure 8 shows weighed feed for each replicate ready to be fed to fish, Figure 9 shows feed being given to the fish.

Monitoring of Water Quality

Water quality parameters were measured weekly in order to ensure that good environmental conditions suitable for the growth and health of fish were maintained. The measurements were done using a multipurpose water quality checker (model: HANNA hi 9829 multi-parameter) at 6: 30 a.m. pH, dissolved oxygen (DO), conductivity, total dissolved solids, salinity and temperature were measured by dipping the probe into each hapa and waiting for the reading to stabilize before values for each parameter were recorded. Debris was often cleared from the water surface with a scoop net in the tanks to help maintain water quality, shown in Figure 10.

Additionally, DO levels were checked at about 4: 00 pm daily during critical periods (i.e. whenever DO level below 2 mg/L was recorded in the morning). This was done in order to put in measures to enhance DO levels where needed. Remedial measures to enhance DO levels were done by releasing some amount of water from the experimental tank and topping up

Figure 8: Weighed treatment feed rations to be given to experimental fish

Figure 9: Administering of treatment diet to experimental subjects

Figure 10: Clearing of debris from water surface to maintain good water quality

with stored fresh water while agitating the water surface to enhance surface water to air interaction thereby improving DO levels.

Determination of Growth, Feed Utilisation and Physical condition of Fish

Monthly measurements of weight, standard length and total length of all fish from each treatment were taken. The weight was measured using a digital scale (model: OHAUS Adventurer ™ PRO) to the nearest 0.01 g, shown in Figure 11. The lengths were measured using measuring board for fish to the nearest 1.0 mm shown in Figure 12. All the length and weight measurements taken were used to calculate the growth feed utilisation and physiological condition of fish. Based on the changes in length and weight of fish, the growth parameters namely mean weight gain, final weight, percentage weight gain, specific growth rate and absolute growth rate were calculated. Based on the feed given and weight gained by fish in each treatment, feed utilization parameters, thus feed conversion ratio and feed efficiency were calculated. Based on the number of fish surviving and their corresponding length and weight, the physical condition parameters of survival rate, length-weight relationship and growth form were calculated. Details of the calculations are described under Data Analyses.

Determination of Blood composition and Biochemical Parameters

Sampling

After 24 weeks of culture, blood samples were drawn from 3 or 4 fish from each replicate of the feeding treatment for the determination of haematological, biochemical and immunological parameters as described by Nya and Austin (2009ab). Feeding of the fishes was suspended for 24 hours

Figure 11: Weighing of fish during sampling

Figure 12: Measuring the length of fish during sampling

prior to collection of the blood samples.

First batch of blood samples from each replicate were collected with sterile 2 mL plastic syringe by caudal vein puncture, lateral approach close to the anus level and quickly transferred into sterile test tubes coated with EDTA (Ethylenediaminetetraacetic acid) as anticoagulant (Grant, 2015). This was done gently to prevent cell lysis during the transfer. About 0.5 to 1.0 mL of blood was collected for this purpose. The blood in the tube was then gently inverted to both ends of the tube eight times to ensure proper mixing of the blood with the anticoagulant. The samples were then kept on ice packs and transported to the laboratory for analyses.

The second batch of blood samples from each replicate were also collected (about 1.5 to 2.0 mL) with 2 mL plastic syringe via the same route on different individuals and transferred quickly into serum separation tubes containing gel separator, taking care not to lyse the cells. The tubes with blood were also inverted eight times and then kept on ice packs before being transported to the laboratory for analyses. Figures 13, 14, 15, 16, 17 and 18 illustrate the blood sampling procedure.

In the laboratory, the second batches of blood samples were centrifuged at 3000 ms^{-2} for 15 minutes in a centrifuge (model: Centurion K240R) shown in Figure 19. After centrifuging, the serum samples which had

been separated clearly from the blood cells by the gel separator were collected using plastic transfer-pipettes into labelled cryogenic tubes accordingly. This is illustrated in Figure 20. The serum to be used for immunological assessment was stored at −20 °C ((Talpur & Ikhwanuddin, 2012) in a deep freezer until use. Further analyses of the samples are described

Figure 13: Anestisized fish

Figure 14: Drawing of blood from fish

Figure 15: Fully drawn blood from fish

Figure 16: Transferring blood from syringe into blood sample container

Figure 17: Blood samples; left one in serum separation tube, right one in EDTA tube

Figure 18: Blood samples placed on ice to be transported to the laboratory

in the following section.

Blood composition analyses

In accordance with previous studies (e.g. Saleh et al., 2015), the following blood constituents were determined: Haematocrit (Hct %) , Haemoglobin (Hgb; g/dl), Total erythrocyte count (RBC), Total leucocyte count (WBC), Platelet count, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC; g/dl).

The method used for analysing whole blood samples was the complete blood count (CBC) (Arnold 2013) . This was carried out using a 3-differential fully automatic blood counter (model: URIT-3000, URIT Medical Electronic Group Co. Ltd.), shown in Figure 21. The operational principle of the analyser is based on electrical impedance for WBC/ RBC/ PLT and photoelectric colorimetry for HGB. The automatic blood counter was used following the manufacturer's instructions. A detailed description of the procedure is outlined in Appendix B. After the procedure, data for the full blood count of each treatment replicate was generated.

Determination of Blood Biochemical Parameters

The following blood biochemical parameters were determined: serum glucose, cholesterol, triglycerides, total protein and albumin as recommended by Saleh et al. (2015) and Arnold (2013). Fresh serum samples were used for the determination of these biochemical parameters. A semi-automated chemistry analyser (illustrated in Figure 22) was used in conjunction with specific reagents the for determining biochemical parameters. A detailed

Figure 19: Operation of centrifuge used for centrifuging blood samples

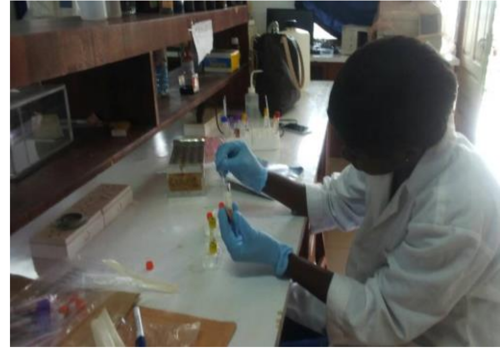

Figure 20: Separation of serum after centrifugation

Figure 21: Haematology analyser used for full blood count

Figure 22: Operation of blood chemistry analyser

description of the procedure can be found in Appendix C.

Determination of Proximate Biochemical Composition of Fish

Two fish per replicate were taken at the end of the feeding trials, degutted and stored in zip-lock bags on ice and transferred to the School of Agriculture, technology laboratory of the University of Cape coast. At the laboratory, the fins and scales were removed, pooled together and homogenized for the determination of the compositions: moisture, total protein, lipid, ash and carbohydrates. These proximate compositions were determined according to the Association of Official Analytical Chemist (AOAC, 2005) standard methodology (see Appendix A for outline of detailed procedure). Figure 23 shows the soxhlet apparatus used for extraction of Lipid in the determination of proximate composition of fish.

Assessment of Immunological Status using Serum Lysozyme Activity

The serum lysozyme activity of the fish was determined using fish lysozyme (LZM) Enzyme Linked Immuno Sorbent Assay (ELISA) kit, Catalogue number: SL0050Fi (96 tests) SUNLONG BIOTECH CO Ltd. for assaying LZM levels in serum, plasma, culture media or any biological fluid. A picture of the ELISA kit is shown in Figure 24.

Sample preparation

The serum samples which had been frozen at -20°C were removed from the freezer on the day of the analyses, allowed to thaw for two hours at room temperature and vortexed at high speed for 3 minutes to ensure a uniform solution. The detailed procedure for the assay is outlined in Appendix D. Figures 25, 26 and 27 illustrate some steps in the assay procedure.

Figure 23: Soxhlet apparatus used in extracting lipid during proximate analyses of fish

Figure 24: ELISA KIT used to assess lysozyme activity in fish serum

Figure 25: Pipetting solution into wells of ELISA plate

Figure 26: Step 5 in the assay procedure; TMP added to substrate to elicit colour change from colourless to blue

Figure 27: ELISA plate reader ready to read the optical absorbance in the wells of the completed assay (depicted by yellow colour in the wells)

Determination of serum lysozyme activity

After the assay, an output of the absorbance values for the standard and sample solution was obtained via the ELISA plate reader (model = Thermo scientific MULTISKAN EX). To obtain the concentration of lysozyme in the samples, regression analyses of the standard concentrations against their corresponding absorbance was first done to estimate the relationship and generate a predictive model equation. The equation generated, Equation 1 was very strong ($R^2 = 0.9106$).

$$Lysozyme\ concentration = (77.712\ X\ Absorbance) - 1.6344 \quad (1)$$

This relationship was then used to calculate the concentration of lysozyme in the samples based on the absorbance recorded for each sample. After the concentration values were obtained, an average was calculated from each duplicate sample to obtain the lysozyme concentration for the 27 samples tested under the nine treatments (3 replicate per treatment).

Determination of cost per kg of supplemented diet

The costs of the basal diets, the supplements and the supplemented diet were determined using mathematical deductions. First, the cost per gram of supplements was determined using Equation 2.

$$Cost\ per\ kg\ of\ supplement = \frac{Cost\ of\ raw\ material\ (GHC)}{Quantity\ of\ powder\ (Kg)} \quad (2)$$

Then the cost of the basal diet was determined using Equation 3.

$$Cost\ per\ kg\ of\ basal\ diet = \frac{Cost\ of\ feed\ (GHC)}{Quantity\ of\ feed\ (Kg)} \quad (3)$$

After constituting the treatment diets, the cost per Kg of the supplemented diets was calculated based on the quantities of basal diet and supplements used using Equation 4.

$$Cost\ of\ 1\ kg\ \ of\ supplemented\ diet = \\ Cost\ of\ required\ Kg\ of\ basal\ diet + Cost\ of\ required\ Kg\ of\ supplement \quad (4)$$

Data Analyses

All data analyses in this study were done using data analyses software's Minitab version 17 and Microsoft Excel 2010. The significance for all analyses was examined at a probability level of 5%. Tukey-pairwise comparison was used to assess differences when a significant difference was found. Table 8 indicates the predictors and response variables used in the analyses of variance (ANOVA) test.

Effect of Feeding Treatments on Growth Performance and Feed Utilization of *S. melanotheron*

The following parameters were used as indicators of growth of the fish during the experiment: mean weight gain (MWG), per cent weight gain (PWG), specific growth rate (SGR), absolute growth rate (AGR), feed conversion ratio (FCR), feed efficiency ratio (FER), condition index (CI), survival rate (SR) and growth form (b). These parameters were calculated using formulae extracted from previous studies (e.g. Stickney 2000; Saleh et al., 2015).

Mean weight gain (MWG) expresses the average weight gained by the fish over the experimental period and was calculated as shown in Equation 5, as recommended by Saleh et al., (2015).

$$MWG = Final\ mean\ weight - Initial\ mean\ weight \quad (5)$$

The mean weight was achieved by averaging the weights of all individuals in a

Table 8: Factors considered to compare effect of ginger and garlic feed supplements using ANOVA on *S. melanotheron*.

Effect examined	Discriminant	Predictor	Response variables
Growth and feed utilisation	Ginger vs. Garlic supplements	Varying % of ginger Varying % of garlic pH dissolved oxygen conductivity total dissolved solids salinity temperature	FMW, MWG, PWG, AGR, SGR, FCR, FER, SR, CI, b
Proximate biochemical composition			Moisture content, Dry weight, fat content, Protein content, Ash content, Fibre content, NFE
Blood composition			RBC, WBC, HCT, MCV, MCH, HGB, MCHC, PCT
Serum biochemical composition			Glucose, total protein, Albumin, globulin, cholesterol, triglyceride
Immune status			Serum lysozyme activity
Cost effectiveness			Cost per kg of feed
Abiotic water parameters			pH, dissolved oxygen, conductivity, total dissolved solids, salinity, temperature

Note: FMW- final mean weight, MWG- mean weight gain, PWG-percentage weight gain, AGR- absolute growth rate, SGR-specific growth rate, FCR-feed conversion ratio, FER-feed efficiency ratio, NFE- nitrogen free extract, RBC- erythrocyte count, WBC – leucocyte count, HCT- haematocrit, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, PCT- platelet count.

particular replicate of each treatment.

Percentage weight gain (PWG) expresses the mean weight gain as a percentage of the initial mean weight of the fish during the experimental

period and was calculated using Equation 6 as recommended by Saleh et al. (2015) :

$$PWG = \frac{Final\ mean\ weight - Initial\ mean\ weight}{Initial\ mean\ weight}\ X\ 100 \qquad (6)$$

Specific growth rate (SGR) is the percentage daily weight gain of the fish. It was calculated using Equation 7 (Stickney, 2000; Saleh et al., 2015):

$$SGR = \frac{\text{Ln}\ final\ mean\ weight - \text{Ln}\ initial\ mean\ weight}{number\ of\ days} \times 100 \qquad (7)$$

AGR is defined as the increment of weight over a known time interval and was calculated using Equation 8 (Stickney, 2000):

$$AGR = \frac{Final\ mean\ weight - Initial\ mean\ weight}{number\ of\ days} \times 100 \qquad (8)$$

Feed conversion ratio (FCR) expresses the quantity of feed needed to produce a unit weight gain by the fish. This was calculated for each treatment using Equation 9 (Stickney, 2000; Saleh et al., 2015):

$$FCR = \frac{Weight\ of\ Feed\ given}{Weight\ gained} \qquad (9)$$

Feed efficiency ratio (FER) indicates how much of the feed given to the fish was actually used to produce flesh. This was calculated for each treatment using the Equation 10 (Stickney, 2000):

$$\text{FER} = \frac{Weight\ gain}{Weight\ of\ feed\ fed} \qquad (10)$$

After calculating the various parameters, the values for the treatments were subjected to one-way analyses of variance (ANOVA, $p < 0.05$) in order to compare the effects of the different feeding treatments on each parameter.

The physical condition of the fish was assessed using the survival rate, condition index and the growth form. The calculations of these parameters are described below.

Survival rate (SR) expresses the percentage of number individuals harvested to the initial number of individual stocked. This was calculated using Equation 11 according to Saleh et al. (2015).

$$SR = \frac{Number\ of\ surviving\ fish}{Initial\ number\ of\ fish\ stocked}\ X\ 100 \qquad (11)$$

The condition index (CI) describes the state of well-being or plumpness of the fish. This was assessed using the Fulton's CI index in Equation 12 as employed by Hassanin et al. (2014):

$$CI = \frac{Weight}{(Standard\ length)^3} \times 100 \qquad (12)$$

The Growth form (b) describes the type of development that is peculiar to a fish species. This growth form according to Froese (2006) is the exponent of the arithmetic form of the length–weight relationship ($W = aL^b$), and the slope of the regression line in the logarithmic form (Log W = Log a + b Log L).

In this study, the b was determined using the relationship between the length (L in mm) and weight (W in g) in the regression Equation below:

$$\log W = \log a + b \log L \qquad (13)$$

where, a is the y- intercept, b – slope of the relationship. To use this equation (13), the lengths and weights recorded in this study were log transformed. This was done to normalise the data. In order to confirm the significance of this

relationship for the different feeding treatments, the slope and the coefficient of determination (R^2) were used. It was assumed that the relationship was significant if the slope was significantly different from zero (Student's t test at alpha = 0.05) and $R^2 \geq 0.5$.

Under natural conditions, *S. melanotheron* grows isometrically, i.e., the length of the fish grows proportionally to its weight (Ndimele, Kumolu-Johnson, Aladetohun & Ayorinde, 2010). For this form of growth, b is expected to be equal to 3 (Froese, 2006). This assertion was investigated for the fish by comparing the gradient observed in this study to 3. The comparison was done using Student's t-test at a significance level of $p < 0.05$.

In order to investigate whether the different treatments affected the growth form of the fish, another Student's *t*-test was carried out to compare the b values observed under each feeding treatment with that of the control. All the tests were performed on the log transformed length-weight data, using Microsoft Excel version 2010.

Effects of Feeding Treatments on Proximate Biochemical Composition of *S. melanotheron*

The effect of the different supplements supplied at five different dosages including the control on the final proximate body composition parameters (moisture, protein, fat, minerals, fibre, and carbohydrate) were compared using a two-way ANOVA. The tests were done at a significance level of $p < 0.05$.

Effects of Feeding Treatments on Physiological Defence Mechanisms of *S. melanotheron*

To assess the effects of the treatments on the composition of blood in the fish, the blood constituents of the different treatments obtained via the complete blood counter were compared using a two-way ANOVA ($p < 0.05$).

The biochemical constituents, i.e. serum glucose, total protein, albumin, globulin, cholesterol and triglyceride levels of the blood extracted from the fish were also compared separately for the different treatments using a one-way ANOVA. The tests were done at a significance level of $p< 0.05$.

Serum lysozyme activity in the fish under the various treatments was also compared using a two-way ANOVA. For this analysis, lysozyme activity measured at the different supplement concentrations constituted the subordinate classification nested within the different feeding treatments. The mean values were considered statistically significantly at $p < 0.05$.

The Cost of supplemented diet versus control

In order to establish the usefulness of the different dietary supplements, the cost effectiveness of the treatments was assessed. This was done by comparing the cost per Kg of control diet and supplemented diets for the three stages of feed used in the experiment. The costs for the different treatments were compared by using one-way ANOVA. The p-value of the analysis was set at 0.05.

Chapter Summary

This chapter began with a description of the study site, followed by the production of fingerlings to the preparation of supplements and treatment diets. How the experiment was set-up and how treatment diets were administered to experimental subjects was also described. Sampling procedures for the assessment of growth, feed utilisation, physical condition, proximate composition, blood composition, serum biochemical and serum lysozyme activity were outlined. Procedures for data analyses were also presented together with the computer software used for analysing data.

CHAPTER FOUR

RESULTS

In this study an experiment was conducted to investigate the effectiveness of using ginger and garlic as alternative dietary supplements for boosting the growth and health status of *S. melanotheron*. This chapter describes the results of the experiment. In intensive aquaculture, synthetic chemicals including antibiotics are commonly used for promoting fish growth and health. The use of these chemicals however suppresses the natural immunity of the fish and may lead to many side effects.

Abiotic Conditions in the Experimental Set-Up

A summary of the abiotic water quality conditions observed from the experimental treatments are presented in Table 9. The observed values were subjected to a one-way ANOVA (see Appendix E for details). Values presented in table are means (± SE) from the weekly records over the experimental period.

The pH recorded in the study ranged from 6.01 to 9.12 with a mean of 7.07 ± 0.06. There was no significant difference between the pH values measured in the different treatment hapas (one-way ANOVA: $F_{674} = 0.07$; $p = 1.00$).

The maximum and minimum DO recorded in the study period was 4.00 mg/L and 1.71 mg/L respectively and averaged 2.41 ± 0.07 mg/L. There was also no significant difference between the DO values recorded in the hapas used for the different feed treatments (one-way ANOVA: $F_{674} = 0.07$; $p = 1.00$).

Table 9: Water quality parameters recorded in the hapa-in-tank system during experimental period

Water quality parameters		Feed Treatments								
		Control	Ginger supplements				Garlic supplements			
		0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
pH	Min.	6.12	6.15	6.08	6.02	6.10	6.15	6.42	6.01	6.04
	Max.	8.89	8.97	8.17	8.97	9.02	8.71	8.95	8.98	8.91
	Mean (± SE)	7.07 ± 0.06	7.05 ± 0.05	7.06 ± 0.05	7.09 ± 0.06	7.07 ± 0.06	7.09 ± 0.05	7.08 ± 0.05	7.07 ± 0.05	7.05 ± 0.06
Dissolved Oxygen (DO) / (mg/L)	Min.	1.73	1.72	1.72	1.72	1.71	1.72	1.72	1.72	1.71
	Max.	4.00	3.99	3.82	3.9	3.98	3.77	3.92	3.91	3.98
	Mean (± SE)	2.41 ± 0.07	2.42 ± 0.07	2.38 ± 0.07	2.48 ± 0.07	2.40 ± 0.07	2.38 ± 0.07	2.41 ± 0.07	2.41 ± 0.07	2.42 ± 0.07
Conduc-tivity (µS/cm)	Min.	231	234	227	216	211	204	213	212	230
	Max	1817	1838	1819	1833	1773	1773	1799	1843	1783
	Mean (± SE)	957.61 ± 54.66	950.51 ± 55.85	956.30 ± 55.03	956.55 ± 55.21	950.75 ± 55.12	948.47 ± 55.88	949.04 ± 55.86	950.49 ± 56.41	956.19 ± 55.09
Total Dissolved Solids (TDS)/ ppm	Min	119	117	114	108	105	111	107	106	115
	Max	809	817	810	815	801	798	801	819	806
	Mean (± SE)	445.19 ± 25.18	440.59 ± 25.53	444.71 ± 25.01	443.66 ± 25.49	442.48 ± 25.34	444.72 ± 25.05	441.22 ± 25.75	441.87 ± 25.42	443.43 ± 25.22
Salinity (PSU)	Min	0.11	0.11	0.11	0.10	0.10	0.11	0.10	0.10	0.11
	Max	0.8	0.81	0.81	0.81	0.8	0.79	0.8	0.82	0.81
	Mean (± SE)	0.43 ± 0.03	0.43 ± 0.03	0.44 ± 0.03	0.43 ± 0.03	0.43 ± 0.03	0.44 ± 0.03	0.43 ± 0.03	0.43 ± 0.03	0.43 ± 0.03
Tempe-rature (^{o}C)	Min	26.59	26.55	26.62	26.59	26.63	26.63	26.59	26.59	26.59
	Max	31.51	31.54	31.50	31.50	31.46	31.45	31.47	31.55	31.39
	Mean (± SE)	29.20± 0.16	29.21 ± 0.16	29.21 ± 0.16	29.21 ± 0.16	29.22 ± 0.16	29.22 ± 0.16	29.21 ± 0.16	29.22 ± 0.16	29.21 ± 0.15

Note: pH- hydrogen ion concentration

Conductivity levels in the tanks for the experiments ranged from 204 μs/cm to 1843 μS/cm with a mean of 953.27 ± 55.86 μs/cm., the differences between the conductivity values in hapas of the different feed treatments were not significant (one-way ANOVA: $F_{674} = 0.00$; $p = 1.00$).

The minimum TDS recorded was 105 ppm, a maximum of 819 ppm and average of 443.10 ± 25.53 ppm with no significant difference between the measurements of the different treatments at 5% level of probability (One-way ANOVA: $F_{674} = 0.00$; $p = 1.00$).

The salinity levels in the culture environment ranged from 0.10 PSU to 0.82 PSU around a mean of 0.43 ± 0.03 PSU and the differences in salinity measured in the hapas used for the treatments were not significant (One-way ANOVA: $F_{674} = 0.01$; $p = 1.00$).

The highest water temperature recorded during the experiment was 31.39^{o}C and the lowest was 26.55^{o}C. The mean water temperature was 29.21 ± 0.16^{o}C. There were no significant differences in water temperature values measured in the hapas used for the different treatments (One-way ANOVA: $F_{674} = 0.00$; $p = 1.00$).

Growth Performance and Feed Utilization of Fish

General Observations

Some observations made on the behaviour of fish in response to feeding and one case of disease incidence was noted and is further described below.

Response to Feeding

Fish response to feed was impressive during the first 4 weeks. It was observed that they readily ingested the basal diet 1 (47% CP, 0.2 - 0.3mm)

irrespective of the feeding treatment. In the second month, feeding aggressiveness was observably reduced, compared to the first month even though they were taking the same feed type. When feed type was changed in the third month to basal diet 2 (40% CP, 2.0 mm), it took some time before the fish adjusted to picking the pellets. When they did, it was not as aggressive as they ingested the basal diet 1. This behaviour continued throughout the rest of the culture period. However there were occasional improvements where in some replicates the fish began to immediately ingest the food items as soon as they were given. In the fourth month, feeding response improved among all the treatments. This declined gradually during the fifth month and stabilised during the last four weeks where greater part of feed given in the morning could remain uneaten throughout the day. It was also notably observed on several occasions that fish picked a sinking feed faster than they did the floating feed. When feed given in an earlier session was still present when the next session was due, feed was not administered to avoid accumulation of uneaten feed which could decompose and cause the water quality to deteriorate.

Incidence of disease

One case of diseased fish was recorded in the ginger 1% group. Symptoms observed were eroded fins, skin lesions and slow movement when catching it indicating morbidity. The diseased fish was removed from the hapa and bathed in saline water (concentration: 2 PSU) for 10 minutes and further isolated in a recovery tank for three weeks. After it recovered (with visible signs of lesion healed, re-developed fins and increased aggressiveness), the fish was returned to the experimental stock. But it had lost weight from 50.25g

to 47.82g even though it was fed the portion of its ration during the recovery time.

The growth performance and utilization of feed fortified with ginger and garlic by the fish is summarised in Table 10. Details of these results are provided below in Appendix F.

Initial mean weight (IMW)

The initial mean weight of fish stocked for the different experimental treatments averaged 2.74 ± 0.10 g with no significant differences between them (One-way ANOVA: $F_{269} = 0.04$, $p = 1.00$).

Final mean weight (FMW)

After administering different concentrations of the supplements to the fish , the highest FMW was recorded in the garlic 1.5% group (FMW = 63.03 ± 3.99g) and the lowest was recorded when the feed contained 1% ginger (FMW = 53.56 ± 2.42g). However, these differences were not significant. There was also no significant differences amongst all the feeding treatments (One-way ANOVA: $F_{261} = 0.79$, $p = 0.61$). This suggests that the fish attained similar final weights under the different treatments.

Mean weight gain (MWG)

The MWG by the fish in the 24 weeks of experimentation ranged from 50.85 ± 6.120g in ginger 1% group to 60.29 ± 6.66g in garlic 1.5% group. However, the differences in the MWG of the treatments were not significant (One-way ANOVA: $F_{26} = 0.20$, $p = 0.99$), suggesting that the effects of the different feeding treatments on the average weight of the fish were similar.

Table 10: Growth and feed utilization parameters (mean ± standard error) of the nine experimental treatments after 24 weeks of feeding supplemented diets

Growth and feed utilization parameter	Feed treatments								
	Control	Ginger supplements				Garlic supplements			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Initial mean wt. (g /fish)	2.75 ± 0.18[a]	2.70 ± 0.18[a]	2.74 ± 0.23[a]	2.78 ± 0.18[a]	2.76 ± 0.18[a]	2.75 ± 0.13[a]	2.74 ± 0.14[a]	2.73 ± 0.15[a]	2.74 ± 0.21[a]
Final mean wt. (g /fish)	60.35 ± 3.04[a]	59.28 ± 3.37[a]	53.56 ± 2.42[a]	59.14 ± 2.40[a]	60.37 ± 3.73[a]	61.76 ± 3.02[a]	61.46 ± 2.50[a]	63.03 ± 3.99[a]	61.57 ± 3.27[a]
MWG (g /fish)	57.20 ± 6.02[a]	58.24 ± 4.14[a]	50.85 ± 6.12[a]	56.43 ± 1.14[a]	58.23 ± 8.05[a]	59.37 ± 7.73[a]	58.77 ± 5.66[a]	60.29 ± 6.66[a]	58.82 ± 7.32[a]
PWG (% /)	2107.04 ± 220.90[a]	2155.20 ± 141.99[a]	1916.66 ± 261.59[a]	2092.68 ± 37.24[a]	2124.86 ± 303.05[a]	2206.26 ± 282.19[a]	2171.68 ± 201.15[a]	2223.31 ± 228.22[a]	2179.82 ± 300.33[a]
SGR (% /day)	3.48 ± 0.10[a]	3.51 ± 0.07[a]	3.38 ± 0.13[a]	3.48 ± 0.02[a]	3.49 ± 0.14[a]	3.52 ± 0.13[a]	3.51 ± 0.09[a]	3.54 ± 0.10[a]	3.51 ± 0.13[a]
AGR (g /day)	0.43 ± 0.12[a]	0.42 ± 0.08[a]	0.38 ± 0.10[a]	0.41 ± 0.07[a]	0.44 ± 0.13[a]	0.44 ± 0.11[a]	0.43 ± 0.09[a]	0.46 ± 0.14[a]	0.44 ± 0.11[a]
FCR	2.39 ± 0.28[a]	2.27 ± 0.11[a]	2.46 ± 0.24[a]	2.27 ± 0.02[a]	2.23 ± 0.28[a]	2.41 ± 0.19[a]	2.41 ± 0.14[a]	2.24 ± 0.20[a]	2.24 ± 0.25[a]
FER	0.43 ± 0.05[a]	0.44 ± 0.02[a]	0.41 ± 0.04[a]	0.44 ± 0.00[a]	0.46 ± 0.05[a]	0.42 ± 0.03[a]	0.42 ± 0.02[a]	0.45 ± 0.04[a]	0.46 ± 0.05[a]

Note: Values with the same alphabets as superscripts on the same row are not significantly different (one-way ANOVA, $p < 0.05$). MWG- mean weight gain; PWG- percentage weight gain; SGR- specific growth rate; AGR – absolute growth rate; FCR – feed conversion ratio; FER- feed efficiency ratio.

Percentage weight gain (PWG)

The maximum PWG was recorded when garlic constituted 1.5% of the feed given to the fish (PWG = 2223.31± 228.22%). The minimum amount of weight gain (PWG = 1916.66± 261.59%) was recorded when 1% of ginger was used. Similar to the MWG observation, there was no significant differences amongst the nine feeding treatments (One-way ANOVA: F_{26} = 0.15; p = 0.99).

Feed conversion ratio (FCR)

The lowest (best) FCR value of 2.24 ± 0.20 was recorded in the garlic 1.5% group and the highest (worst) FCR value 2.46 ± 0.24 was recorded in the ginger 1% group. However, the differences between the FCRs of the nine feeding treatments were not significant (One-way ANOVA: F_{26} = 0.20; p = 0.98).

Feed efficiency ratio (FER)

The highest FER (= 0.46 ± 0.05) among the nine treatments was recorded when both ginger and garlic constituted 2% of the diet.. The least FER (= 0.41 ± 0.04) was recorded in the ginger 1.0% group. Nonetheless, the FER values of the eight supplemented diets were not significantly different from the control group value (One-way ANOVA: F_{26} = 0.24; p = 0.98).

Specific growth rate (SGR)

Generally, the SGR of the fish was higher when the feed given was enriched with garlic. The garlic group attained 0.86 to 1.72% higher SGR than the control. In the ginger group, the remaining dosages were In decreasing order, SGR values recorded for the garlic group were 3.54 ± 0.10 % day^{-1}

(1.5% garlic) > 3.52 ± 0.13% day^{-1} (0.5% garlic) > 3.51 ± 0.09 % day^{-1} (1% garlic) > 3.51± 0.13 % day^{-1} (2% garlic). In the ginger group, the highest increase in SGR of 0.86% over the control SGR was recorded when 0.5% of the supplement was used and the lowest SGR recorded in the 1% ginger was 2.87% lower than the control SGR. These differences in SGRs were however not significant (One-way ANOVA: $F_{26} = 0.18$; $p = 0.99$).

Absolute growth rate (AGR)

As recorded in the SGR, the lowest mean AGR value was obtained by ginger 1% group (11.62% less than the control) and the highest mean AGR recorded by garlic 1.5% group (6.98% more than the control AGR). The apparent differences were however not significant amongst the AGRs of the different feed treatments (One-way ANOVA: $F_{26} = 0.04$; $p = 1.00$).

Physical Condition of Fish

There was one case of disease infection observed throughout the study period (see Figure 28). A total of eight mortalities were recorded from five of the feed treatments (see results under the survival rate). A sample of the mortalities is shown in Figure 29. However there was no observable sign of disease infection on the dead fishes. It can therefore be said that the physical condition of the fish in all the feeding treatments was generally good. Table 11 is a summary of the parameters used as indicators for the physical condition of the fish, i.e., survival rate, condition index and growth form of the fish depicted by the relationship between the length and weight of the animal. Detailed results on these indices are described below.

Table 11: Physical condition indicators (mean ± standard error) of *S. melanotheron* cultured on different dietary supplements

Physical Parameters	Feed Treatments								
	Control	Ginger supplements				Garlic supplements			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
SR (%)	96.67 ± 2.17[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	96.67 ± 2.17[a]	86.67 ± 3.53[a]	96.67 ± 2.17[a]	96.67 ± 2.17[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
CI	3.89 ± 0.07[b]	4.06 ± 0.07 [ab]	3.85 ± 0.05[b]	4.02 ± 0.04[ab]	3.90 ± 0.05[ab]	4.02 ± 0.07[ab]	4.02 ± 0.04[ab]	4.19 ± 0.07[a]	4.04 ± 0.06[ab]
B	3.02	3.10	3.01	3.03	3.01	3.04	3.08	3.07	3.04

NOTE: Means that do not share the same letter as superscripts in a row are significantly different. SR-survival rate; CI- condition index; b – growth form (co-efficient of

Figure 28: Diseased fish observed under ginger 1% treatment

Figure 29: Samples of mortalities recorded in the study

Survival Rate (SR)

Survival rate was 100% in four of the treatment groups, namely ginger 0.5%, ginger 1%, garlic 1.5% and garlic 2% groups. Four groups recorded one mortality each and achieved a survival rate of 96.67 ± 2.17. These were the control, ginger 1.5%, garlic 0.5% and garlic 1.0% groups. Only ginger 2.0% group recorded 4 mortalities during the experimental period and achieved the lowest survival rate of 86.67 ± 3.53%. However, there were no significant differences between the survival rates of the fish raised under the different feeding treatments (One-way ANOVA: $F_{26} = 1.32$; $p = 0.29$).

Condition Index (CI)

The CI of the fish raised on the feed containing 0.5%, 1.5% and 2% ginger as supplements was similar to that of the fish cultured with 0.5%, 1% and 2% garlic as supplements. This suggests that both garlic and ginger had similar effects on the physiological condition of the fish when they constituted 0.5 to 2% of the feed given to the fish. The CI for the 1.5% garlic group was 3.53% higher than the control CI, and this was significantly highest particularly when compared with the control and ginger 1% group (One-way ANOVA: $F_{26} = 3.45$; $p = 0.001$).

Length-Weight relationship (L-WR)

The effect of the different feeding treatments on the morphological growth of the fish was examined using the linear regression model (Equation 13). The model examined the relationship between the length and weight of the fish. The relationship was considered significant if $R^2 \geq 0.5$ and the slope was

significantly different from zero (Student's t test at alpha = 0.05) for the different feeding treatments. Table 12 shows the results of this test.

The length-weight relationship was linear and strong ($R^2 \geq 0.5$) for all the nine experimental treatments (see Table 12). The slopes of the relationship observed under the different treatments were not statistically different; in addition, the y-intercepts for the relationship were similar (t-test at $p < 0.05$). As a result, a single plot was used to represent the length-weight relationship of all the experimental treatments in Figure 30.

The slope (b) estimated for the combined treatments was 3.0493, which was significantly higher than zero (one-sample t-test: t = 488.79, p = 0.00). In addition, over 99% of the variability in weight of the fish could be explained by the changes in their length ($R^2 > 0.99$). Given that the b value is equal to 3 (one sample t-test; $t = 4.20$, p = 0.003; see Appendix M) for all the treatments, the growth of *S. melanotheron* in this study can be said to have been isometric.

Proximate Nutritional Composition of Harvested Fish

The proximate composition of moisture, dry matter, ash, protein, fat, fibre and carbohydrate content of fish harvested from the nine treatments are given in Table 13. A two-way analysis of variance was used to examine the effects of the different concentrations (0%, 0.5%, 1.0%, 1.5% or 2%) of the different supplements (ginger or garlic) used for the culture of the fish (see Appendix G). The levels of the individual biochemical constituents were all different for the different supplements. Similarly, the levels observed under the different concentrations of the supplements were different. As a result an

Table 12: Length-weight relationship parameters of *S. melanotheron* cultured on different dietary supplements

Length-weight relation-ship Parameter	Feed Treatments								
	Control	Ginger supplements				Garlic supplements			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
R^2	0.991	0.991	0.993	0.993	0.992	0.991	0.995	0.991	0.992
t_1-value ($\alpha = 0.05$)	155.05	156.08	174.71	172.42	158.45	157.73	220.61	155.90	161.68
b	3.022	3.102	3.010	3.035	3.016	3.044	3.085	3.072	3.040
t_2-value ($\alpha = 0.05$)	0.00	0.026	0.004	0.004	0.002	0.007	0.021	0.017	0.006
a	-4.816	-4.980	-4.800	-4.842	-4.808	-4.855	-4.940	-4.910	-4.850
N	207	210	210	205	192	209	207	210	210

NOTE: Means that share the same letter as superscripts in a row are not significantly different from the control. R^2- coefficient of determination; t_1-value – students t-test for confirming significant relationship between length and weight (slope ≠ 0); b – growth form; t_2-value - students t-test for comparing slopes of the different treatments with the control; a - y-intercept; n - number of individuals.

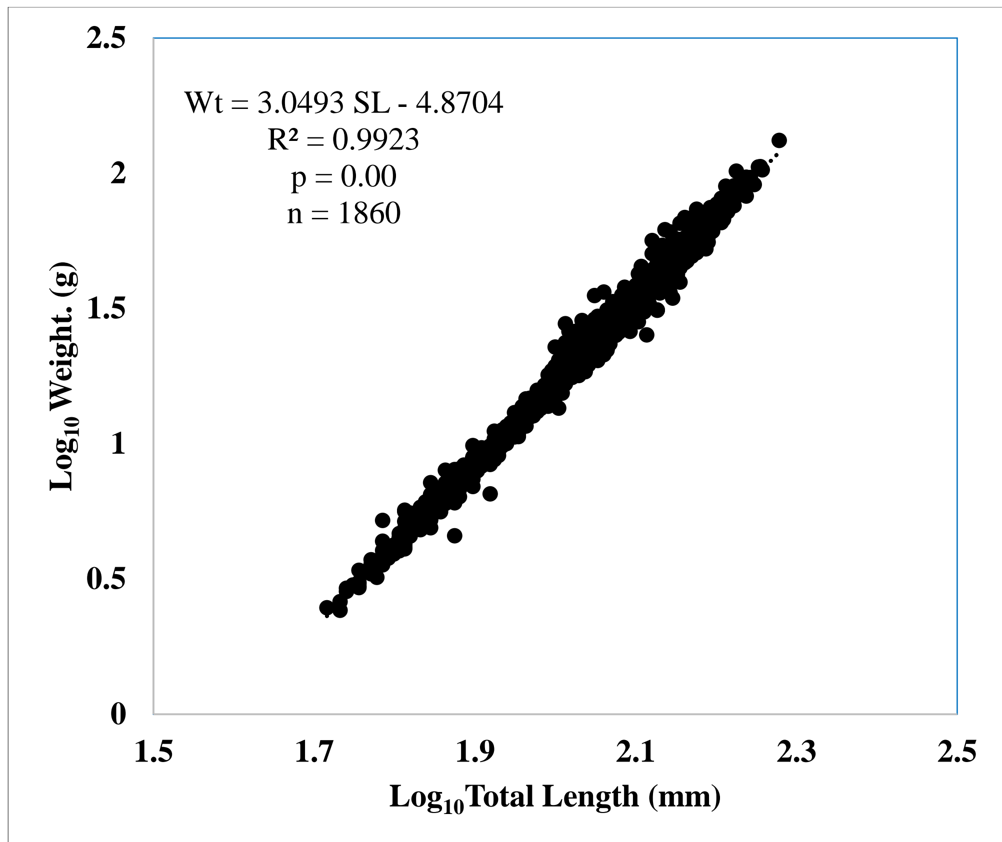

Figure 30: The relationship between the length and weight of black chin tilapia (S. melanotheron) fed on diets either enriched with supplements (0.5 – 2% of ginger and garlic) or without supplements (control) for 24 weeks. n = 1860 observations consisting of 207 observations for control group; 817 observations for ginger group and 836 observations for garlic group.

Interaction between the supplements ant their concentrations had significant effect on all the biochemical constituents of the fish (see Appendix G). The detailed description of these results is provided in the following section.

Moisture and Dry Matter content in fish

The mean moisture content in fresh fish was lowest in garlic 1.5% group being 8.9% less than that of the control and this was significantly lower than the than that of the control and the highest moisture content recorded in ginger 0.5% group (3.6% higher than control group moisture content). Concurrently, the lowest dry matter content of fresh harvested fish from the study (11.3% less than control) recorded in the ginger 0.5% group was significantly lower than the control and garlic 1.5% group (27.6% more than control group) (two-way ANOVA: dosage $F_{29} = 49.70$; $p = 0.00$, supplement $F_{29} = 328.86$; $p = 0.00$, dosage x supplement $F_{29} = 48.15$; $p = 0.00$; see Appendix G).

Ash content in fish

The mean ash content recorded in all the ginger supplemented groups was statistically similar to that of the control group. However, in the garlic groups, 0.5% and 1.5% recorded significantly higher ash content (63.9% and 59.3% respectively more than control group). However, the ash content of the 1% and 2% garlic groups were similar to that of the control (two-way ANOVA: dosage $F_{29} = 21.52$; $p = 0.00$, supplement $F_{29} = 112.73$; $p = 0.00$, dosage x supplement $F_{29} = 16.81$; $p = 0.00$ see Appendix G).

Table 13: The proximate nutritional composition (mean ± standard error) of *S. melanotheron* after dietary supplementation for 24 weeks

Parameters	Feed Treatments								
	Control	Ginger supplements				Garlic supplements			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
% Moisture content	75.65 ± 0.29^{b}	78.39 ± 0.17^{a}	78.31 ± 0.13^{a}	75.71 ± 0.43^{b}	75.21 ± 0.41^{b}	69.32 ± 0.55^{d}	75.82 ± 0.48^{b}	68.92 ± 0.14^{d}	72.33 ± 0.50^{c}
% Dry matter content	24.35 ± 0.29^{c}	21.61 ± 0.17^{d}	21.69 ± 0.13^{d}	24.29 ± 0.43^{c}	24.79 ± 0.41^{c}	30.68 ± 0.55^{a}	24.18 ± 0.48^{c}	31.08 ± 0.14^{a}	27.67 ± 0.50^{b}
% Ash content	2.77 ± 0.09^{bc}	2.64 ± 0.17^{c}	2.26 ± 0.13^{c}	2.55 ± 0.21^{c}	2.38 ± 0.15^{c}	4.54 ± 0.19^{a}	2.53 ± 0.24^{c}	4.43 ± 0.06^{a}	3.44 ± 0.09^{b}
% Protein content	58.68 ± 0.43^{d}	58.77 ± 0.54^{cd}	59.79 ± 0.37^{cd}	65.13 ± 0.42^{a}	60.92 ± 0.45^{bc}	60.40 ± 0.08^{bcd}	56.00 ± 0.21^{e}	62.40 ± 0.49^{b}	60.30 ± 0.68^{bcd}
% Fat content	22.47 ± 0.15^{b}	21.61 ± 0.12^{c}	22.64 ± 0.21^{b}	20.74 ± 0.31^{d}	21.81 ± 0.19^{bc}	23.75 ± 0.17^{a}	23.70 ± 0.08^{a}	23.76 ± 0.10^{a}	23.55 ± 0.07^{a}
% Fibre content	0.75 ± 0.01^{a}	0.48 ± 0.03de	0.50 ± 0.00de	0.54 ± 0.02^{bcd}	0.61 ± 0.01^{b}	0.60 ± 0.01^{bc}	0.74 ± 0.03^{a}	0.53 ± 0.02^{cd}	0.46 ± 0.01e
% NFE content	11.46 ± 0.25^{ab}	12.65 ± 0.37^{a}	11.16 ± 0.22^{ab}	6.70 ± 0.65^{de}	9.69 ± 0.61^{bc}	7.08 ± 0.27^{d}	13.43 ± 0.45^{a}	4.67 ± 0.43^{e}	8.41 ± 0.86^{cd}

Means that do not share a letter as superscript in a row are significantly different (two-way ANOVA, $p < 0.05$). NFE- nitrogen free extract, representing the carbohydrate content.

Protein content in fish

There was a significant difference in the mean protein content of harvested fish (two-way ANOVA: dosage $F_{29} = 53.95$; $p = 0.00$, supplement $F_{29} = 15.65$; $p = 0.00$, dosage x supplement $F_{29} = 12.18$; $p = 0.00$; (see Appendix G) among the various treatments. Two ginger groups (1.5 and 2%) and one garlic group (1.5%) recorded significantly higher protein content in harvested fish than the control ranging from 3.8% to 11.0% more than the control group. The lowest protein content was recorded in garlic 1% group (4.6% less than the control).

Fat content in fish

The mean fat content was significantly higher in all the garlic treatments (ranging from 4.8 % to 5.7 % higher) than the control group content. On the other hand, two ginger groups (1 and 2%) had similar fat content to the control whiles the remaining two groups of ginger (0.5 and 1.5%) recorded significantly lower fat content than the control and garlic treatments (two-way ANOVA: dosage $F_{29} = 8.19$; $p = 0.00$, supplement $F_{29} = 222.22$; $p = 0.00$, dosage x supplement $F_{29} = 22.73$ $p = 0.00$ see Appendix G).

Fibre content in fish

Mean fibre content in the fish harvested was significantly highest in the control group. With the exception of garlic 1% group which had similar fibre content to the control, the other garlic treatments recorded significantly reduced fibre content in the range of 20.0 to 38.7 % less than the control. Comparatively, all the ginger treatments also reduced fibre content significantly in the region of 18.7 to 36.0 % less than the control (two-way

ANOVA: dosage $F_{29} = 73.43$; $p = 0.00$, supplement $F_{29} = 16.77$; $p = 0.00$, dosage x supplement $F_{29} = 46.23$ $p = 0.00$ see Appendix G).

Nitrogen Free Extract (NFE) content in fish

The NFE content which represents the carbohydrate content was reduced significantly in the ginger 1.5% group and garlic 0.5%, 1.5% and 2% groups by 41.5, 38.2, 59.2 and 26.6 % respectively than the control group. The other treatment groups recorded similar carbohydrate content to that of the control group (two-way ANOVA: dosage $F_{29} = 57.60$; $p = 0.00$, supplement $F_{29} = 19.03$; $p = 0.00$, dosage x supplement $F_{29} = 18.11$ $p = 0.00$ see Appendix G).

Blood composition parameters

A summary of the complete count of the different blood constituents, i.e., white blood cells (WBC), red blood cells (RBC), etc. of *S. melanotheron administered* with nine treatments for 24 weeks are shown in Table 14. Of all these various blood constituents, only the platelet large cell ratio (P-LCR %) content was significantly different among the nine feeding treatments (One-way ANOVA: $F_{26} = 3.37$; $p = 0.023$ see Appendix H).

The mean P-LCR % in the ginger 0.5% group (94.10 ± 3.70) was significantly higher than in the garlic 0.5% group (0.00 ± 0.00), but was not significantly different from the control and the other treatment groups.

The mean WBC counts recorded ranged from $56.97 \pm 28.50 \times 10^9$ L^{-1} in the control group to $93.50 \pm 1.59 \times 10^9$ L^{-1} in the garlic 1% group. For the mean RBC counts, they ranged from $2.29 \pm 0.44 \times 10^{12} L^{-1}$ in the garlic 1.5% group to $3.00 \pm 0.16 \times 10^{12}$ L^{-1} in the Garlic 1% group. The lower ginger

doses had a higher RBC count than the control group RBC of 2.57 ± 0.10 x 10^{12} L^{-1}, with the higher dose of 1.5 % ginger recording lower RBC count whiles the 2% ginger group was at par. Similarly, the lower garlic doses (0.5 and 1%) groups had higher RBC counts than the control whereas the higher doses (1.5 and 2%) recorded lower RBC counts.

A similar trend was seen in the mean HGB levels amongst the treatments ranging from 8.3± 1.13 g dL^{-1} in the ginger 1.5% group to 11.30± 1.11 g dL^{-1} in the ginger 1% group, with the control group recording 9.53 ± 0.55 g dL^{-1}.

Mean HCT values ranged from 27.50± 3.86 % in ginger 1.5% group to 41.40 ± 9.30 % in ginger 1% group, and the control group recorded 31.17 ± 0.43%.

The least mean MCV values of 118.0 were recorded jointly in ginger 1.5% and garlic 1% groups followed by the control group recording 121.83 ± 3.15 fL, up to the highest level in garlic 1.5% group with 150.33± 18.71fL.

The mean MCH recorded in the study ranged from 36.14 ± 0.55 pg in ginger 1.5% group to 39.43 ± 2.62 pg in garlic 1.5% group, whiles the MCHC was lowest in the garlic 1.5% group (26.77 ± 2.57 gdL-1) and highest in garlic 1.0% group (31.73 ± 0.88 gdL-1).

The mean red cell distribution width_coefficient of variation (RDW_CV) recorded ranged from 10.47 ± 0.09% in ginger 0.5% group to 13.70 ± 2.99% in garlic 0.5% group, whereas the RDW_SD ranged from 46.17 ± 23.18 fL in the control group to 79.35 ± 13.85 fL in garlic 1.5% group.

Table 14: Blood constituents (mean± standard error) of *S. melanotheron* cultured on diets containing ginger and garlic supplements at different concentrations for 24 weeks

Para-Meters	Feed Treatments								
	Control	Ginger supplements				Garlic supplements			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
WBC ($x10^9 L^{-1}$)	56.97 ± 28.50^{a}	92.10± 2.01^{a}	85.20 ± 1.80^{a}	74.27± 9.77 ^{a}	86.10 ± 8.75^{a}	86.35 ± 2.82^{a}	93.50± 1.59^{a}	79.93 ± 9.92^{a}	78.67± 11.86^{a}
RBC ($x10^{12} L^{-1}$)	2.57 ± 0.10 ^{a}	2.82± 0.02 ^{a}	2.88 ± 0.28^{a}	2.3± 0.38^{a}	2.57 ± 0.25^{a}	2.70 ± 0.04^{a}	3.00± 0.16^{a}	2.29± 0.44^{a}	2.45 ± 0.39^{a}
HGB ($g\ dL^{-1}$)	9.53 ± 055 ^{a}	10.30 ± 0.21 ^{a}	11.30± 1.11^{a}	8.37 ± 1.13^{a}	9.63 ± 1.25^{a}	10.30 ± 0.15^{a}	11.27± 0.37^{a}	8.87 ± 1.50^{a}	9.40 ± 1.62^{a}
HCT (%)	31.17 ± 043 ^{a}	36.87± 2.40 ^{a}	41.40 ± 9.30^{a}	27.50 ± 3.86^{a}	32.40 ± 2.71^{a}	37.87 ± 5.13^{a}	35.47± 0.73^{a}	34.83 ± 9.37^{a}	31.20± 5.37^{a}
MCV (fL)	121.83± 3.15 ^{a}	130.83± 8.41 ^{a}	140.80± 17.61^{a}	118.87± 2.95^{a}	122.20± 2.57^{a}	141.10± 21.37^{a}	118.73± 4.32^{a}	150.33± 18.71^{a}	127.03± 2.41^{a}
MCH (pg)	37.07 ± 0.94 ^{a}	36.50 ± 0.92 ^{a}	39.23 ± 1.59^{a}	36.17 ± 1.62^{a}	37.23 ± 1.38^{a}	38.10 ± 0.12^{a}	37.53 ± 1.08^{a}	39.43 ± 2.62^{a}	38.17 ± 0.55^{a}
MCHC ($g\ dL^{-1}$)	30.53 ± 1.36 ^{a}	28.10± 1.68^{a}	28.67 ± 3.12^{a}	30.43 ± 0.72^{a}	30.47 ± 1.25^{a}	28.17 ± 3.75^{a}	31.73 ± 0.88^{a}	26.77 ± 2.57^{a}	30.10 ± 0.45^{a}
RDW.CV (%)	11.80 ± 0.31 ^{a}	10.47 ± 0.90^{a}	11.53 ± 0.87^{a}	12.10 ± 1.16 ^{a}	11.93 ± 0.34^{a}	13.70 ± 2.99^{a}	12.30 ± 0.80^{a}	11.77 ± 1.47^{a}	11.27 ± 0.19^{a}
RDW.SD (fL)	46.17 ± 23.18 ^{a}	65.47± 1.47^{a}	77.20 ± 5.50^{a}	67.97 ± 4.36^{a}	68.83 ± 2.20^{a}	62.95 ± 5.05^{a}	67.17± 5.89^{a}	79.35 ± 13.85^{a}	68.83 ± 0.83^{a}
PLT ($x10^9 L^{-1}$)	30.33± 15.17 ^{a}	10.00± 2.08 ^{a}	3.67 ± 2.33^{a}	37.33 ± 13.28^{a}	56.00 ± 19.22^{a}	6.00 ± 3.46^{a}	10.67 ± 6.06^{a}	42.33 ± 34.64^{a}	48.33 ± 11.61^{a}
MPV (fL)	15.20 ± 3.20^{a}	27.45 ± 2.25^{a}	0.00 ± 0.00^{a}	16.30 ± 4.05^{a}	13.70 ± 1.99^{a}	14.30 ± 14.30^{a}	21.90 ± 3.70^{a}	10.30 ± 6.04^{a}	14.63 ± 1.78^{a}
PDW (fL)	11.60 ± 3.30^{a}	7.47± 0.85^{a}	3.10 ± 0.00^{a}	6.60 ± 0.10^{a}	10.70 ± 1.27^{a}	8.65 ± 1.55^{a}	9.25 ± 2.15^{a}	3.80 ± 2.00^{a}	9.07 ± 1.07 ^{a}
PCT (%)	0.05 ± 0.02^{a}	0.03 ± 0.00^{a}	0.00 ± 0.00^{a}	0.05 ± 0.01^{a}	0.06 ± 0.02^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.05 ± 0.03^{a}	0.06 ± 0.01^{a}
P-LCR (%)	47.10 ± 11.90^{ab}	94.10 ± 3.70^{a}	0.00 ± 0.00 ^{b}	49.93 ± 15.03^{ab}	38.30 ± 3.98^{ab}	0.00 ± 0.00 ^{b}	41.73 ± 21.76^{ab}	30.20± 17.95^{ab}	47.53 ± 6.80^{ab}
P-LCC ($x10^9 L^{-1}$)	14.67 ± 3.18 ^{a}	9.33 ± 1.33^{a}	2.67± 2.67 ^{a}	14.67 ± 2.73^{a}	19.33 ± 5.93^{a}	6.67± 3.53 ^{a}	6.00 ± 3.06^{a}	13.33 ± 9.21^{a}	21.00 ± 3.00^{a}

NOTE: WBC - white blood cells; RBC - red blood cells; HGB – haemoglobin; HCT – haematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentration; RDW-CV - red cell distribution width_coefficient of variation; RDW-SD - red cell distribution width_ standard deviation; PLT - platelet count; MPV - mean platelet volume; PDW - platelet distribution width; PCT - plateletcrit; P-LCR - platelet large cell ratio; P-LCC - platelet large cell concentration. Means that share the same alphabet as superscript in a row are not significantly different (one-way ANOVA, $p < 0.05$). KEY: dL; deciliter, L;litre, mL; milliliter, fL; femtolitre, pg; pictogram.

The mean PLT, MPV and PDW all recorded lowest values in the ginger 1% group as 3.67± 2.33 x 10^9 L^{-1}, 0.00 fL and 3.10 ± 0.00 fL respectively but their highest values were recorded in different groups as 56.00 ± 19.22 x 10^9 L^{-1}, 27.45± 2.25 fL and 11.60± 3.30 fL in ginger 2%, ginger 0.5% and control groups respectively.

The mean PCT and P-LCC also recorded lowest values in the ginger 1% group as 0.00 % and 2.67± 2.67 x 10^9 L^{-1}, respectively and their lowest and highest values of 0.06 ± 0.02 % and 19.33± 5.93 x 10^9 L^{-1} were both recorded in ginger 2% group.

Serum biochemical parameters

A summary of the serum biochemical composition, i.e., glucose, total protein, albumin, globulin, cholesterol and triglyceride content of *S. melanotheron* administered with nine treatments for a period of 24 weeks is shown in Table 15. A two-way ANOVA was used to examine the effects of the different concentrations (0%, 0.5%, 1.0%, 1.5% or 2%) of the different supplements (ginger or garlic) on the biochemical composition of the serum (see Appendix I). The results showed that neither the supplement type nor their concentrations had a significant difference in their effects on the serum biochemical composition of the fish. Consequently, the supply of the supplements at different concentrations had no effect on the serum composition of the fish. Provided below are detailed descriptions of these results.

Table 15: Serum biochemical parameters (mean ± standard error) in *S. melanotheron* after 24 weeks of dietary supplementation

Parameter	Feed Treatments								
	Control	Ginger supplements				Garlic supplements			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
GLUCOSE (mg dL^{-1})	92.8 5 ± 1 6.23[a]	74.6 4± 1 5.07[a]	98.9 4± 1 5.31[a]	116.9 6± 3 5.81[a]	94.0 1± 2 1.12[a]	92.0 5± 2 4.69[a]	138.3 4± 5. 28[a]	67.6 1± 1 1.29[a]	107.9 1± 1 9.90[a]
TOTAL PROTEIN (g dL^{-1})	3.79 ± 0.24[a]	3.36 ± 0.18[a]	3.10 ± 0.14[a]	3.56 ± 0.30[a]	3.12 ± 0.34[a]	3.64 ± 0.20[a]	3.64 ± 0.12[a]	3.40 ± 0.12[a]	3.3 2± 0.55[a]
ALBUMIN (g dL^{-1})	1.47 ± 0.21[a]	1.04 ± 0.04[a]	1.12 ± 0.25[a]	0.99 ± 0.02[a]	1.09 ± 0.08[a]	1.17 ± 0.28[a]	1.12 ± 0.02[a]	0.94 ± 0.08[a]	1.14 ± 0.09[a]
GLOBULIN (g dL^{-1})	2.32 ± 0.07[a]	2.32 ± 0.15[a]	1.98 ± 0.26[a]	1.98 ± 0.31[a]	2.03 ± 0.38[a]	2.47 ± 0.09[a]	2.52 ± 0.10[a]	2.45 ± 0.12[a]	2.18 ± 0.54[a]
ALBUMIN/ GLOBULIN RATIO	0.63 ± 0.09[a]	0.45 ± 0.02[a]	0.62 ± 0.23[a]	0.40 ± 0.05[a]	0.59 ± 0.16[a]	0.48 ± 0.14[a]	0.45 ± 0.01[a]	0.39 ± 0.05[a]	0.62 ± 0.02[a]
TRIGLYCE RIDE (mg dL^{-1})	88.70 ± 19.52[a]	83.92 ± 15.38[a]	84.82 ± 24.57[a]	84.82 ± 6.12[a]	93.41 ± 13.52[a]	93.41 ± 12.97[a]	115.9 1± 32.07[a]	91.22 ± 20.38[a]	102.8 0± 13.50[a]
CHOLESTE ROL (mg dL^{-1})	168.83 ± 14.26[a]	159.6 8± 4.91[a]	167.0 8± 10.25[a]	143.0 2± 12.33[a]	173.0 3± 8.60[a]	173.0 3± 15.99[a]	177.8 9 ± 7.86[a]	159.9 8± 16.05[a]	173.6 8± 36.42[a]

Means with the same letter as superscript in a row are not significantly different (one-way ANOVA, $p < 0.05$).

The mean serum glucose level recorded in the study ranged from 67.61 ± 11.29 mg dL^{-1} in garlic 1.5% group to 138.34± 5.28 mg dL^{-1} in garlic 1% group. Meanwhile, there was no significant difference in the mean serum glucose levels between the treatment groups and the control.

The serum total protein, albumin and albumin/globulin ratio were all highest in the control group as 3.79 ± 0.24 g dL^{-1}, 1.47 ± 0.21g dL^{-1} and 0.63 ± 0.09 g dL^{-1} respectively whiles their lowest were in ginger 1% group (3.10 ± 0.14 g dL^{-1}), garlic 1.5% group (0.94 ± 0.08g dL^{-1} and 0.39 ± 0.05 g dL^{-1}) accordingly. However, the globulin content was highest in the garlic 1% groups (2.52± 0.10 g dL^{-1}) and lowest globulin recorded jointly in ginger 1% and 1.5% groups (1.98 g dL^{-1}). There were no significant differences in the mean serum protein, albumin, globulin and albumin/globulin ratios among all the treatment groups and the control.

Mean serum triglyceride levels ranged from 83.92± 15.38 in Ginger 0.5% group to 115.91± 32.07 in Garlic 1% group whiles mean serum cholesterol levels ranged from 143.02± 12.33 in Ginger 1.5% to 177.89 ± 36.42 in Garlic 1% group. In spite of the differences, there was no significant difference in the triglyceride and cholesterol levels (two-way ANOVA: $F_{26} = 0.41$; $p = 0.901$).

Serum lysozyme activity

The mean serum lysozyme activity assessed by ELISA at the end of dietary supplementation for 24 weeks is shown in Figure 31. The activity levels were compared using a two-way ANOVA to examine the supplements (ginger or garlic) and their concentrations (0%, 0.5%, 1.0%, 1.5% or 2%)

effects on the fish. The results indicated that administering the supplements at the different concentrations did not significantly affect the serum lysozyme activity in *S. melanotheron*. This resulted in no significant supplement and dosage interaction effect on the serum lysozyme activity in the fish. The details are further described below.

The highest lysozyme activity level was recorded in the Garlic 1% group (65.07 ± 24.31 ~ 49.3% more than Control) which was and followed in the decreasing order as follows: Ginger 2% (63.32 ± 15.02 ~ 45.3% more than

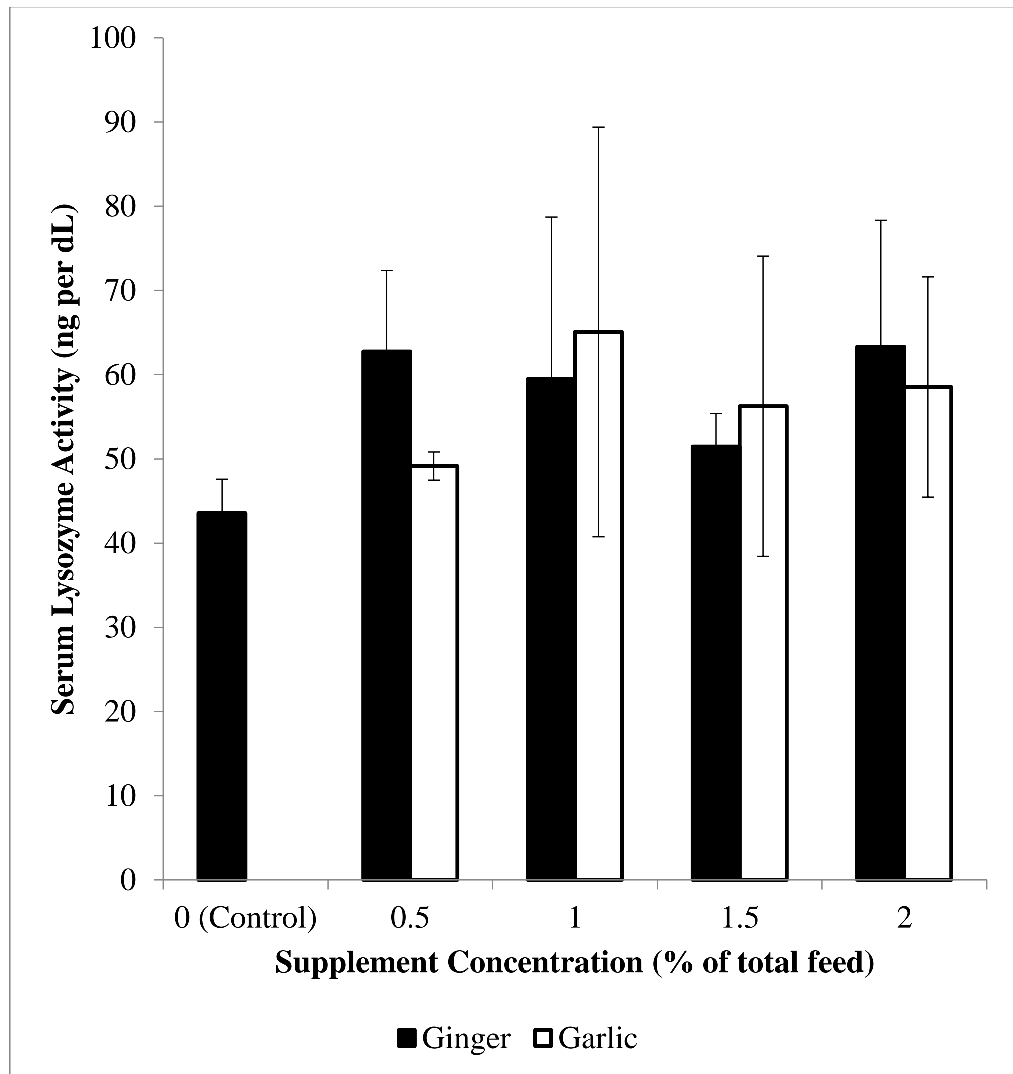

Figure 31: Serum lysozyme activity levels in S. melanotheron fed with ginger or garlic supplemented diets (0.5 to 2%) or control diet (0%) for 24 weeks. Vertical lines on the bars indicate the standard error (±) of the mean; same letter labels indicate there is no significant difference in lysozyme levels of the different treatment groups (two-way ANOVA, $p < 0.0$).

Control) > Ginger 0.5% (62.74 ± 9.65 ~ 44.0% more than Control) > Ginger 1% (59.50 ± 19.21 ~ 36.6% more than Control) > Garlic 2% (58.53 ± 13.06 ~ 34.3% more than Control) > Garlic 1.5% (56.26 ± 17.82~ 29.1% more than Control) > Ginger 1.5% (51.47 ± 3.89 ~ 18.1% more than Control) > Garlic 0.5% (49.14 ± 1.68 ~ 12.8% more than Control) > Control (43.57 ± 4.00) which was the lowest mean lysozyme activity level. Neither the supplement nor dosage showed a significant effect in the serum lysozyme activity across the nine treatments and the dosage verses supplement effect was also not significant (two-way ANOVA: dosage $F_{29} = 0.61$; $p = 0.663$, supplement $F_{29} = 0.04$; $p = 0.853$, dosage x supplement $F_{29} = 0.17$; $p = 0.951$see Appendix J).

Cost of Supplemented Diets

The costs per Kg of the supplemented diets using the three basal diets are shown in table 16. A One-way ANOVA ($F_{26} = 0.05$; $p = 1.00$) conducted showed that the differences in the cost per kg of control (basal) and supplemented diets were not significant.

Chapter Summary

This chapter described the results obtained from the study. The abiotic water parameters monitored throughout the study were similar among all the nine treatment groups. The effects of ginger and garlic supplements on the growth and feed utilization of *S. melanotheron* were not significant compared to the control. This was also the case for haematology, serum biochemical composition and serum lysozyme activity of the fish, where no significant effects were observed. There was however a significant effect of treatments in the condition index and the proximate biochemical composition of fish.

Table 16: Cost per Kg (Gh₵) of basal and supplemented diets administered to *S. melanotheron*

	Feed Treatments								
	Control	Ginger supplemented diets				Garlic supplemented diets			
	0%	0.5%	1%	1.5%	2%	0.5%	1%	1.5%	2%
Average cost of diet (Gh₵)	7.72 ± 3.16[a]	8.27 ± 3.14[a]	8.81 ± 3.13[a]	9.36 ± 3.11[a]	9.91 ± 3.10[a]	8.16 ± 3.14[a]	8.60 ± 3.13[a]	9.04 ± 3.11[a]	9.48 ± 3.10[a]

Means with the same letter as superscript in a row are not significantly different (one-way ANOVA, $p > 0.05$).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

In this chapter, the results of the effects of ginger and garlic supplements on the growth, feed utilisation, proximate nutritional composition, haematology, serum biochemical composition and serum lysozyme activity of *S. melanotheron* are explained and related to the outcomes of similar studies. Possible reasons and deductions are assigned to the outcomes, conclusions are drawn and recommendations made for further studies.

All the environmental parameters (pH, DO, conductivity, turbidity, salinity and temperature) monitored throughout the study period were similar in all the experimental hapas. Hence any observed variations in the results could be attributed to the treatments.

Effect of ginger and garlic supplements on growth performance and feed utilisation of *S. melanotheron*.

Growth in the aquaculture sector is important to ensure food and nutrition security in this era of rapid population growth coupled with static or declining capture fishery stocks. For growth to be sustainable, it is important to adopt best practices which do not adversely impact the environment and compromise the wholesomeness of fish produced (Emeka, Iloegbunam, Gbekele-Oluwa & Bola, 2014). In helping to achieve this, Goda (2008) recommended a number of dietary supplements (which may not be essential nutrients) such as probiotics, amino acids, yeast, carnitine, antioxidants, enzymes, lipid derivatives, colourants, vitamins, nutraceutics, hormones, aromatic compounds, plant extracts and certain organic acids/salts which can

be used in fish nutrition. The aim for using these supplements is to enhance digestion and assimilation process which can improve fish growth and feed efficiency leading to profitability of fish by the sector.

In the present study, the effect of ginger and garlic powder as feed supplements on the growth performance and feed utilization of *S. melanotheron* under culture was assessed. The results showed that the 4 levels (0.5%, 1%, 1.5% and 2%) of both ginger and garlic powder supplemented in the diets did not significantly enhance the growth (SGR, AGR, and MWG) of *S. melanotheron* after 24 weeks of culture although some improvements especially in the 1.5% Garlic group were observed. Contrary to this finding, studies by Venkatramalingam, Godwin and Citarasu, (2007); Nya and Austin (2009b) showed a significant positive effect of ginger on growth rate of *Penaeus monodon* (at 50, 75 and 100% ginger enriched Artemia diet) and *Onchorynchus mykiss* (at 0.5 and 1% ginger enriched diet). Aly and Mohamed (2010) and Nya and Austin (2009a) reported significant improvement in the growth parameters of Nile tilapia (at 10 and 20g garlic powder per kg of feed) and rainbow trout (at 0.5 and 1% garlic powder). In addition, Gholipour Kanani et al. (2014) recorded significant increase in the specific growth rate and body weight gain of *Huso huso* administered with 1% ginger supplements but no significant difference with 1% garlic supplements. Shalaby, Khattab and Abdel Rahman (2006) reported that there was a significant increase in weight gain of Nile tilapia fed garlic powder supplemented diets at 30.0 g/kg of feed.

In agreement with the present study, Nwabueze (2012) reported that garlic supplemented diet did not have any significant effect on weight gain of

Clarias gariepinus compared with the control group. Labrador, Guiñares and Hontiveros (2016) also showed that garlic powder (at 2, 4 and 6%) supplemented diet did not have any significant effect on weight gain (%), and final weight (g) of *Litopenaeus vannamei*. In contrast to both scenarios of either a significant positive effect on weight gain or no significant effect on weight gain, Ndong and Fall (2011) reported that garlic-supplemented (0.5 and 1%) diets reduced weight gain in juvenile hybrid tilapia (*O.niloticus* x *O. aureus*).The contradicting results obtained from the different studies may be attributed to the different species and their life stage, the dosages of supplementation applied, the route and duration of administering the supplements and also the conditions in the culture environment as well as the variety of the ginger and garlic used in each case. With the myriad of factors and probable combination of factors that may contribute to the realization of possible effects, conducting further studies, focusing on higher doses of the supplement may yield results necessary to harness any beneficial effects for the aquaculture industry.

For the growth performance of *S. melanotheron*, SGR of 3.38 to 3.54 % / day is comparable to SGR value of 3.09 reported by Ahmed, Sultana, Shamsuddin and Hossain (2013) for *O. Niloticus* in a pond system. This is an improvement on previous records of 1.3 % / day recorded by Tseku (2016) on this species. The DO (average 2.41 ± 0.07) for the period of culture in this work was persistently below the optimal recommended level (5 mg/l) for most freshwater species. This sub-optimal condition may have posed an additional stress on the fish thereby hindering its optimal growth performance and may

not have favoured the effects of the supplements in the diets (see Stickney, 2005).

Feed utilization and efficiency indicators (FCR, FER) are important economic parameters in any fish culture establishment because it has a direct impact on the operations cost (cost of feed), the environment (waste produced) and the final price of fish produced. Measures to improve feed efficiency and utilization in order to minimize these impacts are therefore critical for the successful farming of any species. According to Bulfon, Volpalti and Galeotti (2013), some authors suggest that dietary supplementation with plants can help modulate lipid metabolism, enhance reactivity of some digestive enzymes (e.g., trypsin), and consequently lead to an efficient feed digestion and nutrient absorption. In support of this view, various studies have reported an increase in the feed efficiency ratio (FER) and protein productive value (PPV) as well as energy retention in different fish species that received supplemented diets (see, for example, Bulfon et al., 2013).

Previous studies on *S. melanotheron* reported FCR values of 5.27 to 8.47 (Tseku, 2016), 15.04 (Ayoola, Oluwatoyin & Akapo, 2012) and 11.0 (Legendre & Ecoutin, 1983). These FCR values are rather high compared to the acceptable range of FCR (i.e., 1.5 to 2.0) considered good for culture of most fish species (Houlihan et al., 2001). An FCR of for e.g. 2 means that a fish requires 2 g of feed in order to produce 1 g of flesh, so the higher the FCR, the less efficient the fish converts the feed. Hence the results of previous studies were not favourable for commercial culture of this species. In this study, the FCR obtained for *S. melanotheron* ranged from 2.24 to 2.46 which is an improvement over the previous records and closer to the acceptable

range. This suggests a new direction that with proper adjustments in feed type and quality given to *S. melanotheron*, it is possible to achieve an FCR suitable for commercial culture of this species. However, the groups of fish fed on ginger and garlic supplements in this study did not show a significant improvement in the FCR and FER values when compared with those fed the control diet (commercial feed without supplement). Contrarily, Labrador, Guiñares and Hontiveros (2016) reported significantly ($p < 0.05$) higher FCR values (1.94 ± 0.03) when they cultured the fry of *L. Vannamei* without garlic supplement (their Control group). The concentration of garlic supplement in their study was even higher (2, 4 and 6%) than the levels used in this study. This suggests that the levels of active ingredients in garlic supplements at the higher doses better influenced feed utilization in *L. vannamei* fry than the lower doses did in *S. melanotheron* and thus requires further studies. The care taken not to overfeed the fish (feed for a session not given when there was presence of feed from the previous session) could have contributed to the lower FCRs obtained but this same precaution could have affected the treatments.

Effect of ginger and garlic supplements on physical condition of *S. melanotheron*

The physical condition of fish expressed in terms of their survival rate, condition index and growth form demonstrates in physical terms their resilience, general well-being and normal behaviour patterns. In this study, there was no significant difference in the survival rate and growth forms of all the supplemented groups compared with the control. This finding shows that the supplements did not confer any positive advantage to survival of the fish

(see, Table 11). This observation does not agree with the findings of Shalaby et al. (2006) on *O. niloticus* fed with 3% of the supplement, Nya and Austin (2009a) on *O. Mykiss* fed with 0.5 and 1%, and Talpur and Ikwhanuddin (2012) on *L. calcarifer* fed with 1% of the supplement. In these studies, diets enriched with garlic significantly increased survival of the experimental fish compared with the control. Additionally, white leg shrimp are known to significantly increase their survival rates when fed on diets enriched with garlic powder (at 2, 4 and 6%) (Labrador, Guiñares & Hontiveros, 2016). The survival rates recorded in the present study were over 87%; it is interesting to note that the lower doses of ginger supplements (0.5% and 1%) and the higher doses of Garlic (1.5% and 2%) recorded 100% survivals (see Table 11). This notwithstanding, the results also indicate that the supplements were not harmful to the fish at any of the dosage levels used.

In terms of their growth form, there was a strong positive correlation between the length and weight of the fish in this study (see Figure 30). This shows that the increase in the weight of fish was proportional to increase in fish length: the growth form of the fish was isometric ($b = 3$). This observation concurs with the general observation stated by Froese (2006) that the length of a fish grows proportionally to the weight and the degree of this relationship depends on the species. The range of b values obtained in this study (3.0108 to 3.1024) fell within the recommended range of 2 - 4 for fresh water fishes by Bagenal and Tesch (1978). Generally, values of b above 3 occur in stress-free environment (Bagenal & Tesch, 1978). Similar to this finding, Tseku (2016) also recorded isometric growth of *S. melanotheron* in a reservoir system with b values below 3, indicating a higher level of stress on the fish which was

evident in their lower growth rate recorded in that study. These observations suggest that *S. melanotheron* is sensitive to environmental stress but can readily compensate its growth in order to survive under such conditions.

The condition index (CI) is an indication of the general well-being of a fish and values of CI above 1 suggests that fish are in good condition. In this study, values of CI ranged from 3.85 to 4.19, implying that all the fish under study were in good condition. Additionally, the CI was significantly enhanced in the 1.5% garlic supplemented group over the control group CI while all the other levels of supplementation were not significantly different from the control. Ogunji, Toor, Schulz and Kloas, (2008) stated that, the higher the CI value, the better the physiological state of the fish. This suggests that supplementing garlic in the diet of *S. melanotheron* could have helped to alleviate stress and put fish in a better physiological state. It is also interesting to note that this group (Garlic 1.5%) recorded the highest values in the growth parameters and the best values in the feed utilization and efficiency even though they were not significantly different from the control. In the case of *Huso huso*, Gholipour Kanani et al. (2014) observed that dietary treatments with ginger and garlic at 1% dose each significantly improved condition factor in the ginger treatment group but not the garlic group. This suggests that different species of fish may react differently to plant supplements and the dose applied.

Effect of ginger and garlic supplements on proximate nutritional composition of *S. melanotheron*

According to the High Level Panel of Experts on World Food Security (2014), the nutritional sufficiency of farmed fish is a major concern as

aquaculture production is gradually substituting wild fish stocks. The nutritional composition of fish is also important to the nutritionist, processor and the consumer. In this study, there was a significant difference in the proximate nutritional composition of the treated fish harvested after the experiment compared with the control suggesting that ginger and garlic supplementation affected the nutritional quality of *S. melanotheron*. However, some studies have reported no significant differences in chemical body composition of fish supplemented with plant products (Hassaan and Soltan, 2016; Abd El Hakim, 2010).

According to Murray and Burt (2001), moisture usually constitutes approximately 70 (fatty fish) to 80% (lean fish) of fish flesh. The moisture content of fish harvested from this study ranged from 68.92% in garlic 1.5% group to 78.39% in ginger 0.5% treatment groups. Ginger supplementation (at 0.5 and 1%) increased moisture levels and reduced dry matter significantly whereas garlic supplementation (at 0.5, 1.5 and 2%) reduced moisture levels and increased dry matter significantly from the control. From the moisture levels, it can be seen that the garlic treatments produced more fatty fish whiles the ginger treatments produced leaner fish. The levels of the other nutritional components could throw more light on the dry matter proportions in the fish.

Beginning with the ash content, ash represents the crude minerals content in the fish and this was significantly enhanced in 0.5% and 1.5% garlic groups but all ginger supplemented groups had significantly lower mineral content from the control. Comparably, Metwally (2009) showed that ash was increased significantly in Nile tilapia following treatment with garlic enriched diet. The higher minerals content in the garlic groups could have limited

excess water uptake by the fish through osmoregulation thereby reducing moisture content. Whereas the ginger groups which contained lesser minerals maximised water uptake by the fish hence increased moisture content in flesh. This also implies that ginger groups needed to spend more energy to expel excess water from their body.

The whole body composition in this study recorded significantly higher fat content in all garlic (0.5%, 1%, 1.5% and 2%) treatment groups compared to the control. In the case of ginger supplemented diet groups, 0.5% and 1.5% recorded significantly reduced lipid content in the fish. In this regard, Lee, Ra, Song, Sung and Kim (2012) also reported that supplementing garlic extract (1.0%) in diet of Sterlet sturgeon *Acipenser ruthenus* resulted in more lipid content in fish body than the control. However, Maniat, Ghotbeddin, and Ghatrami (2014) reported that garlic in diets of benni fish (*Mesopotamichthys sharpeyi*) induced higher protein and lower fat content. This may be attributed to the assertion that an increase in the protein utilization for the fish could limit the role of lipid as fish prefer to use dietary protein as energy source compared to lipid or carbohydrate for growth activities (Lee et al., 2012). In contrast to this finding, Shalaby et al. (2006) as well as Talpur and Ikhwanuddin (2012) reported significantly decreased ($p < 0.05$) total lipids in the body of Nile tilapia and Sea bass when they were treated with garlic enhanced feeds. Similarly, Metwally (2009) and Maniat et al. (2014) reported that garlic in diets of Nile tilapia (garlic oil 250 mg per kg of feed) and Benni fish (garlic powder 10 g per kg diet) respectively induced lower fat content. Considering the fact that some fish oils are important in

human nutrition, further investigation into the classes of lipid content in the treated fish may elucidate whether it can be beneficial or not.

Supplementing ginger (at 1.5 and 2%) and garlic (at 1.5%) in the diet significantly induced higher protein content of fish compared to the control and the other levels of supplementation. This finding agrees with results from previous studies. For example, Lee et al. (2012) found that *A. ruthenus* increases its nitrogen utilization and protein retention (PRE, %) when fed with garlic enriched diet. Likewise, Maniat et al. (2014) reported that garlic (at 10g per kg of feed induced higher protein content in benni fish. Additionally, Metwally (2009) showed that crude protein was increased significantly in *O. niloticus* after garlic oil administration. Saleh, Michael and Toutou (2015) also demonstrated from their study that protein content in whole body of *O. mykiss* was increased significantly at all inclusion levels of garlic powder (1, 2 and 3%). They suggested that the garlic powder may have caused an increase in the level of free amino acid in the muscle which stimulated protein synthesis. Gabor et al. (2012) also reported an increment in crude protein contents of *O. mykiss* when fed 3% garlic supplemented diet. The higher protein content in garlic supplemented fish group could be attributed to the protein retention capacity of four bioactive components found in garlic namely garlic oil, diallyl sulphide (DAS), diallyl trisulphide (DATS) and diallyl disulphide (DADS) reported by Wu, Sheen, Chen, Tsai and Lii (2001). They found that these components increased the protein content of cytochrome (iron containing hemi proteins responsible for generation of ATP in the mitochondria) in cells. Increased nitrogen digestibility, retention and absorption have also been reported in other species such as ruminants and rats

administered with garlic than the control without garlic although nitrogen intake was similar (Wanapat, Khejornsart, Pakdee & Wanapat 2008; Oi, Imafuku, Shishido, Kominato, Nishimura & Iwai 2001). The effect of garlic supplement on fish protein content in this study is therefore consistent with existing knowledge.

The amount of protein in fish muscle is estimated to be in the range of 15 to 20% (Murray & Burt 2001). The protein content of *S. melanotheron* recorded in this study ranged from 12.70 to 19.39%. The highest protein level was obtained by the garlic 1.5% treatment group which is comparable to protein content of 19.13% recorded for the same species by Fashina-Bombata and Megbowon (2012) in the Epe Lagoon, Lagos Nigeria, while the control group recorded a protein content of 14. 29%. This suggests that dietary garlic supplementation can improve the nutritional value of *S. melanotheron* under culture.

The active ingredients of ginger (gingerols) render it as a rich source of antioxidant capable of scavenging free radicals, lowering lipid peroxidation and raising the levels of antioxidant enzymes resulting in enhancing the antioxidant status of the host tissue (Butt & Sultan, 2011). In addition, ginger has been implicated in increasing the activity of digestives enzymes such as amylase, protease and lipase (Venkatramalingam et al., 2007). All these properties could have contributed to the positive effect of ginger on protein content in whole fish by improving protein synthesis and protecting host tissue from damage leading to increased protein content.

The carbohydrate content in fish was significantly highest in the control fish and was reduced significantly in ginger 1.5% and garlic 0.5, 1.5

and 2% groups (see Table 13). Likewise, fibre was also highest in the control group but reduced significantly in all supplemented groups except 1% garlic group. The higher proportion of protein and fat in some ginger and garlic groups could account for the lower proportion of carbohydrate and fibre. It can therefore be said that the supplements possibly played a significant role in digestion and assimilation as well as energy balances to achieve a better nutritious fish than the control.

Effect of ginger and garlic supplements on blood parameters of *S. melanotheron*

Bulfon et al. (2013) noted that definite reference ranges for haematological and blood biochemical indices in fish species have not been well defined. Moreover, the correlation that characterizes changes in blood parameters with the occurrence of diseases and disorders are also not fully known. Nonetheless, Shalaby et al. (2006) reported that haematological analysis may provide valuable knowledge for assessment of fish health status and to monitor the fish physiological status in response to therapeutic and dietary treatments, environmental changes and stress.

According to Houston (1997), the red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are haematological indices, which show the erythrocyte status and the fish's oxygen carrying ability. In this study, the RBC, HGB, MCV, MCH and MCHC values were statistically similar across all the treatments and the control (see Table 14) indicating that the treatments did not significantly improve these indices in *S. melanotheron*. In this regard,

Soltan and El-Laithy (2008) also reported no significant effect of garlic powder on HCT and HB values for *O. niloticus*. Contrary to these findings, significant increase in RBC, HGB and HTC of *O. niloticus* fed with garlic was reported by Shalaby et al. (2006) at 30g per kg of feed. Additionally, Nya and Austin (2009a, 2011) reported that the dietary administration of garlic (at 0.5 and 1%) in rainbow trout enhanced the maturation and haemoglobin content of erythrocytes. Elevated levels of RBC and HCT, WBC were observed by Nya and Austin (2009b) with a ginger supplementation (at 0.5%) in rainbow trout. A significant increase ($p < 0.05$) in HGB percentage was observed by Talpur and Ikhwanuddin (2012) in all garlic treatment groups (0.5, 1, 1.5 and 2%) of Asian sea bass, with the higher HGB levels in groups fed garlic-added feed at 15 g/kg feed (1.5%) and 20 g/kg feed (2%) compared with those of control. Gholipour Kanani et al. (2014), observed highest levels of erythrocyte and hematocrit in ginger fed fingerlings (at 1%) after 60 days. In the present study, the highest levels of erythrocytes count ($3.00 \pm 0.16 \times 10^{12} L^{-1}$) was seen in the Garlic 1% group while the highest haematocrit (41.40± 9.30%) and haemoglobin (11.30 ± 1.11 g dL^{-1}) levels were observed in Ginger 1% group. Fazlolahzadeh, Keramati, Nazifi, Shirian and Seifi (2011) attributed the decrease or increase in blood indices to a defense reaction against garlic, which occurs by stimulation of erythropoiesis. The different species, dosage and duration of the supplementation may all contribute to the different results achieved by these studies.

The white blood cell count (WBC) indicates the leucocyte levels in the blood. Leukocytes play an important role in non-specific or innate immunity and their count can be considered as an indicator of the health status of fish

(Fazlolahzadeh et al., 2011). Thus the increase in number of leukocytes produced is essential for the modulation of a protective response against infectious agents and chemicals (Harikrishnan, Balasundaram, Kim, Kim, Han & Heo, 2010). The WBC count in the present study was not significantly increased in the supplemented groups compared to the control. Nonetheless, there were slightly elevated levels in the supplemented groups' while the control recorded the least WBC count. With this finding, the present study partly agrees with Nya and Austin (2009a, 2011) that the dietary administration of garlic in rainbow (0.5 and 1%) trout enhanced the number of total leucocytes. They also observed elevated RBC, HCT, WBC, lymphocytes, monocytes and neutrophils after supplementation with ginger at 0.5% (Nya & Austin 2009b).

Effect of ginger and garlic supplements on serum biochemical composition of *S. melanotheron*

The serum proteins include albumin, globulins and various humoral factors of the innate immune system Magnadottir, Lange, Gudmundsdottir, Bøgwald and Dalmo (2005). According to Nya and Austin (2009a), albumin functions to regulate the osmotic pressure that is needed for the distribution of body fluids and is essential as a plasma carrier or as a non-specific ligand which has many binding domains. On the other hand, serum globulins are the source of immunoglobulins, therefore their levels in blood indicate the amount of antibodies and as a result the general immune status of the fish (Goda, 2008). In the present study, the total protein content of blood serum, albumin and globulin were statistically similar in the nine treatments administered, thus the supplements did not affect them. But in other studies, significant increase

in ginger 1% group for globulin and in garlic 1% group for albumin were observed (Gholipour Kanani et al., 2014). Verdegem, Hilbrands and Boon (1997) reported that plasma proteins reflected the protein levels fed to fish. In this regard, the control group in the present study recorded the highest serum total protein (3.79 ± 0.24) and albumin (1.47± 0.21) content, but the highest globulin content was observed in the Garlic 1% group (2.52 ± 0.10). This finding contradicts that of Shalaby et al. (2006) of an increase in total proteins of Nile tilapia after feeding diets supplemented with garlic and the highest level achieved at a concentration of 10 g per kg of feed(1%). Total proteins, albumin and globulin were also enhanced in *O. mossambicu*s after the dietary administration of ginger acetone extract (at 1%w/w) (Immanuel et al., 2009) which does not concur with the results of the present work. In addition, Nya and Austin (2009b) recorded significant increase in total protein and globulin levels after dietary ginger (0.5%) supplementation in the rainbow trout. Again the different species, method of administration, and duration could account for the different results achieved.

In fish, blood glucose concentration is often used to indicate the presence of a non-specific stress. This is because during a stressful condition, there is a quick rise in the cortisol concentration in the blood which causes glycogen to breakdown from the liver and results in a rise in the glucose concentration in the blood (Bulfon et al., 2013). In the present study, there was no significant difference in blood glucose levels of the supplemented diets and the control. Bulfon et al. (2013) indicated that administering medicinal plants to fish can decrease blood sugar significantly to limit the effect of stressors but the present result did not back that assertion. Talpur and

Ikhwanuddin (2012) also found that plasma glucose concentration reduced significantly in fish fed on diets containing garlic. Considering that the highest and lowest levels of glucose were both observed in groups fed with supplemented diets suggests that probably the supplements at certain dosage could slightly induce or relieve stress in the fish. These were observed in the Ginger 1.5% group recording the highest mean glucose level (116.96± 35.81) and the Garlic 1.5% group recording the lowest level (67.61± 11.29). This observation may partly support Ahmed and Sharma (1997) that application of garlic decreases blood glucose which results in an increase in insulin levels. Contrary to this, blood glucose was significantly reduced in Nile tilapia (Shalaby et al., 2006) after the dietary administration of garlic at 3%.

Ji, Jeong, Im, Lee, Yoo and Takii (2007), suggested that medicinal plants can modulate lipid metabolism that will catalyse the use of body fatty acids as the main source of energy for metabolic processes whiles sparing proteins for better growth performance. In the present study, cholesterol levels ranged from 143.02± 12.33 in Ginger 1.5% group to 177.89 ± 7.8in Garlic 1% group whiles the control recorded 168.83± 14.26 and there were no significant differences in serum cholesterol levels of the nine treatment groups. Triglyceride levels recorded a similar trend with the highest level (115.91± 32.07) also in the Garlic 1% group whiles the lowest was in Ginger 1.5% group (84.82 ± 6.12). This finding contradicts with Shalaby et al. (2006) that Nile tilapia fed diets containing garlic (at 40g per kg of feed) recorded significantly decreased total lipids. However, it partly agrees with Immanuel et al. (2009) that triglycerides and cholesterol were reduced in *O. mossambicus* fed a diet with acetone extract of *Z. Officinale* (at 1% w/w). Perhaps the

acetone extract of ginger provided a more effective treatment compared to the crude ginger powder supplement used in the present study. The fact that all garlic groups recorded higher lipid levels and ginger groups recorded lower lipid levels than the control group demonstrates that ginger could better catalyse the use of fatty acids in *S. melanotheron* than garlic. It is interesting to note that an acute study conducted by Weidner and Sigwart (2000) using ginger extract (25–100 mg/kg of feed) did not significantly impact blood glucose, blood pressure, heart rate or blood coagulation in normal rats. However, when hyper-glycaemic rats or those given high lipid diets were used by Fuhrman, Rosenblat, Hayek, Coleman and Aviram (2000), the ginger supplements significantly lowered blood glucose, serum total cholesterol and triglycerides. This suggests that in the present study, the levels of glucose and lipid in the fishes could be in normal levels hence no impacts of supplements were seen. Additionally, the long term supplementation did not cause any detrimental effects on the fish as the values of these indices were similar to the control.

Effect of ginger and garlic supplements on serum lysozyme activity of *S. melanotheron*

According to Magnadottir (2006), "lysozyme is a bactericidal enzyme that hydrolyses the b-1,4glycosidic linkage between N-acetyl glucosamine and N-acetyl muramic acid of bacterial cell wall peptidoglycan, thereby causing bacteriolysis and preventing the growth of bacteria". Additionally, lysozyme is known to activate the complement system and phagocytes by acting as an opsonin, and also displays anti-viral and anti-inflammatory properties (Saurabh & Sahoo, 2008). The results of the present study showed slightly

elevated levels in serum lysozyme activity in all supplemented groups especially Garlic 1% group (65.07 ± 24.32) which was not significantly different from the control (43.57 ± 4.01) which recorded the least value. This contradicts the findings of Nya and Austin (2009ab; 2011) that serum lysozyme activity was enhanced in rainbow trout fed diets supplemented with garlic (0.5 and 1%) and ginger (0.5%). Talpur, Ikhwanuddin and Ambok, (2013) also found that garlic supplemented diets (0.5 and 1%) significantly increased ($p < 0.05$) lysozyme activity over the control group in *L. calcarifer* before and after challenge with *Vibrio harveyi*. The finding in the present study emphasizes the point made by Harikrishnan et al. (2011) that the effects of immuno-stimulants on fish are species specific. Nonetheless, the slight improvement in serum lysozyme activity levels in all the supplemented groups compared to the control is promising to the effect that a corresponding increase in defence response could be achieved in a disease causing situation.

Regarding the period of supplementation, Matsuo and Miyazano (1993) reported that peptidoglucan supplementation for 28 days in the rainbow trout showed increased protection but the protective effect declined after feeding for 56 days. Additionally, in the African catfish, the NBT-positive cells were enhanced after dietary supplementation with glucan for 30 days but not when used over 45 days (Yoshida, Kruger & Inglis 1995). Furthermore, Nya and Austin (2011) reported a declining immune modulation effect of rainbow trout after the cessation of garlic treatment. In view of this, the immune modulatory effects of the dietary ginger and garlic supplements in this study could have reached optimum level at a point within the 24 weeks of their administration. Therefore, supplementing diets for effective short term

periods intermittently within the 6-months culture period could modulate immunity that will offer the fish a lasting protection against disease causing situations for the duration of culture. This requires further studies.

Cost-effectiveness of supplementing ginger and garlic in fish diets

Plant products present a cheaper source of immuno-stimulants for disease prevention and control compared to vaccines, probiotics, etc. (Reverter et al., 2015). Unlike vaccines which are effective against pathogens of a particular disease, plants also present an advantage of modulating the non-specific immunity to target a wider range of stressful and pathogenic situations (Sakai, 1999; Harikrishnan et al., 2011; Wang, Sun, Liu & Xue, 2016). The costs of supplemented diets in this study were not significantly higher than the control (see Table 16) suggesting that they are affordable to use. In order to appeal to consumers who prefer organic and environmentally friendly food, the use of affordable plant supplements such as ginger and garlic which have proved to have some beneficial effect when administered to cultured fish, can encourage public confidence to consume aquaculture products.

Conclusions

The results of the present study show that supplementing ginger and garlic in the diets of *S. melanotheron:*

1. Did not significantly impact growth parameters (MWG, SGR and AGR).
2. Did not significantly impact feed utilization and efficiency parameters (FCR and FER)
3. Significantly improved condition index (CI) of treated fish at 1.5% garlic dose, but did not significantly affect survival (SR) and growth form (b).

4. Significantly affected proximate nutritional composition of the harvested fish (moisture, dry matter, protein, ash, fats/oils, fibre and carbohydrate content) resulting in a higher protein, lipid and ash but less moisture, carbohydrate and fibre fish for garlic supplements; and higher protein and moisture but less lipid, ash, fibre and carbohydrate fish for ginger supplements than the control group.
5. Did not significantly impact blood parameters: erythrocyte count RBC, leucocyte count, WBC; mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH; mean corpuscular haemoglobin concentration, MCHC; haematocrit, HCT and platelet count, PLT.
6. Did not significantly affect serum biochemical composition (total protein, albumin, globulin, glucose, cholesterol and triglyceride).
7. Did not significantly modulate innate immunity (Serum lysozyme activity).
8. The most promising supplementation level for both ginger and garlic was 1.5% supplementation level for improving apparent condition and performance of *S. melanotheron* under culture.

Recommendations

From the outcome of this study, it is recommended that;

1. Further studies could focus on an appropriate dose (such as 0.5% to 1.5% ginger to garlic ratio, 1% to 1% ginger to garlic ratio) of a ginger and garlic mixture for a possible synergistic, additive or antagonistic effect with/without other plant products on *S. melanotheron* which can enhance growth and immune response.

2. The effective administration period of ginger and garlic supplements as immunnostimulants should be investigated for *S. melanotheron*.
3. An immune challenge should be conducted at the end of treatment to confirm immunocompetence of *S. melanotheron* and efficacy of the immunnostimulants.

Chapter Summary

In relating the outcome of the present study to similar works, it was realised that there were many studies whose findings per the literature used, that did not agree with the present study in terms of growth, feed utilization, survival, serum biochemical and immune modulation via serum lysozyme activity. Meanwhile, others also recorded similar results with the present studies concerning the stated parameters. The nutritional composition of fish realised in this study backed some existing knowledge that ginger and garlic supplementation can affect nutritional quality of fish. The condition index that was enhanced also points to the fact that garlic supplements possibly played a significant role in the digestion and assimilation of feed. The experiment concluded that ginger and garlic supplements affected the nutritional composition and condition index of fish but did not affect growth, feed utilization, haematology, and serum biochemical and serum lysozyme activity. Recommendations were made for further trials using the supplements.

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APPENDICES

APPENDIX A

Proximate composition of feed and fish.

Proximate analyses of fish and feed samples according to AOAC (2005).

Determination of moisture content

Porcelain crucibles were washed, oven-dried and weighed.

Procedure

1. 10 g of fresh fish samples were weighed on a digital scale in a pre-weighed crucible and recorded.
2. The crucibles containing the sample were spread over the base of the oven to ensure equal distribution of heat. They were then placed in a drying oven at 105°C for 48 hours to dry to a constant weight.
3. At the end of the period the samples were removed, cooled in a desiccator and reweighed. Each sample was done thrice.
4. The moisture content was then calculated as the percentage water loss by the sample by the calculation below:

$$\% \, Moisture\ content = \frac{(B-A)-(C-A)}{(B-A)} \times 100$$

Where: A = weight of clean, crucible (g)

B = weight of crucible + wet sample (g)

C = weight of scale pan + dry sample (g)

Determination of protein content

Determination of total nitrogen was carried out employing the Micro-Kjedahl Method by distillation of Sulphuric acid – Hydrogen Peroxide Digestion.

Procedure

1. The digestion mixture comprised of 350 ml of hydrogen peroxide, 0.42 g of selenium powder, 14 g Lithium Sulphate and 420 ml sulphuric acid.
2. 0.2g of oven-dried (at 60°C for 48 hours) ground sample was weighed into a 100ml Kjeldahl flask and 4.4ml of the digestion reagent was added and the samples digested at 360°C for two hours.
3. Blank digestions (digestion of the digestion mixture without a sample) were carried out in the same way.
4. After the digestion, the digests were transferred quantitatively into 50mL volumetric flasks and made up to the volume.
5. A steam distillation apparatus was set up and steam passed through it for about 20 minutes. After flushing out the apparatus, a 100ml conical flask containing 5ml of boric acid indicator solution was placed under the condenser of the distillation apparatus.
6. An aliquot of the sample digest was transferred to the reaction chamber through the trap funnel.
7. 10ml of alkali mixture was added to commence distillation immediately and about 50ml of the distillate was collected.
8. The distillate was titrated against 1/140 M HCL from green to the initial color of the indicator (wine red). Digestion blanks were treated the same way and subtracted from the sample titre value.
9. The percentage nitrogen was calculated using the formula below:

$$\%\ \text{Nitrogen content} = \frac{(\text{S} - \text{B}) \times \text{M} \times 14.007}{\text{Sample weight (mg)}} \times 100$$

Where:

M = Molality of Acid

S = Sample titre value

B = Blank titre value

10. The percent protein content was obtained as follows:

$$\% \text{ Protein content} = \% \text{ Nitrogen content } \times 6.25$$

Determination of Crude Fat/Lipid content using ether extract method

In this method, the fats are extracted from the sample with petroleum ether and evaluated as a percentage of the weight before the solvent are evaporated.

Procedure

1. 15g of the ground samples were weighed into a 50 ×10 mm soxhlet extraction thimble. This was transferred to a 50ml capacity soxhlet extractor.
2. A clean dry 250 mL round bottom flask was weighed. 150 mL Petroleum spirit was added and connected to the soxhlet extractor and extraction was done for 6 hours using a heating mantle as a source of heating.
3. After the 6 hours the flask was removed and placed in an oven at 60°C for 2 hours. After the 2 hours, the round bottom flask was removed and cooled in a desiccator before weighing.
4. The percentage fat/oil was calculated as follows:

$$\% \text{ Crude fat} = \frac{\text{Weight of oil}}{\text{Sample weight}} \times 100$$

Determination of Ash content by calcination method.

Ash is considered as the total mineral or inorganic content of the sample.

Procedure

1. 5 g of dry sample was weighed in a crucible previously calcined and brought to constant weight.
2. The crucible with the samples were placed in a furnace and heated at 550°C overnight.
3. After 12 hours, heating was reduced and left to cool and later transferred to a dryer.
4. The crucible with the ash was then weighed again and the ash content was calculated as a percentage of the original sample as follows:

$$\%\, Ash\ content = \frac{A - B}{C} \times 100$$

Where: A = weight of crucible with sample (g)

B = weight of crucible with ash (g)

C = weight of sample (g)

Determination of Crude Fibre

Procedure

1. 0.50g of the sample was weighed and placed in a boiling flask. 100ml of the 1.25% sulphuric acid solution was added and boiled for 30mins.
2. After the boiling, filtration was done in a numbered sintered glass crucible. The residue was transferred back into the boiling flask and 100ml of 1.25% sodium hydroxide solution was added and boiled for 30minutes.
3. Filtration continued after the boiling and the residue was washed with boiling water and methanol.
4. The crucible was dried in an oven at 105°C overnight, weighed and then placed in a furnace at 500°C for about 3 hours. The crucible was then finally cooled to room temperature in a desiccator and weighed.

$$\%CP = \frac{Weight\ loss\ through\ ashing}{Sample\ weight} \times 100$$

Determination of Nitrogen Free Extract (NFE)

The % NFE represents the carbohydrate content of the sample this was calculated as follows: $\%\ \mathrm{NFE} = \%\mathrm{DM} - (\%\ \mathrm{EE} + \%\ \mathrm{CP} + \%\mathrm{CF} + \%\mathrm{Ash})$

Where: DM - Dry Matter;

EE - Ether Extract;

CP - Crude Protein

CF - Crude

APPENDIX B

Complete blood count

Procedure for complete blood count using a 3- differential fully automatic blood counter (model: URIT-3000 URIT Medical Electronic Group Co. Ltd.).

1. To do this, it was ensured that the analyser was in good condition and functioning properly.

2. Then it was washed and pre-set to the general mode to remove any errors since it is used mainly for human samples.

3. When all was set, the test tube containing the blood sample was fixed to the nozzle of the analyser which pumped 20 μL of the sample into the system,

4. After pumping, it took approximately 60 seconds for the machine to generate a print out with the information about the full blood count displayed for the blood sample.

This was repeated for all the 27 samples and the system was washed after each sample analysis.

APPENDIX C

Serum biochemical analyses.

Procedure for serum biochemical analyses using a semi-automated chemistry analyzer.

Glucose content determination

The serum glucose concentration was determined using Liquizone Glucose-MR (GOD-POD) kit, for the quantitative estimation of glucose in human serum/ plasma. It is designed based on Trinder's method in which Glucose Oxidase (GOD) and Peroxidase (POD) are used along with Phenol and 4-aminoantipyrine.

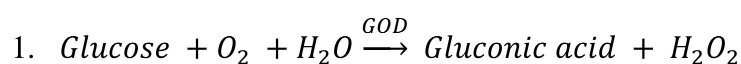
Procedure for each sample

1. Three 1.0 ml eppendorf tubes were set up and labelled as blank (B), standard (S) and test (T).
2. 1.0 mL of the Glucose reagent was pipetted into the "blank", "standard" and "test" eppendorf tubes.
3. 10 µL of the standard glucose solution ($conc^n$:100 mg/dL) was then added to the "standard" tube and mixed well using a pipette. The colour of the solution then changed from colourless to red.
4. 10 µL of the test sample was added to the "test" tube and also mixed well using the pipette. There was also a colour change from colourless to red.
5. All the solutions were incubated for 15 minutes at room temperature, (timing started when reagent was put in the "blank" tube).
6. Meanwhile, the analyser was washed with distilled water and set to the glucose determination, "End Point" method mode. The absorbance was set to

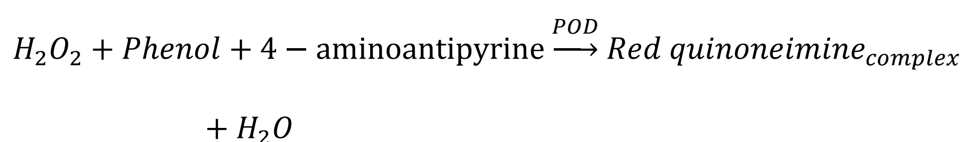
505 nm as directed by the Glucose kit manual. The analyzer displayed "pump blank please" on the LCD screen indicating that it was ready for the analyses.

7. After 15 minutes, the blank was first pumped to the analyzer and the absorbance was read and recorded by the analyser. After it was done, it displayed "pump standard please" on the LCD screen.
8. The standard solution was then pumped to the analyzer and the absorbance was also read and recorded by the analyser, After the waiting time, it displayed "pump sample please".
9. The test sample solution was then pumped to the analyzer and the absorbance was recorded by the analyser, after the waiting mode, it generated the concentration of cholesterol in the sample based on the absorbance of the standard and test against reagent blank.
10. The concentration of glucose in the sample displayed was then recorded.

- Principle for glucose determination



1. $Glucose \; + O_2 \; + H_2O \xrightarrow{GOD} Gluconic\ acid \; + \; H_2O_2$



$$H_2O_2 + Phenol + 4 - \text{aminoantipyrine} \xrightarrow{POD} Red\ quinoneimine_{complex} + H_2O$$

Determination of total cholesterol

The total cholesterol concentration was determined using Liquizone Cholesterol-MR (CHOD-POD method) kit for the quantitative estimation of cholesterol in human serum and plasma.

Procedure for each sample

1. Three 1.0 ml eppendorf tubes were set up and labelled as blank (B), standard (S) and test (T).

2. 1.0 mL of the cholesterol reagent was pipetted into the “blank”, “standard” and “test” eppendorf tubes.

3. 10 µL of the standard cholesterol solution ($conc^n$: 200 mg/dL) was added to the “standard” tube and mixed well using a pipette. The colour of the solution then changed from colourless to pink.

4. 10 µL of the test sample was also added to the “test” tube and also mixed well using the pipette. There was also a colour change from colourless to pink.

5. All the solutions were incubated for 10 minutes at room temperature, (timing started when reagent was put in the “blank” tube)

6. Meanwhile, the analyser was washed with distilled water and set to the cholesterol determination, “End Point” method mode. The absorbance was set to 505 nm as directed by the cholesterol kit manual. The analyzer displayed “pump blank please” on the LCD screen indicating that it was ready for the analyses.

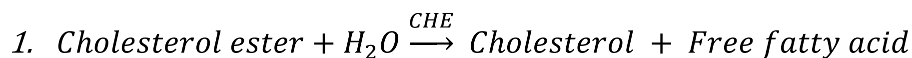
7. After 10 minutes, the blank was first pumped to the analyzer and the absorbance was read and recorded by the analyzer. After it was done, it displayed “pump standard please” on the LCD screen.

8. The standard solution was then pumped to the analyzer and the absorbance was also read and recorded by the analyzer, After the waiting time, it displayed “pump sample please”.

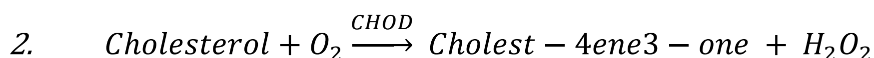
9. The test sample solution was then pumped to the analyzer and the absorbance was recorded by the analyzer, after the waiting mode, it generated the concentration of cholesterol in the sample based on the absorbance of standard and total cholesterol against the blank.

10. The concentration of total cholesterol in the sample displayed was then recorded.

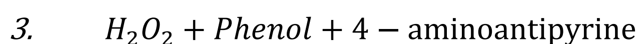
- Principle for total cholesterol



1. $Cholesterol\ ester + H_2O \xrightarrow{CHE} Cholesterol\ +\ Free\ fatty\ acid$



2. $Cholesterol + O_2 \xrightarrow{CHOD} Cholest - 4ene3 - one\ +\ H_2O_2$



3. $H_2O_2 + Phenol + 4 - \text{aminoantipyrine}$

$\xrightarrow{POD} Red\ quinoneimine_{complex} + H_2O$

Determination of triglyceride content

The triglycerides concentration was determined using Liquizone Triglyceride -MR (GPO-POD method) kit for the quantitative estimation of triglycerides in human serum or plasma.

Procedure for each sample

1. Three 2.0 ml eppendorf tubes were set up and labelled as blank (B), standard (S) and test (T).

2. The analyzer was washed with distilled water and set to the triglycerides determination, “End Point” method mode. The absorbance was set to 505 nm as directed by the tryglycerides kit manual. The analyzer displayed “pump blank please” on the LCD screen indicating that it was ready for the analyses.

3. 1.0 mL of the triglyceride reagent was pipetted into each of the “blank”, “standard” and “test” eppendorf tubes.

4. 10 μL of the standard triglyceride solution (conc^n : 200 mg/dL) was added to the "standard" tube and mixed well using a pipette. The colour of the solution then changed from colourless to pink.

5. 10 μL of the test sample was added to the "test" tube and also mixed well using the pipette. There was also a colour change from colourless to pink.

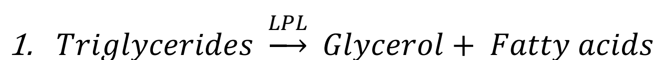
6. Following immediately the blank was first pumped to the analyzer and the absorbance was read and recorded by the analyzer. After it was done, it displayed "pump standard please" on the LCD screen.

7. The standard solution was then pumped to the analyzer and the absorbance was also read and recorded by the analyzer, After the waiting time, it displayed "pump sample please".

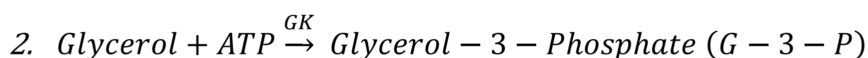
8. Following, the test sample solution was pumped to the analyzer and the absorbance was recorded by the analyzer, after the waiting mode, it generated the concentration of triglycerides in the sample based on the absorbance of standard and test against the blank.

9. The concentration of triglycerides in the sample displayed was then recorded.

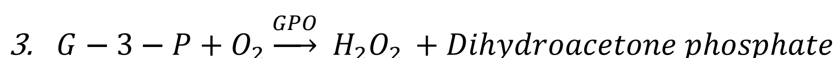
- Principle for triglycerides



1. $Triglycerides \xrightarrow{LPL} Glycerol + \ Fatty\ acids$

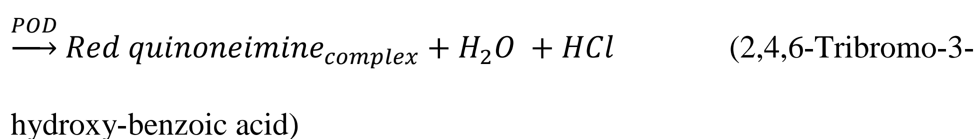


2. $Glycerol + ATP \xrightarrow{GK} \ Glycerol - 3 - Phosphate\ (G - 3 - P)$



3. $G - 3 - P + O_2 \xrightarrow{GPO} \ H_2O_2 \ + Dihydroacetone\ phosphate$

4. $H_2O_2 + 4 - \text{aminoantipyrine} \ + \text{TBHA}$



$\xrightarrow{POD} Red\ quinoneimine_{complex} + H_2O \ + HCl$ (2,4,6-Tribromo-3-hydroxy-benzoic acid)

Determination of total protein content

The total protein concentration was determined using Liquizone Total Protein (Biuret Method) kit for the quantitative estimation of total protein in human serum and plasma.

Procedure for each sample

1. Three 2.0 ml eppendorf tubes were set up and labelled as blank (B), standard (S) and test (T).
2. 1.0 mL of the biuret reagent was pipetted into the "blank", "standard" and "test" eppendorf tubes.
3. 50 µL of the standard total protein solution (conc^n : 5.5 g/dL) was added to the "standard" tube and mixed well using a pipette. The colour of the solution then changed from colourless to pink.
4. 50 µL of the test sample was added to the "test" tube and also mixed well using the pipette. There was also a colour change from colourless to pink.
5. All the solutions were incubated for 20 minutes at room temperature, (timing started when reagent was put in the "blank" tube)
6. Meanwhile, the analyser was washed with distilled water and set to the total protein determination, "END POINT" method mode. The absorbance was at 555 nm as directed by the cholesterol kit manual. The analyzer displayed "pump blank please" on the LCD screen indicating that it was ready for the analyses.
7. After 20 minutes, the blank was first pumped to the analyzer and the absorbance was recorded by the analyzer. After it was done, it displayed "pump standard please" on the LCD screen.

8. The standard solution was then pumped to the analyzer and the absorbance was also recorded by the analyzer, After the waiting time, it displayed "pump sample please".

9. The test sample solution was then pumped to the analyzer and the absorbance was recorded by the analyzer, after the waiting mode, it generated the concentration of total protein in the sample based on the absorbance of standard and test against the blank.

10. The concentration of total protein in the sample displayed was then recorded.

- Principle for total protein

The total protein is estimated based on the biuret reaction.

Determination of Albumin content

The albumin concentration was determined using Liquizone Albumin (BCG- Method) kit for the quantitative estimation of albumin in human serum and plasma.

Procedure for each sample

1. Three 2.0 ml eppendorf tubes were set up and labelled as blank (B), standard (S) and test (T).
2. The analyser was washed with distilled water and set to the albumin determination, "End point" method mode. The absorbance was set at 630 nm as directed by the cholesterol kit manual. The analyzer displayed "pump blank please" on the LCD screen indicating that it was ready for the analyses.
3. 1.0 mL of BCG reagent was pipetted into the "blank", "standard" and "test" eppendorf tubes.

4. 10 μL of the standard solution (conc^{n} : 3.5 g/dL) was added to the "standard" tube and mixed well using a pipette. The colour of the solution then changed from colourless to green.
5. 10 μL of the test sample was added to the "test" tube and also mixed well using the pipette. There was also a colour change from colourless to green.
6. Afterwards, the blank was first pumped to the analyzer and the absorbance was recorded by the analyzer. After it was done, it displayed "pump standard please" on the LCD screen.
7. The standard solution was then pumped to the analyzer and the absorbance was also recorded by the analyzer, After the waiting time, it displayed "pump sample please".
8. The test sample solution was then pumped to the analyzer and the absorbance was recorded by the analyzer, after the waiting mode, it generated the concentration of albumin in the sample based on the absorbance of standard and test against the blank.
9. The concentration of albumin in the sample displayed was then recorded.

- Principle for Albumin

For albumin estimation, the ionic dye Bromocresol green (BCG) binds selectively to albumin in a buffered medium to produce a green coloured complex. The intensity of the colour is directly proportional to the albumin concentration.

Determination of Globulin content

The globulin content was determined by the formula:

$$Globulin\ content = Total\ protein\ content - Albumin\ content$$

(Talpur & Ikhwanuddin, 2012)

APPENDIX D

Immune assessment

Assessment of lysozyme activity in fish serum

- Principle

This ELISA kit uses the Sandwich-ELISA as the method. The microelisa stripplate provided in the kit has been pre-coated with an antibody specific to LZM. Standards or samples are added to the appropriate microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for LZM is added to each microelisa stripplate well and incubated. Free components are washed away. A TMB substrate is added to each well and only those wells containing IL-10 and HRP conjugated LZM antibody will appear blue in colour and then turn to yellow upon addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of LZM. The concentration of LZM is then calculated by comparing the values of samples to the standard curve.

Assay Procedure

All reagents, working standards, and samples were prepared as directed in the instructions manual. Position of standards and samples to be assayed were recorded in a plate layout to guide in the distribution of solutions.

1. Standards dilution:

Ten wells were set for standards in the microelisa stripplate.

- ✓ In well 1 and well 2, 100 µL Standard solution (SS) and 50 µL Standard Dilution buffer (SD) were added and mixed well using the pipette.
- ✓ In well 3 and well 4, 100 µL solution from well 1 and well 2 are added respectively. Then 50 µL SD were added and mixed well. After mixing, 50 µL solution were discarded from well 3 and well 4.
- ✓ In well 5 and well 6, 50 µL solution from well 3 and well 4 are added respectively. Then 50 µL SD were added and mixed well.
- ✓ In well 7 and well 8, 50 µL solution from well 5 and well 6 are added respectively. Then 50 µL SD were added and mixed well.
- ✓ In well 9 and well 10, 50 µL solution from well 7 and well 8 are added respectively. Then 50 µL SD were added and mixed well. After mixing, 50 µL solution were discarded from well 9 and well 10.

After the dilution, the total volume in all the 10 wells are 50 µL and the concentrations are 36 ng/L (well 1 and well 2), 24 ng/L (well 3 and well 4), 12 ng/L (well 5 and well 6), 6 ng/L (well 7 and well 8), and 3 ng/L (well 9 and well 10).

2. Two blank wells (control) were set at well 11 and well 12 without any solution.

3. To the sample wells (well 13 to well 66) 40µL of Sample dilution buffer and 10µL sample were added (dilution factor of 5) per well in duplicates, according to the plate layout. Care was taken to load samples to the bottom without touching the well wall. The solution was mixed well with the pipette, then by gently shaking for 60 seconds.

4. After mixing, wells were covered with closure plate membrane and incubated for 30 minutes at 37°C.

5. While incubating, the concentrated washing buffer was diluted with distilled water 30 times: 580 mL was added to the 20-mL stock solution.

6. After the incubation, the closure plate membrane was carefully peeled off and each well was aspirated with plastic transfer pipettes, the wells were refilled with Wash Buffer using a squirt bottle and letting it stand for 30 seconds. The wash buffer was discarded whiles ensuring complete removal of liquid at each step for good performance. Washing was done five times. After the last wash, remaining Wash Buffer was removed by decanting and inverting the plate to blot it against clean paper towels.

7. Following, 50µl of HRP-conjugate reagent were added to each well immediately except the Blank control well.

8. Wells were covered with closure plate membrane and incubated for 30 minutes at 37°C.

9. Following incubation, the wells were washed again as described in step 7.

10. Afterwards, 50µL of Chromogen Solution A and 50µL of Chromogen Solution B were added to each well and mixed by gentle shaking while protecting from light. There was a colour change to blue in all the wells. Then the plate was incubated again for 15 minutes at 37°C.

11. Finally 50µl of Stop Solution were added to each well to terminate the reaction, changing the colour from blue to yellow. Thorough mixing was ensured by gently tapping the plate.

12. Optical density of each well was determined within 10 minutes after adding the stop solution using a ELISA plate reader set to 450 nm.

APPENDIX E

Abiotic water parameters

Method: One-way ANOVA; testing for differences in water quality parameters of nine groups of *S. melanotheron* cultured on different food supplements at various levels.

Null hypothesis All means are equal

Alternative hypothesis At least one mean is different

Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	9	Control, 0.5% Ginger, 1.0% Ginger, 1.5% Ginger, 2.0% Ginger, 0.5% Garlic, 1.0% Garlic, 1.5% Garlic, 2.0% Garlic

Table 17: Statistics for Analysis of variance for water quality parameters

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
pH							
Factor	8			0.121	0.01512	0.07	1.000
Error	666			151.335	0.22723		
Total	674			151.456			
DO							
Factor	8	0.203	0.08%	0.203	0.02539	0.07	1.000
Error	666	247.267	99.92%	247.267	0.37127		
Total	674	247.470	100.00%				
Conductivity							
Factor	8	6706	0.00%	6706	838	0.00	1.000
Error	666	153332359	100.00%	153332359	230229		
Total	674	153339065	100.00%				
TDS							
Factor	8	1633	0.01%	1633	204.1	0.00	1.000
Error	666	32055878	99.99%	32055878	48131.9		

Total	674	32057512	100.00%				

Salinity

Factor	8	0.0047	0.01%	0.0047	0.000587	0.01	1.000
Error	666	32.6762	99.99%	32.6762	0.049063		
Total	674	32.6809	100.00%				

Temperature

Factor	8	0.04	0.00%	0.04	0.00488	0.00	1.000
Error	666	1208.80	100.00%	1208.80	1.81502		
Total	674	1208.84	100.00%				

APPENDIX F

Growth and feed utilization parameters

Method: One-way ANOVA; ; testing for differences in growth and feed utilization parameters of *S. melanotheron* cultured on different food supplements at various levels.

Null hypothesis: All means are equal

Alternative hypothesis: At least one mean is different

Significance level: $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	9	Control, 0.5% Ginger, 1.0% Ginger, 1.5% Ginger, 2.0% Ginger, 0.5% Garlic, 1.0% Garlic, 1.5% Garlic, 2.0% Garlic

Table 18: Statistics for analysis of variance for growth and feed utilization parameters

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Initial Stocking Weight							
Factor	8	0.0982	0.14%	0.0982	0.01228	0.04	1.000
Error	261	72.3477	99.86%	72.3477	0.27719		
Total	269	72.4459	100.00%				
Final Harvest Weights							
Factor	8	1791	2.43%	1791	223.9	0.79	0.613
Error	253	71793	97.57%	71793	283.8		
Total	261	73584	100.00%				
Mean Weight Gain							
Factor	8	183.5	8.10%	183.5	22.93	0.20	0.987
Error	18	2081.4	91.90%	2081.4	115.63		
Total	26	2264.9	100.00%				
Percentage Weight Gain							
Factor	8	200524	6.35%	200524	25065	0.15	0.995
Error	18	2957920	93.65%	2957920	164329		
Total	26	3158444	100.00%				

Feed Conversion Ratio

Factor	8	0.2003	8.05%	0.2003	0.02504	0.20	0.988
Error	18	2.2890	91.95%	2.2890	0.12717		
Total	26	2.4893	100.00%				

Feed Efficiency Ratio

Factor	8	0.007830	0.000979	0.24	0.977
Error	18	0.073470	0.004082		
Total	26	0.081299			

Specific growth rate

Factor	8	0.05059	0.006324	0.18	0.990
Error	18	0.61589	0.034216		
Total	26	0.66649			

Absolute growth rate

Factor	8	0.01223	1.91%	0.01223	0.001528	0.04	1.000
Error	18	0.62627	98.09%	0.62627	0.034793		
Total	26	0.63850	100.00%				

Survival Rate

Factor	8	429.6	36.94%	429.6	53.70	1.32	0.296
Error	18	733.3	63.06%	733.3	40.74		
Total	26	1163.0	100.00%				

Condition Index

Factor	8	2.617	9.83%	2.617	0.32716	3.45	0.001
Error	253	24.016	90.17%	24.016	0.09492		
Total	261	26.633	100.00%				

Tukey Pairwise Comparisons for CI.

Table 19: Grouping Information Using the Tukey Method

Factor	N	Mean	Grouping
Garlic 1.5%	30	4.1903	A
Ginger O.5%	30	4.0644	A B
Garlic 2%	30	4.0394	A B
Ginger 1.5%	29	4.0228	A B
Garlic 0.5%	29	4.0188	A B
Garlic 1%	29	4.0172	A B
Ginger 2%	26	3.8981	B
Control	29	3.8906	B
Ginger 1%	30	3.8462	B

Means that do not share a letter are significantly differ

APPENDIX G

Proximate composition of fish

Method: General Linear Model; testing for differences proximate composition of *S. melanotheron* cultured on different food supplements at various levels.

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Supplements	Fixed	2	garlic, ginger
Dosage	Fixed	5	dosages 0%, 0.5%, 1%, 1.5%, 2%

Table20: Statistics for analysis of variance for the proximate nutritional composition of *S. melanotheron*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Moisture Content					
supplements	1	135.229	135.229	328.86	0.000
dosage	4	81.744	20.436	49.70	0.000
supplements*dosage	4	79.204	19.801	48.15	0.000
Error	20	8.224	0.411		
Total	29	304.401			
Dry Matter (%)in Fresh Fish					
supplements	1	135.229	135.229	328.86	0.000
dosage	4	81.744	20.436	49.70	0.000
supplements*dosage	4	79.204	19.801	48.15	0.000
Error	20	8.224	0.411		
Total	29	304.401			
Ash Content (%) in Fish					
supplements	1	7.856	7.85603	112.73	0.000
dosage	4	6.020	1.50489	21.59	0.000
supplements*dosage	4	4.686	1.17151	16.81	0.000
Error	20	1.394	0.06969		
Total	29	19.955			

Protein Content (%) in Fish						
Supplements	1	9.060	9.060	9.0603	15.65	0.001
Doses	4	124.889	124.889	31.2223	53.95	0.000
Supplements*Doses	4	28.207	28.207	7.0516	12.18	0.000
Error	20	11.575	11.575	0.5787		
Total	29	173.731				

Fat/Oil Content (%) In Fish

Supplements	1	19.106	19.106	19.1056	222.22	0.000
Dose	4	2.816	2.816	0.7041	8.19	0.000
Supplements*Dose	4	7.816	7.816	1.9541	22.73	0.000
Error	20	1.720	1.720	0.0860		
Total	29	31.458				

Fibre Content (%) In Fish

Dose	4	0.20710	0.051776	73.43	0.000
Supplement	1	0.01179	0.011787	16.72	0.001
Supplements*Dose	4	0.13039	0.032597	46.23	0.000
Error	20	0.01410	0.000705		
Total	29	0.36338			

NFE Content (%) in Fish

Supplement	1	13.07	13.0735	19.03	0.000
Dose	4	158.26	39.5647	57.60	0.000
Supplement*Dose	4	49.76	12.4389	18.11	0.000
Error	20	13.74	0.6869		
Total	29	234.83			

Tukey Pairwise Comparisons:

Response = Moisture, Dry matter, Protein, Fat, Ash, Fibre, NFE.

Term = supplements*dosage

Table 21: Grouping Information Using the Tukey Method

Factor	N	Moisture		Dry matter		Protein		Fat		Ash		Fibre		NFE	
		Mean	GP	Mean	GP	Mean	GP	Mean	GP	Mean	GP	Mean	GP	Mean	GP
Control	3	75.65	B	24.35	C	58.678	D	22.41	B	2.773	BC	0.748	A	11.46	AB
Ginger 0.5%	3	78.4	A	21.61	D	58.767	CD	21.61	C	2.641	C	0.482	CD	12.65	A
Ginger 1%	3	78.31	A	21.69	D	59.791	CD	22.64	B	2.264	C	0.505	CD	11.16	AB
Ginger 1.5%	3	75.71	B	24.29	C	65.126	A	20.74	D	2.547	C	0.538	BC	6.07	DE
Ginger 2%	3	75.21	B	24.79	C	60.916	BC	21.81	BC	2.383	C	0.61	B	9.69	BC
Garlic 0.5%	3	69.32	D	30.68	A	60.40	BCD	23.76	A	4.54	A	0.602	B	7.08	DE
Garlic 1%	3	75.82	B	24.18	C	56.00	E	23.70	A	2.535	C	0.743	A	13.43	A
Garlic 1.5%	3	68.92	D	31.08	A	62.40	B	23.77	A	4.433	A	0.533	BCD	4.68	E
Garlic 2%	3	72.33	C	27.67	B3	60.30	BCD	23.55	A	3.445	B	0.455	D	8.41	CD

APPENDIX H

Complete blood count

Method: General Linear Model; testing for differences in blood constituents of S. *melanotheron* cultured on different food supplements at various levels.

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Treatments	Fixed	9	Control, Garlic 0.5%, Garlic 1%, Garlic 1.5%, Ginger 1.5%, Ginger 2%, Garlic 2%, Ginger 1%, Ginger O.5%

Table 22: Statistics for analysis of variance for blood constituents parameters

Source	DF	Adj SS	Adj MS	F-Value	P-Value
WBC					
TREATMENTS	8	2897	362.2	0.78	0.625
Error	16	7410	463.1		
Total	24	10307			
HGB					
TREATMENTS	8	24.06	3.008	0.95	0.500
Error	18	56.81	3.156		
Total	26	80.87			
HCT					
TREATMENTS	8	421.9	52.74	0.61	0.756
Error	18	1548.8	86.04		
Total	26	1970.7			
MCV					
TREATMENTS	8	3123	390.3	0.95	0.505
Error	18	7424	412.4		
Total	26	10546			
MCH					
TREATMENTS	8	30.51	3.813	0.67	0.712
Error	18	102.67	5.704		
Total	26	133.17			
MCHC					

TREATMENTS	8	60.28	7.535	0.59	0.771
Error	18	228.67	12.704		
Total	26	288.95			

RCW-CV

TREATMENTS	8	18.16	2.270	0.46	0.870
Error	18	89.33	4.963		
Total	26	107.49			

RDW-SD

TREATMENTS	8	1981	247.6	0.94	0.511
Error	16	4210	263.1		
Total	24	6191			

PLT

TREATMENTS	8	9570	1196.3	1.65	0.178
Error	18	13016	723.1		
Total	26	22586			

MPV

TREATMENTS	8	705.4	88.18	1.28	0.338
Error	12	826.7	68.89		
Total	20	1532.1			

PDW

TREATMENTS	8	175.6	21.948	2.29	0.080
Error	15	143.9	9.596		
Total	23	319.5			

PCT

TREATMENTS	8	0.01102	0.001377	1.76	0.164
Error	15	0.01172	0.000781		
Total	23	0.02273			

P-LCR

TREATMENTS	8	13117	1639.7	3.37	0.023
Error	14	6813	486.7		
Total	22	19930			

P-LCC

TREATMENTS	8	928.3	116.04	1.97	0.111
Error	18	1062.7	59.04		
Total	26	1991.0			

APPENDIX I

Serum Biochemical Parameters

Method: General Linear Model; testing for differences in serum biochemical parameters *S. melanotheron* cultured on different food supplements at various levels.

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Treatments	Fixed	9	Control, Garlic 0.5%, Garlic 1%, Garlic 1.5%, Garlic 2%, Ginger 1%, Ginger 1.5%, Ginger 2%, Ginger O.5%

Table 23: Statistics for analysis of variance for Serum Biochemical Parameters

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Glucose					
TREATMENTS	8	10899	1362	1.13	0.390
Error	18	21685	1205		
Total	26	32584			
Total Protein					
TREATMENTS	8	1.385	0.1732	0.75	0.646
Error	18	4.133	0.2296		
Total	26	5.518			
Albumin					
TREATMENTS	8	0.5501	0.06876	0.98	0.484
Error	18	1.2667	0.07037		
Total	26	1.8168			
Globulin					
TREATMENTS	8	1.078	0.1347	0.62	0.750
Error	18	3.908	0.2171		
Total	26	4.986			
Triglyceride					

TREATMENTS	8	2825	353.2	0.33	0.944
Error	18	19417	1078.7		
Total	26	22243			

Cholesterol

TREATMENTS	8	2680	335.1	0.41	0.901
Error	18	14758	819.9		
Total	26	17439			

Albumin/Globulin Ratio

TREATMENTS	8	0.2457	0.03071	0.61	0.760
Error	18	0.9095	0.05053		
Total	26	1.1552			

APPENDIX J

Serum Lysozyme activity

Method: Two-way ANOVA; testing for differences in lysosome activity of S. melanotheron cultured on different food supplements (ginger or garlic) supplied at different dosages (0%, 0.5%, 1.0%, 1.5% and 2.0%) in the feeding experiment.

Table 24: Statistic for analysis of variance for Serum lysozyme activity

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Supplement	19.3454	1	19.3454	0.0355	0.8525
Dosage	1323.0077	4	330.7519	0.6065	0.6626
Supplement * Dosage	373.5016	4	93.3754	0.1712	0.9506
Error	10907.3681	20	545.3684		
Total	12623.2228	29			

APPENDIX K

Cost of treatment diets

Method: One-way ANOVA; testing for differences in cost of supplemented and basal diets administered to *S. melanotheron* in the experiment.

Null hypothesis All means are equal

Alternative hypothesis At least one mean is different

Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	9	Control, Ginger O.5%, Ginger 1%, Ginger 1.5%, Ginger 2%, Garlic 0.5%, Garlic 1%, Garlic 1.5%, Garlic 2%

Table 25: Statistics for analysis of variance for Cost of supplemented diets

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Factor	8	11.96	2.22%	11.96	1.495	0.05	1.000
Error	18	527.05	97.78%	527.05	29.280		
Total	26	539.01	100.00%				

APPENDIX L

Length-Weight relationship

Method: Regression analyses; testing for the significance of the relationship between the length and weight of nine groups of *S. melanotheron* fed on dietary supplements of ginger and garlic at different levels for 24 weeks.

Table 26: Regression Statistics for length-weight relationship of *S. melanotheron*

Treatments	Multiple R	R Square	Adjusted R Square	Std. Error	N
Control	0.996	0.992	0.992	0.034	207
Ginger 0.5%	0.996	0.992	0.992	0.036	210
Ginger 1%	0.997	0.993	0.993	0.028	210
Ginger 1.5%	0.997	0.993	0.993	0.030	205
Ginger 2%	0.996	0.992	0.992	0.033	192
Garlic 0.5%	0.996	0.992	0.992	0.033	209
Garlic 1%	0.998	0.996	0.996	0.024	207
Garlic 1.5%	0.996	0.992	0.992	0.034	210
Garlic 2%	0.996	0.992	0.992	0.034	210
Combined treatments	0.996	0.992	0.992	0.032	1860

Table 27: Statistics for the intercept and slope of the regression analysis.

Treatment	Variable	*Coefficients*	*St. Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Control	Intercept	-4.81679	0.040072	-120.205	4.9E-192	-4.8958	-4.73779
	Slope	3.021966	0.01949	155.0492	1.9E-214	2.983538	3.060393
Ginger 0.5%	Intercept	-4.98021	0.040676	-122.437	8.2E-196	-5.0604	-4.90002
	Slope	3.102422	0.019877	156.08	1.7E-217	3.063236	3.141609
Ginger 1%	Intercept	-4.80057	0.035298	-136.003	3.5E-205	-4.87016	-4.73098
	Slope	3.010757	0.017233	174.7063	1.3E-227	2.976783	3.044732
Ginger 1.5%	Intercept	-4.84203	0.0361	-134.128	3.8E-200	-4.91321	-4.77085
	Slope	3.035434	0.017605	172.4182	4.3E-222	3.000721	3.070146
Ginger 2%	Intercept	-4.80871	0.038926	-123.534	2E-183	-4.88549	-4.73193
	Slope	3.016237	0.019036	158.453	9E-204	2.978689	3.053785
Garlic 0.5%	Intercept	-4.85534	0.039742	-122.173	6.7E-195	-4.93369	-4.77699
	Slope	3.044002	0.019298	157.7337	1.3E-217	3.005955	3.082048
Garlic 1%	Intercept	-4.94061	0.028796	-171.572	2.1E-223	-4.99738	-4.88384
	Slope	3.085491	0.013986	220.6066	1.2E-245	3.057915	3.113067
Garlic 1.5%	Intercept	-4.91038	0.040437	-121.431	4.5E-195	-4.9901	-4.83066
	Slope	3.072567	0.019709	155.896	2.1E-217	3.033712	3.111422
Garlic 2%	Intercept	-4.85043	0.038529	-125.892	2.7E-198	-4.92639	-4.77447
	Slope	3.040306	0.018804	161.6814	1.2E-220	3.003235	3.077377
Combined treatments	Intercept	-4.87043	0.012799	-380.525	0	-4.89554	-4.84533
	Slope	3.049286	0.006238	488.7958	0	3.037051	3.061521

Table 28: Statistic for the analyses of variance for the length-weight relationship

Treatments	Control	*df*	*SS*	*MS*	*F*	*Sig. F*
Control	Regression	1	27.59	27.59	24040	2E-214
	Residual	205	0.2353	0.0011		
	Total	206	27.825			
Ginger	Regression	1	30.968	30.968	24361	2E-

0.5%						217
	Residual	208	0.2644	0.0013		
	Total	209	31.232			
Ginger 1%	Regression	1	24.36	24.36	30522	1E-227
	Residual	208	0.166	0.0008		
	Total	209	24.526			
Ginger 1.5%	Regression	1	27.4	27.4	29728	4E-222
	Residual	203	0.1871	0.0009		
	Total	204	27.587			
Ginger 2%	Regression	1	27.074	27.074	25107	9E-204
	Residual	190	0.2049	0.0011		
	Total	191	27.279			
Garlic 0.5%	Regression	1	26.409	26.409	24880	1E-217
	Residual	207	0.2197	0.0011		
	Total	208	26.629			
Garlic 1%	Regression	1	27.144	27.144	48667	1E-245
	Residual	205	0.1143	0.0006		
	Total	206	27.258			
Garlic 1.5%	Regression	1	28.399	28.399	24304	2E-217
	Residual	208	0.2431	0.0012		
	Total	209	28.642			
Garlic 2%	Regression	1	29.657	29.657	26141	1E-220
	Residual	208	0.236	0.0011		
	Total	209	29.893			
Combined Treatments	Regression	1	249.58	249.58	238921	0
	Residual	1858	1.9409	0.001		
	Total	1859	251.52			

APPENDIX M

Comparison of slopes

Method: student's t-test; testing for differences in the slopes of eight treatments administered to *S. melanotheron* with the control treatment.

Table 29: Statistics for the comparison of slopes of nine groups of *S. melanotheron* fed different levels of ginger and garlic supplemented diets

Treatment	SS	t	Df	A	p-value	t-critical
0.5% GINGER	0.028	0.026	417	0.05	0.979	1.966
1.0% GINGER	0.026	0.004	417	0.05	0.997	1.966
1.5% GINGER	0.026	0.004	412	0.05	0.996	1.966
2.0% GINGER	0.027	0.002	399	0.05	0.998	1.966
0.5% GARLIC	0.027	0.007	416	0.05	0.994	1.966
1.0% GARLIC	0.024	0.021	414	0.05	0.983	1.966
1.5% GARLIC	0.028	0.017	417	0.05	0.987	1.966
2.0% GARLIC	0.027	0.006	417	0.05	0.995	1.966

APPENDIX N

Growth form

Test of b = 3 versus b ≠ 3

Method: one sample t-test; testing whether the slopes (b) of nine groups of *S. melanotheron* administered with different feeding treatments is equal to 3.

Table 30: Statistics for comparing growth form of *S. melanotheron* under culture to the isometric growth pattern.

Statistic	Coefficients
Mean	3.0466
Variance	0.001035
N	9
StDev	0.0333
SE Mean	0.0111
95% CI	(3.0210, 3.0722)
t-stat	4.20
P	0.003