CHAPTER ONE

INTRODUCTION

1.1 Tilapia

Tilapia is a generic name of the group of cichlids. This group consists of three aquaculturally important genera such as *Oreochromis, Sarotherodon* and *Tilapia*. Several characteristics distinguish these three genera, but possibly the most prominent criteria is the reproductive behaviour (Gunasekara *et al*., 1995). In *Oreochromis* species, only the female practice mouth brooding, while in *Sarotherodon* species either the male or both sex are mouth brooders (Abdelghany, 2003). The scientific names of the Nile tilapia are also known as *Tilapia nilotica, Sarotherodon niloticus*, and currently known as *Oreochromis niloticus* (Popma and Masser, 1999).

Oreochromis niloticus is also an omnivore fish which is the most popular species cultured throughout the world. Its fast growth, tolerance to environmental conditions and favourable breeding characteristic are only some of the traits which make *Oreochromis niloticus* a desired fish for culture (Abdelghany, 2003). The protein range for tilapia is between 30-40% of diet depending on the particular life span. On the other hand, it has a good reproductive system (Gunasekara *et al*., 1995, 1996).

Morphologically, tilapias are easily identified by an interrupted lateral line characteristic of the Cichlid family of fishes (Abdelghany, 2003). They are laterally compressed and deep-bodied with long dorsal fins. The forward portion of the dorsal fin is heavily spine. Spines are also found in the pelvis and anal fins. There are usually wide vertical bars down the sides of fry and fingerlings (Popma and Masser, 1999).

1.2 Feeding behaviour and nutrition requirements

A wide improvement of fish culture in current years lead to the development of nutritious fish feeds and a better feed utilization, whereby the feed cost may increase the fish culture cost by 50–80% (Cavalheiro *et al*., 2007). Commonly, tilapias consume natural food organisms such as plankton, some aquatic macrophytes, planktonic, benthic aquatic invertebrates, larval fish, detritus, and decomposing organic matter (Popma and Masser, 1999). In general, natural food organisms increase the tilapias growth by 30-50% (Abdelghany, 2003). Tilapias are filter feeders whereby they efficiently harvest plankton from water. Yet, tilapias do not physically filter water through gill rakers as other filter feeders such as gizzard shad and silver carp. Tilapia gills secrete a mucous that traps plankton and it's then swallowed (El-Sayed and Teshima, 1991). Digestion and assimilation of plant material occurs along the length of the intestine (usually at least six times the total length of the fish) (NRC, 1993). The two mechanisms that help tilapia digest the filamentous and planktonic algae and succulent higher plants is by physical grinding of plant tissues with fine teeth; and an acidic stomach condition with pH below 2 that ruptures the cell walls of algae and bacteria (Fitzsimmons, 2000). Upon feeding, tilapias do not disturb the bottom part of the fish tank as aggressively as common carp which may affect water quality as well as other parameters. They also effectively browse on live benthic invertebrates and bacteria-laden detritus (Bhikajee and Gobin, 1998).

The nutritional value of the natural food supply for tilapia is very important even for commercial operations. In highly fed tanks with little or no water exchange, natural feed may supply one-third or more of total nutrients for growth (Dutta, 1994). In common, tilapia ingests plant protein and fibrous materials in feeds more efficiently compared to channel catfish. However, digestion of animal protein in tilapia is similar to catfish (Kubaryk, 1980). Tilapia requires the same ten essential amino acids as other warm water fish and the requirements for each amino acid are similar to those of other fish. Protein requirements for maximum growth are as high as 50% of the diet for small fingerlings (Jauncey and Ross, 1982). Tilapia may have a dietary requirement for fatty acids of the linoleic (n-6) family (Stickney, 1996). Tilapias appear to have similar vitamin requirements as other warm water fish species. Vitamin and mineral premixes similar to those added to catfish diets are also incorporated in commercial tilapia feeds (Al Hafedh, 1999). The feeding behaviour of tilapia allows them to use an unpelletized feeding more efficiently than the catfish or trout, but most of the commercial tilapia feeds are pelletized to reduce nutrient loss.

1.3 Tilapia nutrition

Nile tilapia is an important food fish in developing countries which is widely used in commercial farming systems for intensive aquaculture (Fitzsimmons, 2000). The high level of intensification requests improved production technology, including efficient low-cost and low-pollution feeding systems (NRC, 1993). One of the great advantages of tilapia for aquaculture is that they feed on a low tropic level. The members of the genus *Oreochromis* are all omnivores, feeding on algae, aquatic plants, small invertebrates and detritus material (Al Hafedh, 1999). The individual species may have preferences between these materials and are more or less efficient depending on species and life stages in grazing on these feeding (Abdelghany, 2003). They are all opportunistic and will exploit any or all of these feeds when they exist.

In extensive aquaculture, the fish will be able to grow by eating algae and detritus particles (NRC, 1993). In intensive systems, tilapias have the advantage that they can be fed with prepared feed which includes a high percentage of plant proteins (NRC, 1993). Apart from that, a carnivorous fish require fish meal or other animal proteins in their diets which are more expensive than plant proteins. Nutritional studies which substitute plant proteins supplemented with specific amino acid supplements may appear cheaper, but still not achieve the tilapia diets level (El-Sayed and Teshima, 1991).

1.4 Environmental conditions

In general, dissolved oxygen (DO) concentration, water temperature, pH and photoperiod are main influences of feed consumption, energy expenditure and metabolic rate, and growth of poikilothermic vertebrates as well as fish species (Dutta, 1994; Bhikajee and Gobin, 1998). Thus, the effects of these environmental factors on fish growth and metabolism deserve meticulous study. Tilapias are more tolerant to high water temperature, low dissolved oxygen, and high ammonia concentration compared to farmed freshwater fish. All tilapia are tolerant to brackish water (Jensen, 2003). The Nile tilapia is the least saline tolerant of the commercially important species but grows well at salinities up to 15ppt. The Blue tilapia grows well in brackish water up to 20ppt salinity and the Mozambique tilapia grows well at salinities near or at full strength seawater (NRC, 1993).

An optimal water temperature for tilapia growth is about 29ºC to 31ºC. Growth at this optimal temperature is three times greater than at 22ºC (Popma and Masser, 1999). The intolerance of tilapia to low temperatures is a serious constraint for commercial culture in clement regions. The lower lethal temperature for most species is from 10ºC to 11ºC for a few days but the Blue tilapia tolerates temperatures up to 9ºC (Dato-Cajegas and Yakupitiyage, 1996). Tilapia generally stops feeding when water temperature falls below 17ºC. Disease-enhanced mortality after handling critically restrains sampling, harvest and transport below 18ºC. Reproduction is excellent at water temperatures higher than 27ºC and does not occur at temperature below 20ºC (Jensen, 2003).

Tilapia survival in morning dissolved oxygen (DO) concentration with less than 0.3ppm which is considerably below the tolerance limits for most other cultured fish (Diana *et al*., 1997). Nile tilapia grew better when aerators were used to prevent morning DO concentration from falling below 0.7 to 0.8ppm. Although tilapia can survive from drastic decline of DO concentration for several hours, yet tilapia tanks should be managed to maintain DO concentration above 1ppm (Beketov, 2004). At the same time, tilapia can survive in pH level ranging from 5 to 10 but a greater in pH level from 6 to 9 (Popma and Messer, 1999).

Massive fatality occur within a few days when fish are transferred or exposed to water with unionized ammonia concentration greater than 2mg/L (Camargo *et al*., 2005). However, when gradually acclimated to sub lethal levels, approximately 50% of the fish will survive for 3 or 4 days at unionized ammonia concentration as high as 3mg/L (Buttner *et al.*, 1993). Prolonged exposure especially for several weeks to unionized ammonia concentration greater than 1mg/L causes high mortality rate, especially among fry and juveniles in water with low DO concentration. The first mortalities from prolonged exposure may begin at concentration as low as 0.2mg/L. Unionized ammonia begins to depress food consumption at concentration as low as 0.08mg/L (Camargo *et al*., 2005).

High nitrite concentration tends to be toxic to many aquaculture species because nitrite reduces the hemoglobin function as oxygen carrier. However the chloride ions in water may reduce the toxicity (Jensen, 2003; Alonso and Camargo, 2008). Tilapias are more tolerant to nitrite than other cultured freshwater fish. The nitrite concentration in freshwater culture should be maintained below 27mg/L. As a precaution against nitrite toxicity in recirculation systems, chloride concentration is often maintained at 100 to 150mg/L (Jensen, 2003).

1.5 Fish feed

Commonly, fish meal is the major protein source used in poultry, swine industries and aqua feeds. Meanwhile, fish meal is highly demanded and costly with the availability, together with risk factors associated with diseases (El-Sayed *et al*., 2003). Nutritionists have to study on the alternative sources for fish meal replacement to the diets of freshwater and marine species. The growing aquaculture industry can't continue to rely on finite stocks of wild-caught fish because a number of which are already classified as fully exploited, over-exploited or depleted (NRC, 1999).

1.6 *Pleurotus sajor-caju*

Pleurotus sajor-caju (Basidiomycetes) is the grey oyster mushroom. This mushroom is easy to cultivate on various plant waste materials such as mixture of wheat straw, paddy straw; and hulled maize; animal bedding and rice (Kandaswamy and Ramasamy, 1978; Roxon and Jong, 1977). The fruiting bodies, which are edible and are used as protein-rich food in India and Southeast Asia (Bano *et al*., 1963), are appear approximately 20 - 25 days after the inoculation.

Pleurotus sajor-caju is the most important cultivated mushroom in Malaysia. Nutritionally, it is considered to be high in protein (%), fiber, carbohydrates, vitamins and minerals. The most important aspects of *Pleurotus* spp. is the use of ligninolytic enzymes for a variety of applications such as the bioconversion of agricultural wastes into valuable products for animal feed and other food products (Cohen *et al*., 2002). *Pleurotus* spp. is also known for pharmaceutical properties which include hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation actions (Mattila *et al*., 2000).

1.7 Objective of study

This study aimed to observe the ability of juvenile *Oreochromis niloticus* to utilise supplement diets of powdered *Pleurotus sajor-caju* stalks (waste) in formulated pellets.

The objectives of the present study were:

a) To determine the effects of feeding powdered *Pleurotus sajor-caju* stalks as supplement in a commercial diets on growth and survival of juvenile *Oreochromis niloticus* in 8 weeks of feeding trial.

b) To determine whether the pH, temperature, ammonia, nitrite, nitrate and dissolved oxygen (DO) concentrations in water influence by the diet given to juvenile *Oreochromis niloticus* in 8 weeks of feeding trial.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tilapia

Tilapia culture has been conducted since the beginning of human history. For instance, ancient Egyptians raised tilapia for human consumption about 2000–2500 BC (Chimits, 1957; Bardach *et al*., 1972). Currently, tilapia culture is widely developed in many tropical and subtropical regions of the world. In general, more than 22 tilapia species are being cultured worldwide (Tsadik and Bart, 2007). Though, Nile tilapia (*Oreochromis niloticus)*, Mozambique tilapia (*O. mossambicus)*, blue tilapia (*O. aureus)*, *O. macrochir*, *O. hornorum*, *O. galilaeus*, *Tilapia zillii* and *T. rendalli* are the most industrially cultured tilapia species (Thankur *et al*., 2004). The Nile tilapia, *O. niloticus* grows and reproduces in a various range of environmental conditions and tolerates stress. Culture practices of tilapias may lead to extensive, semi-intensive and intensive culture systems. Although tilapias are now the most popularly farmed fish after carps, a consistent supply of quality seed remains a major limitation to further expansion (Tsadik and Bart, 2007).

In terms of prepared diet for tilapia, the physical properties of pelleted tilapia feeds, especially size of the pellet and water stability are important. Tilapia required smaller pellets than salmonids and channel catfish of similar size (Kubaryk, 1980). The most common pellet size for feeding tilapia to a marketable size of 0.5kg is 3 to 5mm in diameter. Tilapia responds to frequent feeding than channel catfish and salmonids because of their continuous feeding behavior and smaller stomach capacity. Kubaryk (1980) found that Nile tilapia grows faster when fed four times daily rather than twice or fed eight times a day.

2.2 Nutrient requirement

The most important factor to enhance growth and production of reared fish is by feeding of prepared diets (Thankur *et al*., 2004; Liti *et al*., 2005). Generally, necessities for each nutrient are reliant on several factors. Including factors such as the age, size, condition, and reproductive state of the fish, the environmental factors such as water pH, dissolved oxygen, temperature, water quality, and photoperiod are also affect requirements; and feeding management which includes the amount, and frequency of feeding. Furthermore, the diet including the amount and quality of protein, energy, and the technique of processing will influence the necessities.

Commonly tilapia fed with a balanced diet with appropriate mix of protein, carbohydrates, lipids, vitamins, mineral and fiber will give the best growth rate (Jauncey and Ross, 1982; Stickney, 1996). The nutritional requirements of each species vary slightly depending on life stage. Fry and fingerling fish require a diet with higher protein. Protein requirement for a maximum performance of tilapia during larval stages is relatively high (30 - 45%), and decreases with increasing fish size (Table 2.1). For tilapia juveniles, the protein requirements range from 30-40%, while adult tilapias require 20-30% of dietary protein for optimum performance. On the other hand, tilapia brood stocks require 35-45% dietary protein for optimum reproduction, spawning efficiency, and larval growth and survival.

Table 2.1: Protein requirements of cultured tilapia (*O. niloticus*)

Life stage	Weight (g)	Protein source	Requirement (%)	References
Fry	0.012	Fish meal	45	El-Sayed & Teshima, 1992
	0.51	Fish meal	40	Al Hafedh, 1999
	0.80	Fish meal	40	Siddiqui *et al*., 1988
Fingerlings	2.4	Casein/Gelatin	35	Abdelghany, 2000
	3.50	Casein	30	Wang *et al*., 1985a
	6.1-16.5	Fish meal	30	De Silva & Radampola,1990
	45-264	Fish meal	30	Al Hafedh, 1999
Brood stock	-	Fish meal / Soybean Meal	40	El-Sayed *et al*., 2003
	-	Fish meal	45	Siddiqui *et al*., 1998
	-	Casein/Gelatin	35-40	Gunasekera *et al*., 1996

Fish growth is determined through the combined effects of feed quantity and quality. The quantity of feed consumed is regulated through the appetite to satisfy the energy requirements of fish. Several studies have been conducted to determine the optimal protein: energy (P/E) ratio for some cultured fishes (Catacutan and Coloso, 1995; Webster *et al*., 1995; Tibaldi *et al*., 1996; Hernandez *et al*., 2001). However, the optimal protein range may vary depending on the fish species, fish size, diet formulation and culture system. Limited food availability, that does not allow full appetite satisfaction results in growth rates below maximum potential. The failure to provide an adequate amount of protein and energy in the diets have also contributed to poor growth, while excessive quantities of energy might cause an unwanted fat deposition or reduced feed consumption (NRC, 1993).

Previously, Dutta, (1994) reported that the fish growth is dependent on feed consumption, nutrients absorption and adaptation into body tissues such as muscle, fat, epithelial and connective tissue. The percentage of protein or fat adapted in these tissues is extremely dependent on the diet (NRC, 1993). The fish is unable to synthesize the protein; therefore a proper ratio of essential amino acids has to be provided in the diet to induce the protein synthesizing (Regost *et al*., 1999; Cheng *et al*., 2003; Gomez- Requeni *et al*., 2004). In general, tilapia requires nutrients with an essential growth unit for both tissue expansion and protein synthesis (nitrogen retention) (De Silva and Perera, 1995; Abdelghany, 2003).

The description of growth is a basic problem in population biology (Sumagaysay and McGlone, 2003). This is because in monitoring growth, the data set involves size at various ages. This size-age data points is difficult to interpret. The common factors of growth are stocking density (Zonnevels and Fadholi, 1991; Wu, 1995), stocking size (Wohlfarth and Wedekind, 1991), appropriate diet (Dong *et al*., 1993), quality of diet (Abdelghany, 2003), feeding frequency (De Silva and Perera, 1995; Gunasekera *et al*., 1996), effect of diet substances (El-Sayed *et al*., 2003) and source of energy (El-Sayed and Garling, 1988). One the other hand, the excessive usage of non-protein energy in fish have some disadvantages such as inhibition of other nutrients function (Ulloa- Rojas and Weerd, 1997); generates fatty fish (Fu *et al*., 2001); affects feed consumption (Kissil *et al*., 2000). Therefore usage of non-protein energy in the diets must be closely evaluated.

In general, two factors mainly used to determine the nutritional value of diet are specific growth rate (SGR) and feed conversion ratio (FCR). SGR can be calculated as percentage of mean weight gain per day (Kissil *et al*., 2000). SGR increases at maximum rate of feeding and the utilization of nutrients in diet. The proper nutrients in diet help to increase the growth rates and cut down the costs of diet per unit of production while reducing the potential accumulations of net solids and chemical oxygen demand

(Jirsa *et al*., 1997). Apart from that, Ai *et al*., (2004) found that the genetic and environmental factors would also affect the growth and body composition of juvenile Japanease sea bass, *Lateolobrax japonicas.*

2.3 Major nutrient components of feedstuff

The major nutrient components of feedstuff are moisture, fat, protein, carbohydrate, vitamins and minerals that are needed for fish during the growth process (El-Sayed *et al*., 2003).

2.3.1 Moisture

Moisture (water) is important diluents of the nutrients in feedstuffs. Moisture derived from the atmosphere is generally low, 6 – 10% by weight, and a slight significance was attributed to these low concentrations in diets or ingredients (De Silva and Perera, 1995). Low-moisture concentrations allow good storage and handling. In contrast, feeds with ≥12% moisture are highly at risk of spoilage by microorganisms (Fu *et al*., 2001).

2.3.2 Fat

Dietary lipids are known as main sources of energy and of essential fatty acids (EFA) which are required for normal growth and development. They assist in the absorption of fat-soluble vitamins. Lipids are also important factors in the palatability of feeds because feeds are made from purified ingredients rather than that the type of ingredient normally used in commercial animal feed (Tacon *et al*., 1990). Takeda *et al.*

(1975) reported the lower protein concentration in yellowtail diets from 70 to 55% without any depression in growth rate by elevating the lipid substance. This finding proven that all the diets must be formulated not only to meet the optimum ratio of energy to protein, but also contain an adequate amount of lipid for that species.

2.3.3 Protein

Dietary protein is a main feature in obtaining efficient fish production and its desires should contain fish requirements due to age/weight (Dong *et al*., 1993). Protein is the most costly element in prepared feeds and therefore it should be carefully formulated to meet the needs of the cultured organism (Abdelghany, 2003). The basic principle in fish culture management is by knowing the protein requirement of fish throughout the growth period contribute to maximize the efficiency of feed conversion, cheap, and decreased nutrient release into the aquatic ecosystem (Abdel-Tawwab and Ahmad, 2009).

A lot of leguminous or cereal plants and by-products are able to be utilized as partial protein sources for tilapia. For example, leucaena leaf meal (LLM, 30% crude protein), brewery wastes, corn products (gluten, gluten feed, distiller's grain, co-products), cassava leaf meal, green gram legume, lima bean and leaf protein concentrates are the most important sources (El-Sayed *et al*., 2003). Though, the majority of leguminous or cereal plants are lacking in few essential amino acid such as arginine, threonine, isoleucine, histidine, and methionine. This LLM might consist of antinutrients such as mimosine which tend to be a toxic non-protein amino acid (Lim and Dominy, 1991).

The fishery by-products such as fish silage and shrimp meals have been commercially used for tilapia diet. Previously, several studies have shown the use of fishery by-products as fish meal substitute in diet of tilapia. The results confirmed that fish silage in range from 30 to 75% can be successfully integrated in tilapia feed, depending on fish species and size, silage source, and diet composition (Fagbenro, 1994; Fagbenro *et al*., 1994). Apart from that, animal by-products such as poultry by-product meal (PBM), blood meal (BM), hydrolyzed feather meal (HFM) and meat and bone meal (MBM) are also largely utilized as protein sources for tilapia, because these by-products are rich in protein content and good essential amino acids (EAA) profiles (Tacon, 1993). Nevertheless, these by-product might be lacking in some of the EAA such as lysine (in PBM, HFM), isoleocine (BM) and methionine (MBM, BM, HFM) (NRC, 1983; Tacon and Jackson, 1985). The quality of these diets can be improved by adding the by-products in the feed at appropriate ratios (Tacon *et al*., 1983; Davies *et al*., 1989).

Some researchers have considered other oilseed by-products (groundnut, sunflower, rapeseeds, sesame seeds, copra, macadamia, cocoa cake and palm kernel), as a good protein sources for tilapia. Jackson *et al*. (1982) proved that rapeseed meal could effectively replace up to 75% of fish meal protein in *O. mossambicus* diets. In contrast, Davies *et al*. (1990) indicated that only 15% rapeseed meal could successfully replace fish meal or soybean meal in *O. mossambicus* diets, whereas higher levels contributed to reduction of growth and feed efficiency. This might be due to the high content of glucosinolate (antinutrient) in rapeseed. Almost the same findings were presented with respect to the use of macadamia press cake as a protein source for tilapia (Fagbenro, 1993; Balogun and Fagbenro, 1995).

2.3.4 Fiber

Fiber supplies physical form to the fish feed. Commonly, cellulose and hemicellulose were used as diluents and binding agents in experimental fish diets. Dietary fiber enhanced gastric clearance time of rainbow trout fed with purified diets (Hilton *et al*., 1983). Buhler and Halver (1961) indicated that small quantity of supplemental cellulose improves growth and the efficiency of protein consumption in experimental diets. Fish species can tolerate up to 8% of fiber in their diets. However higher concentrations (8 to 30%) may affect the growth (Hilton *et al*., 1983; Poston, 1986).

2.3.5 Carbohydrate

The nutritional value of carbohydrates is differing between fish species (Balogun and Fagbenro, 1995). Warm-water fish can utilize higher volume of dietary carbohydrate compared to cold-water and sea water fish (Davies *et al*., 1990). The absence of carbohydrates in diet may cause other compounds, such as lipids and protein, are catabolized for energy and for the synthesis of various biologically important compounds is typically resulting from carbohydrates (El-Sayed *et al*., 2003). Therefore, it is important to supply the proper concentration of carbohydrate in the diet.

The utilization of dietary carbohydrates by fish differs and appears to be related with the complexity of the carbohydrate. Presence of glucose, maltose, and sucrose contribute to the excellent growth rates, followed by descending order of dextrin and fructose, galactose and potato starch, and glucosamine when a variety of carbohydrate sources were fed to young chinook salmon at a concentration of 10% of the diet (Buhler and Halver, 1961). Apart from that, the rainbow trout have been reported to utilize 30 % of glucose in a 45% of protein diet, while a concentration of 30% of glucose in a 30% of protein diet had a negative effect on the growth and feed efficiency (Bergot, 1979).

2.3.6 Minerals and vitamins

Fishes differ from other animals, where they can absorb a number of minerals (inorganic elements) from their external aquatic environment and not only from their diets (Watanabe *et al.,* 1997). Concentration of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) from the water are also important elements of the fish nutritional requirements. On other hand, sulfates and phosphates could be gained from feed sources (Lall, 1989). Inorganic elements are required for the normal life stage of fish. A part from that, the natural food, fertilizer, lime, and water also could be source of minerals (Dato-Cajegas and Yakupitiyage, 1996). Summary of required minerals for fish is listed in Table 2.2 (Watanabe *et al.,* 1997; El-Sayed and Teshima 1991).

Calcium and phosphate composition are also important aspects, which need to be considered during diet formulations for use in aquaculture. Phillips *et al*., 1959 reported that fishes have the ability to absorb calcium from their environment. Fishes rely fully on presence of calcium in water during dietary calcium deficiency (Ogino and Takeda, 1978; Ichii and Mugiya, 1983). In addition to its structural functions, calcium is very useful in formation of blood clot, muscle contraction, nerve impulse transmission, activation of some enzymes and the maintenance of cell reliability and acid-base stability. Table 3 shows of minerals requirement of fish (Watanabe *et al.,* 1997; El-Sayed and Teshima 1991)

Vitamins are known as organic compounds, which is distinct from amino acids, carbohydrates, and lipids, that are required in trace amounts in the diets for normal growth, reproduction, and health of the fish species (Dato-Cajegas and Yakupitiyage, 1996). Small volume of vitamins is mixed in feedstuff from a different unit of other major and minor feed substance. Vitamin deficiencies may be due to low content in feeds, environmental or physiological stresses and diseases especially arising in the premature development stages (Lall, 2000). Vitamins might be lost upon processing and prolonged storage of feed, often due to the oxidation of lipids caused by the addition of a sub-optimum level of antioxidant (Lall, 2000).

Table 2.2: Summary of minerals requirement of fish (Watanabe *et al.,* 1997; El-Sayed and Teshima, 1991)

Minerals	**g/kg**
Calcium (Ca)	3.0
Phosphorus (P)	7.0
Magnesium (Mg)	0.5
Iron (Fe)	0.15
Zinc (Zn)	0.20 (Note: Should not be above 0.3 (30 ppm)
Copper (Cu)	0.003
Manganese (Mn)	0.013
Selenium (Se)	0.0004
Iodine (I)	0.001

2.4 Water quality

Survival and growth of organisms in aquatic environment are also determined into a large extent, by the physical and chemical properties of the water (Rogan and Cross, 1996; Diana *et al*., 1997). Generally, water quality in aquatic ecosystem is affected by the biochemical processes which is associated with the normal metabolism of the organisms present (Milstein, 1990; Milstein and Svirsky, 1996), as well as external inputs related to aquaculture management (Milstein *et al*., 2001; Jha *et al*., 2003). Parameters used to check water quality are temperature, dissolved oxygen (DO), pH, ammonia, nitrite, nitrate, phosphate, salinity and many others. Water quality parameters were monitored in most of the studies involving fish nutrition. Table 2.3, reflects the pertinent points in some of the studies which were done earlier.

Dissolved oxygen (DO) is a critical factor in intensive fish culture, and success or failure in fish farming often depends upon the ability of the farmer to cope with problems caused by the lack of DO (Dutta, 1994). It is difficult to predict rates of oxygen in the fish tank because dissolved oxygen concentrations are constantly changing as these experiments are run in the laboratory. DO concentration above 5.0ppm enhances the growth (NRC, 1993). Meanwhile, feeding capability was decreased if DO concentration falls between 3.0-5.0ppm and at below 3.0ppm for extended period, fish stopped to consume feeds (Jensen, 2003). DO concentrations can be maintained with a proper aeration system. Previous studies reported the suitable water temperatures in tank were within range from 26-34°C and pH were within 5.2 – 7.9 (Table 2.3).

Table 2.3: Water quality assessment for fish from several studies

Parameter	**Pertinent Points**	**Source**
pH Temperature Dissolved oxygen (DO) Nitrite-nitrogen Ammonia-nitrogen Alkalinity	7.6; Growth in red drum (*Scia-nenops ocellatus*) 28.5ºC 6.9mg/L 0.32mg/L 0.9mg/L 159.5 mg/L	McGoogan, 1996
pH Temperature Dissolved oxygen (DO) Total phosphate Ammonia-nitrogen Alkalinity Plankton biomass	7.5; Growth in Nile tilapia *O. niloticus* 29ºC - 2.6 (0.6-6.3 for 6h)mg/L - 6.2 (1.8-10.6 for 18h)mg/L 0.25 (0.05-0.59)mg/L 0.62 (0.01-2.03)mg/L 128 (53-160)mg/L 7 (1.26)mg/L	Dato-Cajegas and Yakupi-tiyage, 1996
pH Temperature Dissolved oxygen (DO) Ammonia-nitrogen Alkalinity	7.8; Growth in Red tilapia *O. ni-loticus x O. mossambicus)* 26.2ºC 6.3mg/L 1.35mg/L 251mg/L	Abdelghany, 2003
pH Temperature Dissolved oxygen (DO) Nitrite-nitrogen Ammonia-nitrogen Alkalinity Carbon dioxide (CO_2)	5.18-7.47; Growth *Acipencer oxyrinchus* in cement tanks 26.7-33.53ºC 4.54-9.96mg/L 3.96-33.25µg/L 0.086-800.43µg/L 38.75-51.05mg/L 2.8-4.4mg/L	Gangadhara *et al*., 2004
pH Temperature Dissolved oxygen (DO) Nitrite-nitrogen Ammonia-nitrogen Salinity	7.6; Growth in red drum 28.5ºC 5.9mg/L 0.07mg/L 0.10 mg/L 33.5%	Thoman *et al*., 2004

Elevated concentrations of inorganic nitrogen compounds (i.e., ammonia, nitrite, and nitrate) in freshwater ecosystems can induce eco-toxicological processes because they are toxic to aquatic animals (Alcaraz and Espina, 1994; Beketov, 2004; Camargo *et al*., 2005). Ammonia production is directly related to feeding and depends on the quality of feed, feeding rate, fish size, and temperature. Therefore, feeding activity leads to the increase of ammonia concentration. In most fish species, ammonia production will be high at 4–6 hours after feedings. High protein feeds in intensively cultured fish stock excrete high concentrations of total ammonia-nitrogen (TAN) into the water. Aquatic ecosystems get worse quickly due to TAN accumulation causing lack of productivity. The amount of TAN that exists in the toxic un-ionized form increases with escalating water temperature as well as pH (Buttner *et al.*, 1993).

Nitrite (NO_2^-) is a natural element from the nitrogen cycle in aquatic ecosystems (Wetzel, 2001; Philips *et al*., 2002). Nitrite is an intermediate oxidation form between ammonia and nitrate, which can be traced in unpolluted freshwater ecosystems at concentrations between 0.001 and 0.005ppm NO_2^-N (Kelso *et al*., 1997; Wiesche and Wetzel, 1998). On the other hand, pollution sources such as, industrial wastewater effluents, municipal sewage effluents, excess from agriculture, emissions to the atmosphere and the subsequent atmospheric depositions, also increased the concentrations of nitrite and as well as the ammonia and nitrate concentrations in freshwater ecosystems (Gleick, 1993; Smil, 2001). Aquatic animals absorb nitrite from water through their chloride cells, located in the gills (fish and crustaceans) and in the anal papillae (*Chironomus* larvae, Diptera) (Lewis and Morris, 1986; Alcaraz and Espina, 1994; Jensen, 1995). Higher external nitrite concentrations reduce the rate of chloride active uptake, and this is due to higher affinity of NO_2^- for the Cl^- uptake mechanism (Jensen 1995, 2003).

Nitrite can cause dysfunctions of the oxygen carrying pigments, leading to hypoxia and finally death after diffusion in the animal bodies (Jensen 1995; Camargo and Alonso 2006). The oxidation of respiratory pigments seems to be active in hemoglobin (fish pigment) and being less important in hemocyanin (crustacean pigment) (Jensen 1995 & 2003). Compared to ammonia and nitrite, nitrate is relatively non-toxic to organisms in the aquatic ecosystem. Nevertheless, high nitrate concentrations can influence the growth of commercially cultured aquatic organisms, such as eel (Kamstra and van der Heul, 1998); shrimp (Muir *et al*., 1991) and octopus (Hyrayama, 1966). Maximum nitrate concentrations vary among recirculation systems and mainly recorded by water exchange rates and the extent of nitrification and nitrate removal.

Fishes could partly absorb phosphorus from the aquatic ecosystem. A low concentration of dissolved phosphorus (0.005- 0.05mg/l) is inadequate to meet their requirement (Lall, 1989 and 1991) while total phosphate concentration of the culture increased from 0.05mg/l to 0.59mg/l (Lall, 1991). Temperature range of 26.5 – 32°C, dissolved oxygen about 7mg/l and salinity from 32 to 36% was presented by Ai *et al*., (2004); pH of 7.65, dissolved oxygen about 6.93mg/l, temperature at 18.3°C, total ammonia of 0.06mg/l, and salinity of 37% growth in abalone had been presented by Sales and Britz (2002). Khan, (1994) reported water quality in tank at temperature from 25 to 28°C, dissolved oxygen from 6.60 – 8.30mg/l an pH from 7.63- 8.25, ammonia-nitrogen from 0.05 to 0.09mg/l and nitrate nitrogen from 1.0 to 1.15mg/l.

2.5 Nutritional value of *P. sajor-caju*

Mushrooms are a good source of vitamins and minerals and are preferred due to its special flavor and aroma in many countries. Increasing consumption of mushroom is good for preventing malnutrition; although mushrooms cannot be an alternative protein source to replace meat, fish, and egg (Garcha *et al*., 1993) Mushroom species also has functional properties such as the richness in vitamin B complex and vitamin D and anti-tumor, anticancer and antiviral activities due to lentinan. Shiitake, which contains lenti-nacin and lentysine, has serum cholesterol lowering effect (Mattila *et al*., 2000).

There are few amounts of scientific data on *P. sajor-caju* and the research carried out are mostly on nutrients and volatiles and some properties of *L. edodes* and *Pleurotus* spp. Patrabansh and Madan (1999) investigated those minerals of *P. sajor- caju* in different kinds of biomass. Their results showed that there are differences between various substrates of cultivation and minerals of fruiting bodies *P. sajor-caju* increase in substrates containing high mineral content. *Pleurotus* spp. is a good source of B complex vitamins, minerals and protein, and can contribute to human diet (Timmel & Kluthe, 1997; Latiff *et al*., 1996).

2.6 Application of *Pleurotus* spp. in animal feed

The use of *Pleurotus* spp. is related to the utilization of their ligninolytic system for a variety of applications, such as the bioconversion of wastes from agricultural practices into animal feed and other foodstuffs. In addition, their ligninolytic enzymes are also used for the biodegradation of organopollutants, xenobiotics and industrial contaminants (Cohen *et al*., 2002). The direct use of lignocellulosic residues as ruminant animal feed, or as an element of such feeds, represents one of its oldest and most widespread applications and, as such, it plays an important role in the ruminant diet. The use of white rot fungi is to improve the digestibility of lignocellulosic material for ruminants.

Some species of *Pleurotus* are able to colonize different type of vegetable wastes such as cotton plant stalks (Platt *et al*., 1984), corn stover (Commanday and Macy, 1985), natural lignino-cellulosic wastes (Rajarathnam and Bano, 1989), rice and wheat straw (Zhang *et al*., 2002), water hyacinth (Mukherjee and Nandi, 2004), and coffee pulp and wheat straw (Salmones *et al*., 2005) and increase their digestibility. Previous studies have shown the feasibility of using spent wheat straw (Calzada *et al*., 1987) and wheat straw (Adamovic *et al*., 1998) to produce animal feed and as substrate for mushroom production (Yildiz *et al*., 2002). Upon harvesting the mushroom, the spent mushroom substrates still consists of all the nutrients and medicinal compounds which are similar to the mushrooms. Therefore, Adamovic *et al*., 1998 suggested the spent mushroom substrate also can be a best feed product for livestock.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Feed formulation and preparation

This experiment was designed by using three different dietary compositions with powdered *Pleurotus sajor-caju* (Oyster mushroom) waste (stalk) in combination with a commercial fish diet. These diets are based on commercial feed which were incorporated with 25% powdered *P. sajor-caju* and 50% powdered *P. sajor-caju* respectively and a control diet where only pure commercial feed was used (Table 3.1). The initial preparation was done by cleaning, washing and air drying of the *P. sajor-caju* mushroom waste stalks at ambient temperature. Once it has dried completely, it was transformed into powder using a Continuous Chinese Herbs Grinding Machine (Model: DF-20) to be added into commercial feed. These three diets were then mixed and pelletized before being dried at room temperature. *P. sajor-caju* waste stalks in this study were obtained from a local mushroom farm named Vita Agrotech Sdn. Bhd., which was located in Tanjung Sepat, Selangor.

Table 3.1: Pelletized tilapia diets formulation.

Feed	**Formulation**
Diet 1	25% of Powdered mushroom stalks (30g) + 75% of commercial feed (90g) + water (56.25ml)
Diet 2	50% of Powdered mushroom stalks (60g) + 50% of commercial feed (60g) + water (56.25ml)
Diet 3	Commercial feed (120g)

3.2 Pellet preparation

Bovine gelatine which played as a binder in this diet was used to prepare the pellets for this study. The production of pellets were done by mixing the bovine based gelatine with the prepared powdered *P. sajor-caju* stalks and finely ground powdered commercial feeds which were then mixed with a mixture of cooled (lower than 70°C) gelatine (21.26g) prepared in a container and stirred thoroughly until dissolved completely in boiling water (112.5ml). The ingredients were then prepared by mixing the formulation which were then added with water (56.25ml) and the gelatine solution, after which the dough was hand rolled and shaped into different forms. A simple machine such as pasta or 'traditional murukku' maker was then used to extrude and shape the dough into fine noodles. The extrusion was then air dried and cut to the desired length thus producing the pellets (Julie-Anne and Frank, 2003).

3.3 Proximate analysis

The diets were sent to the food analysis laboratory, UNIPEQ, located at National University of Malaysia, Bangi for proximate analyses. These analyses were done to evaluate the amount of basic nutrients such as protein, fat, carbohydrates, ash, and moisture. These analyses were carried out using in-house modified method from AOAC 16th edition. The methodology to determine the Quantity of crude fibre was carried out individually following the original AOAC method, 1995. The values of each nutrient were stated in percentage (weight/total dry weight). The proximate analysis was summarised on Table 3.2.

Table 3.2: Summary of proximate analysis of all diets

Nutrient	**Sample**		
	P. sajor-caju 25%	**P. sajor-caju 50%**	**Commercial fish feed**
Protein	26.45	27.20	19.65
Fat	0.9	0.80	2.5
Carbohydrate	48.3	44.20	57.75
Ash	9.1	9.10	13.5
Moisture	15.25	19.20	6.6
Energy	307	307	332

3.4 Maintenance of fish

The juvenile *Oreochromis niloticus* (2 weeks old; 3.7 ± 0.4cm total length) for this study were obtained from Banli Fish Farm, Ipoh, Perak. This feeding trial was carried out in Rimba Ilmu, University of Malaya. Experimental fishes were maintained in nine small plastic fish tanks (32cm x 15cm x 20cm) filled with 10 liters of dechlorinated freshwater. Each tank was stocked with 20 fishes at ambient temperature. Aeration through air stone diffusers on the mid-bottom of the tanks was provided (Ng *et al.*, 2000). The fishes were acclimatised to the system for one day prior to feeding trial (Day 0). The feeding trial commenced from day 1. The aquaria feed dietary were randomly arranged to diet in triplicate. Fishes were fed twice in a day (0900h and 1500h) for a consecutive 8 weeks. Half of the tank water was changed every alternate day, during the weekly length measurement procedure. The walls of each tank was scrubbed and washed before refilling with dechlorinated freshwater and releasing the fish back to the tank.

3.5 Water quality monitoring

During the experiment, water quality was maintained by constantly changing the water on alternative days. Unconsumed feed particles and faeces that settled at the bottom of the fish tanks were removed by using a siphoning tube whereas the floating unconsumed pellets or debris was collected by using a small fish net. A proper aeration was ensured by using a centralised air compressor connected with plastic tubing attached to the air stone diffuser to the respective tanks. The physico-chemical characteristic of water such as dissolved oxygen (DO) concentration (ppm) was monitored using digital oxygen meter (DO-MA 841Portable Dissolved Oxygen Meter); temperature and pH were monitored using pH meter (Martini Instruments: MI 106). Both parameters were measured on alternative days. Meanwhile ammonia, nitrite and nitrate concentration were measured once a week by testing water sample using a digital water quality analytical instrument (DR/2010 Spectrophotometer) in the IPS Laboratory, University of Malaya. The analytical methods used to determine the ammonia, nitrite and nitrate concentrations are known as the Salicylate method, Diazotization, NED Rapid Liquid method and Cadmium reduction method. A water sample from each tank has to be collected in clean plastic bottles were being left in ambient temperature upon analyses and if the samples were not analyzed on time, they were stored for up to 48 hours at 4°C (39 °F).

3.6 Growth

Growths performances of juvenile tilapia were observed total length (cm) of fish from week one until week eight of feeding trial. Prior to the length measurement, a small amount (25-30%) of existing water from respective tank need to be transferred into a new clean tank. Then juvenile tilapia was picked randomly from the respective experimental tank and put on dry and clean plastic dish or plate. The total length of each juvenile tilapia was measured and was then immediately released to the new tank containing (25-30%) of transferred water to prevent shock. Specific growth rate was calculated using the following formula:

$$\text{Specific growth rate, (SGR) \% day}^{-1} = \frac{\text{In final weight-In initial weight}}{\text{56 days}} \times 100$$

3.7 Survival

The survival of juvenile tilapia was determined every day. This was done by counting the number of surviving population through observation. Dead fish were removed daily.

3.8 Statistical analysis

The data on growth, survival rate and water qualities were analysed using one-way analysis of variance (ANOVA) and compared Duncan Multiple Range (DMR) Test. The differences were considered statistically significant at the probability level $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Survival

Table 4.1 shows the survival of fish from week 0 to week 8 which were fed with different diets. In the 8th week, the fish fed with Diet 1 gave survival of 85.18 ± 0.85% followed by Diet 3 (79.63 ± 0.64%) and Diet 2 (72.22 ± 1.11%). All the results were significantly different ($P<0.05$).

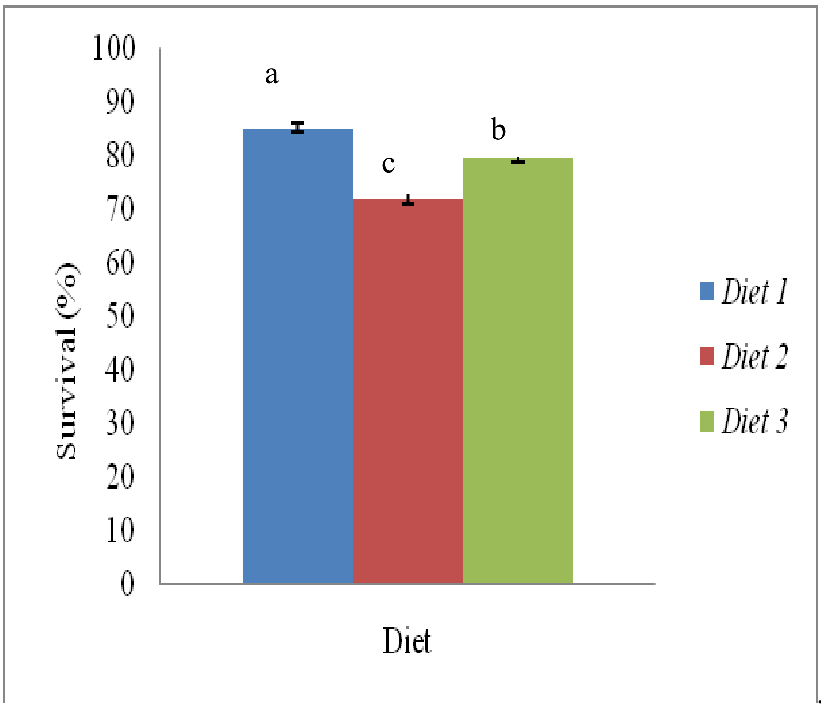

Figure 4.1: The mean of survival (%) of juvenile *O. niloticus* during the eight weeks feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a,b,and c] In the histogram shows significant differences ($P < 0.05$) with various *P. sajor-caju* incorporated diets.

4.2 Growth and Specific growth rate (SGR)

Growth performance in term of total length and specific growth rate of tilapia are presented in Figure 4.2 and Figure 4.3. Effects of Diet 1, Diet 2 and Diet 3 were analyzed during eight weeks using manual measurement by measuring the length (cm), initial mean body weight (g), final mean body weight (g). The results for the growth performance in term of length and specific growth rate of tilapia were used to test significance at the level of the probability $P < 0.05$. Figure 4.2 shows the highest total growth length was measured by Diet 1(4.40 ± 0.03cm) followed by Diet 2 (4.26 ± 0.04cm) and Diet 3 (4.21 ± 0.01cm). It showed that, the tilapia fed with Diet 1 was increased in length compared to Diet 2 and Diet 3. The growth performance in length for Diet 2 and Diet 3 were significant lower ($p < 0.05$) towards that of Diet 1.

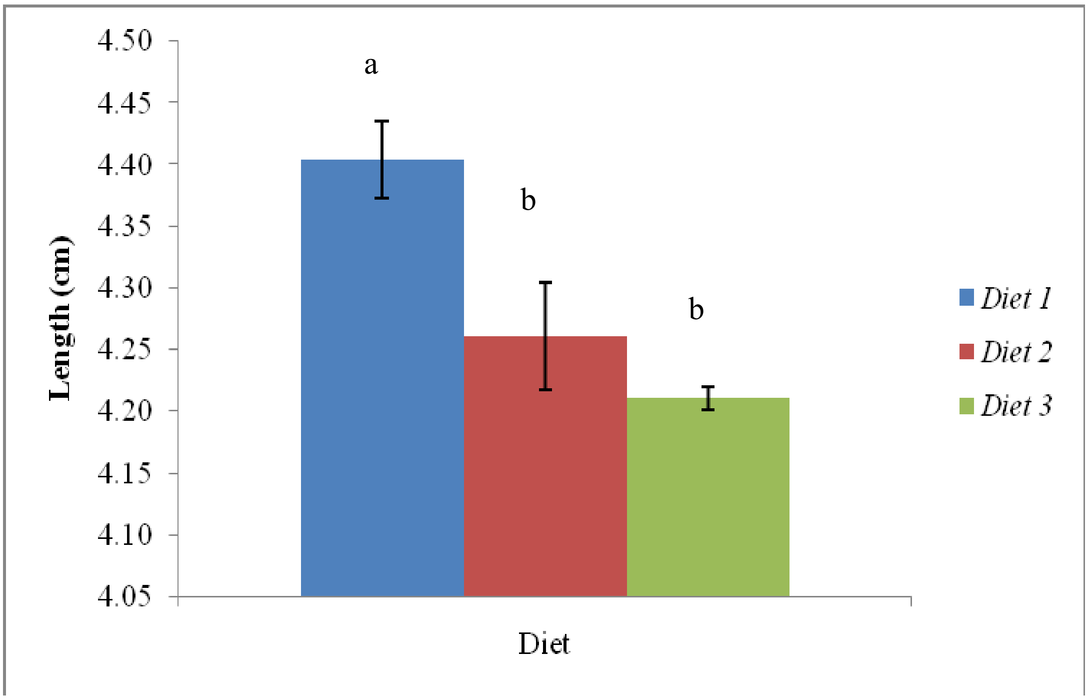

Figure 4.2: The mean of total growth (cm) level of *O. niloticus* during the eight weeks feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a, and b] In the histogram shows significant differences ($P < 0.05$) with various *P. sajor-caju* incorporated diets.

The specific growth rates for each diet Figure 4.3. SGR value for Diet 1 was 1.65 ± 0.01 % day^{-1}, Diet 2 was 1.5 4± 0.02 % day^{-1} and Diet 3 was 1.54±0.02 % day^{-1}. Difference superscript against the mean of specific growth rate of Diet 2 and Diet 3 showed significant difference ($P < 0.05$) towards Diet 1. However, at the end of the study, the tilapia fed by Diet 1 showed higher SGR compared to other diets.

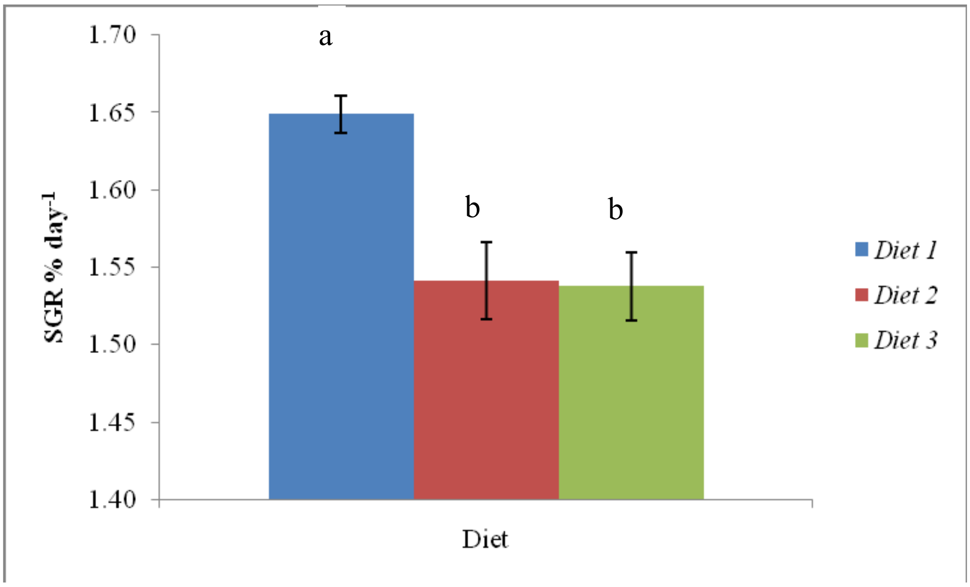

Figure 4.3: The mean of specific growth rate of *O. niloticus* in percentage of weight gain in a day, during the eight weeks feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a and b] In the histogram shows significant differences ($P < 0.05$) with various *P. sajor-caju* incorporated diets.

4.3 Water quality

Mean and significant values related to eight weeks of feeding trial of Diet 1, Diet 2 and Diet 3 for water quality measurements are summarized in Figure 4.4 to Figure 4.9. Water quality although difficult to control in comparison to other extrinsic factors such as stocking density, diet (feeding), and size of the fish plays an important part in determining growth and survival. This was proven by the mean value of different diets for water quality parameters of pH, temperature, and dissolved oxygen (DO), ammonia, nitrite and nitrate concentrations that were within the tolerable levels. Besides that, alkalinity or acidity, and carbon dioxide (CO_2) values were also not extremely high. This is because of the tolerable water quality management and environmental conditions in hatchery at Rimba Ilmu, University of Malaya where consistent water flowing in fish tanks with placement of powerful aerators that assisted in sustaining water quality. Results of water quality between groups of diet and within groups of triplicate tanks were not much difference for all parameter studied. The eight weeks of feeding trial was completed without any interruption or apparent problems. The mean of water quality parameters has been recorded.

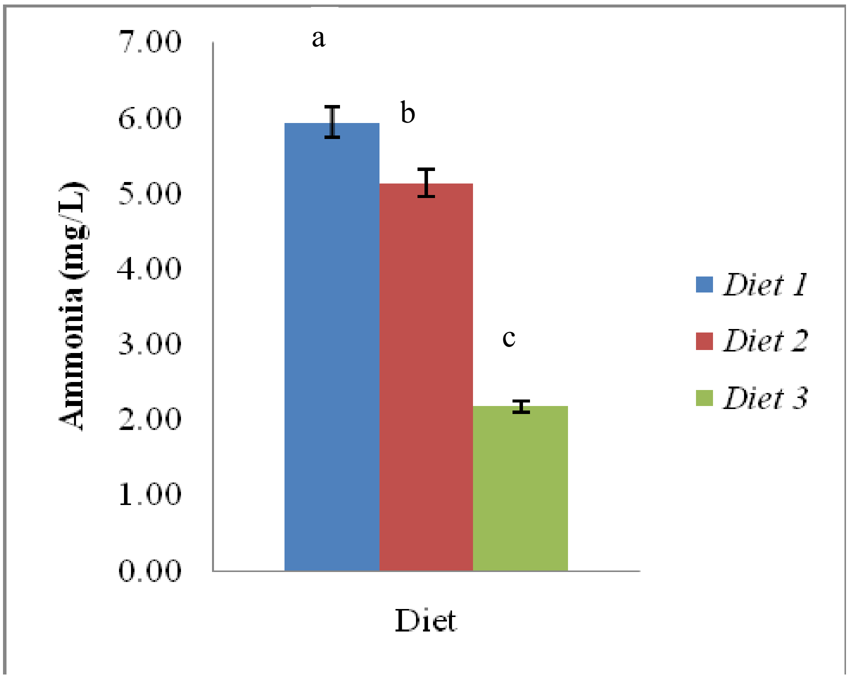

Figure 4.4: The mean value of ammonia concentration in fish tank water during the eight weeks feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a,b,and c] In the histogram shows significant differences ($P < 0.05$) with various *P. sajor-caju* incorporated diets.

Figure 4.4 shows the mean value of ammonia concentration in fish tank water during the eight weeks feeding trial. Ammonia concentration for each three diets with Diet 1 was 5.95 ± 0.19mg/L, Diet 2 was 5.14 ± 0.17mg/L and Diet 3 was 2.18 ± 0.07mg/L. The concentration of ammonia for Diet 1 was significantly high compared to Diet 2 and the least ammonia concentration was observed in Diet 3. This might be due to protein value was higher in Diet 1 compared to Diet 2 and Diet 3. Overall, the ammonia concentration fluctuated every week due to certain intrinsic and extrinsic factors. However, the Diet 3 showed the least value of ammonia concentration which was the least cloudy and water quality was well maintained compared to Diet 1 and Diet 2. Therefore ammonia concentration over eight weeks of feeding trial shows that there is significant difference (P < 0.05) between all the three diets.

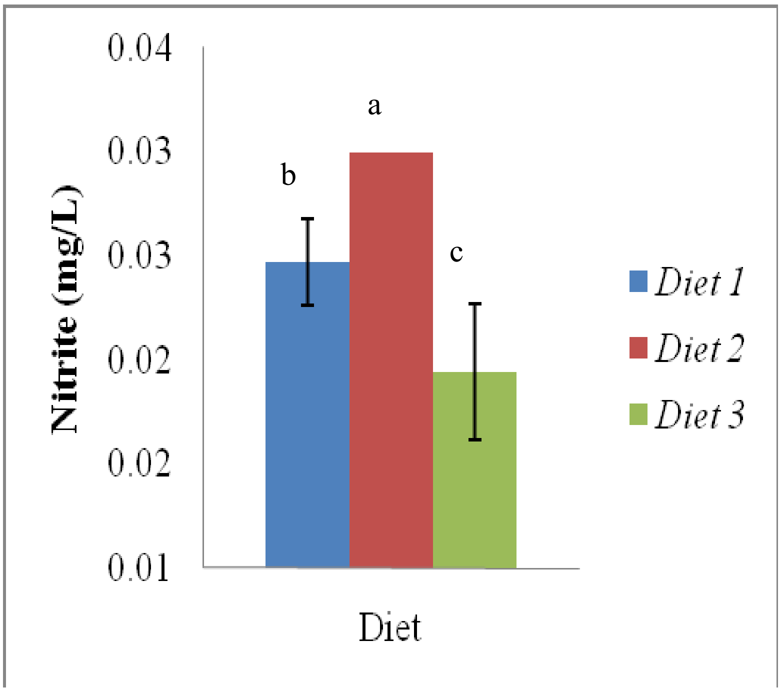

Figure 4.5: The mean of nitrite concentration in fish tank water during the eight weeks of feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a,b,and c] In the histogram shows significant differences (P < 0.05) with various *P. sajor-caju* incorporated diets.

The 8 weeks of feeding trial for nitrite concentration on three various diets was successfully completed and the results was plotted in Figure 4.5. The least nitrite concentration was found in the Diet 3 and Diet 1 with 0.02 ± 0.002mg/L and 0.22 ± 0.003mg/L respectively compared to Diet 2 with 0.03 ± 0.00mg/L. Although during the earlier weeks, the nitrite concentration was higher for Diet 1 due to assimilation of fish to new environment and other factors, but at the final week the nitrite concentration was similar to the Diet 3 due to fish tolerate for that environment. Meanwhile, Diet 2 showed a highest mean of nitrite concentration for overall eight weeks of feeding trial. Nevertheless there was a significant difference ($P < 0.05$) in nitrite concentration over eight weeks of feeding trial for all Diet 1, Diet 2 and Diet 3.

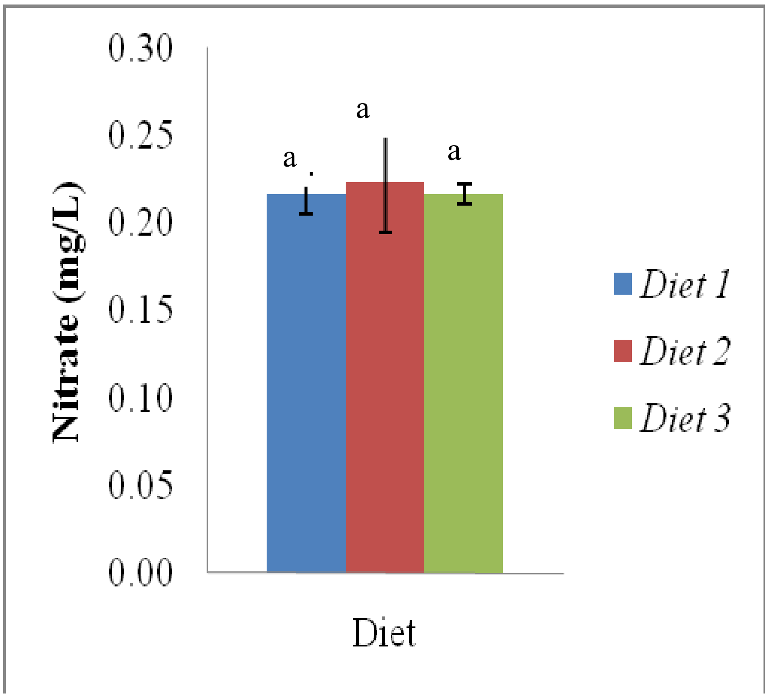

Figure 4.6: The mean of nitrate concentration in fish tank water during the eight weeks of feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a,] In the histogram shows no significant differences ($P > 0.05$) with various *P. sajor-caju* incorporated diets

Figure 4.6 shows a good water quality for nitrate concentration has been recorded for Diet 1 even though the nitrate concentration was higher at first week only, compared to other diets. In general, all the three diets, Diet 1, Diet 2, and Diet 3 showed similar nitrate mean value with 0.02 ± 0.01mg/L, 0.02 ± 0.03mg/L and 0.02 ±0 .05mg/L respectively. Therefore the nitrate concentration over eight weeks of feeding trial shows no significant differences ($P > 0.05$) for all three diets.

Meanwhile, Figure 4.7 shows the temperature level in fish tank water during eight weeks of feeding trial. Although the temperature of fish tank water was measured every morning, the difference in timing during measurement and also the location of the tank could explain the variation in the temperature of fish tank water. The changes on the temperature depend on the climates. When the weather in the morning was cool and humid, water temperature level was decreased. But during the hot and sunny weather, water temperature level was increased. For fish tank water with Diet 1, mean of the temperature level was 26.3 ± 0.00°C. For fish tank water with Diet 2 and Diet 3, the temperature levels were 26.5 ± 0.00°C and 26.57 ± 0.12°C respectively. Overall there are not drastic changes in the temperature for all the tanks with those three diets. There is significant difference ($P<0.05$) between Diet 2 and Diet 3 towards Diet 1.

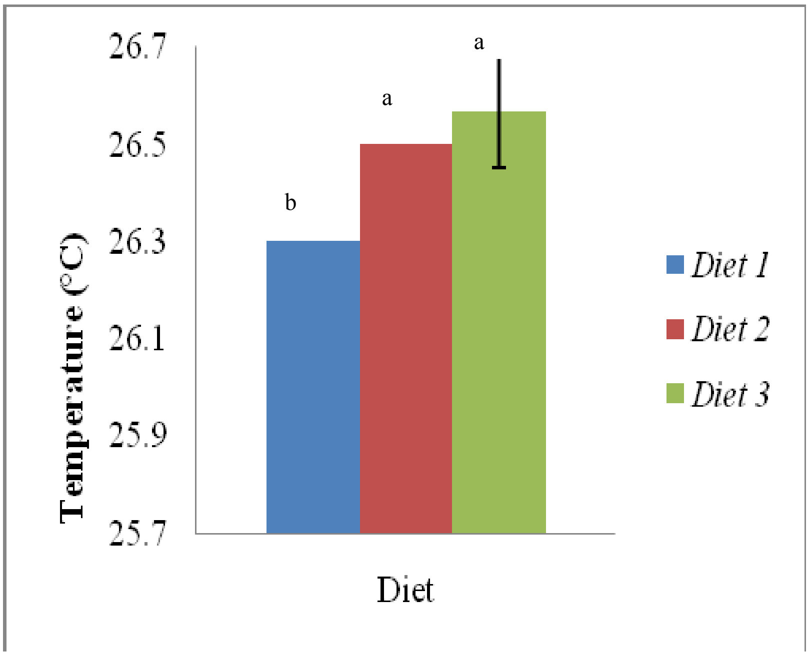

Figure 4.7: The mean of temperature level in fish tank water during the eight weeks of feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a and b] In the histogram shows significant differences ($P < 0.05$) with various *P. sajor-caju* incorporated diets.

Figure 4.8**,** shows the pH value in fish tank water during the eight weeks of feeding trial. Overall the total pH mean value of water sample for each diet was 7.31 ± 0.06. It also showed that the trend of pH changes was similar for all diets throughout the experiment period. The mean of pH for Diet 1 was 7.34 ± 0.08, Diet 2 was 7.33 ± 0.05 and Diet 3 was 7.26 ± 0.01. During the feeding trails, there were drastic drop in daily pH value. This was because of the accumulation of toxic waste and uneaten food which lead to pH increasing. In general, the pH value fluctuates with minor changes for each diet due to population number of juvenile fish may adapt to the environment and stabilized easily. The pH began to rise steadily at lower rate due to release of nitrate and ammonia by the fish. During this experiment, the overall pH value in the fish tank from beginning until the end fluctuated slightly compared to other parameters. The utilization of *Pleurotus* spp. as a supplement contributes to a good result with a standard pH value which similar to commercial feed without any significant difference (P > 0.05).

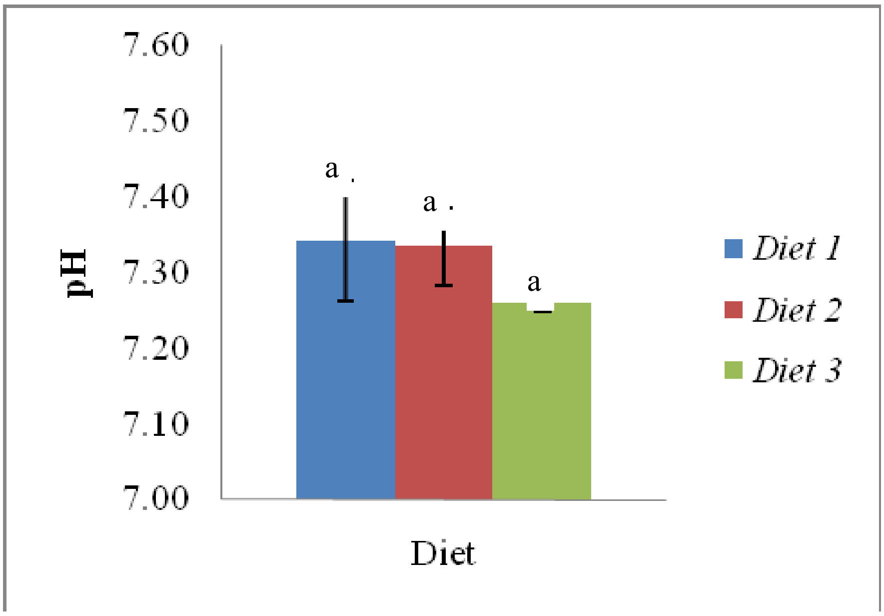

Figure 4.8: The mean of pH value in fish tank water during the eight weeks of feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a] In the histogram shows no significant differences (P > 0.05) with various *P. sajor-caju* incorporated diets.

Figure 4.9, shows the dissolved oxygen (DO) level in fish tank water during the eight weeks of feeding trial. The overall DO result for all three diets showed fluctuations but the mean value was maintained. The total of DO mean value of the sample water for overall diet was 5.58 ± 0.42ppm. The DO levels were 5.81 ± 0.13ppm, 5.70 ± 0.63ppm and 5.24 ± 0.15ppm for fish tank water fed with Diet 1, Diet 2 and Diet 3 respectively. The DO mean value in middle weeks showed fluctuating with minor changes. This could be due to the tilapias started to stabilize themselves and the growth maintained the strength and oxygen consumption of the tilapias. There was no significant difference (P > 0.05) in DO measurement.

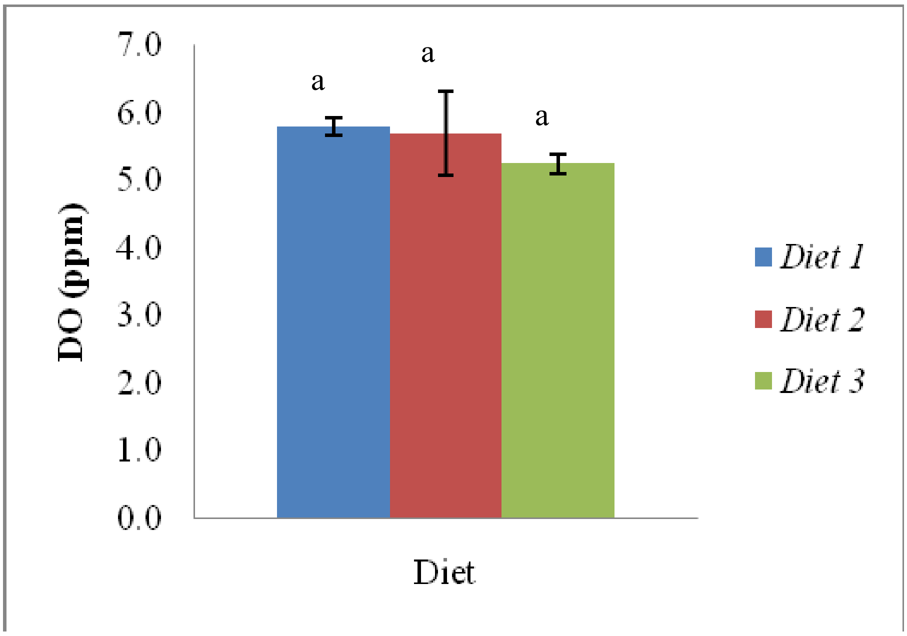

Figure 4.9: The mean of dissolved oxygen level in fish tank water during the eight weeks of feeding trial on various *P. sajor-caju* incorporated diets and commercial diet.

[a] In the histogram shows no significant differences (P > 0.05) with various *P. sajor-caju* incorporated diets.

4.4 Proximate analysis

The results of proximate analysis which was carried out by food analysis laboratory, UNIPEQ based in National University of Malaysia, Bangi was stated in Figure 4.10. These analyses consist of few basic nutrients such as protein, fat, carbohydrates, ash, and moisture. Protein level in Diet 1 was slightly higher than Diet 2, which was 27.45% and 27.2%. Compared to Diet 3, protein content in both Diet 1 and diet 2 were very high in range value. The total amount of fat and carbohydrates found to be higher in Diet 3 compared to other diets with the value of 2.5% and 57.75% respectively. Apart from that, crude fiber content in Diet 1 and Diet 3 were similar with amount of 46.25% but low level in Diet 2 which was 41.85%. Moisture level in both Diet 1 and Diet 2 were in range of 16.3-19.2% but in Diet 3 moisture level showed very low in range with the value of 6.6%. In other hand, the level of ash in Diet 2 (9.1%) was slightly lower than both Diet 1 (9.45%) and Diet 3 (13.5%).

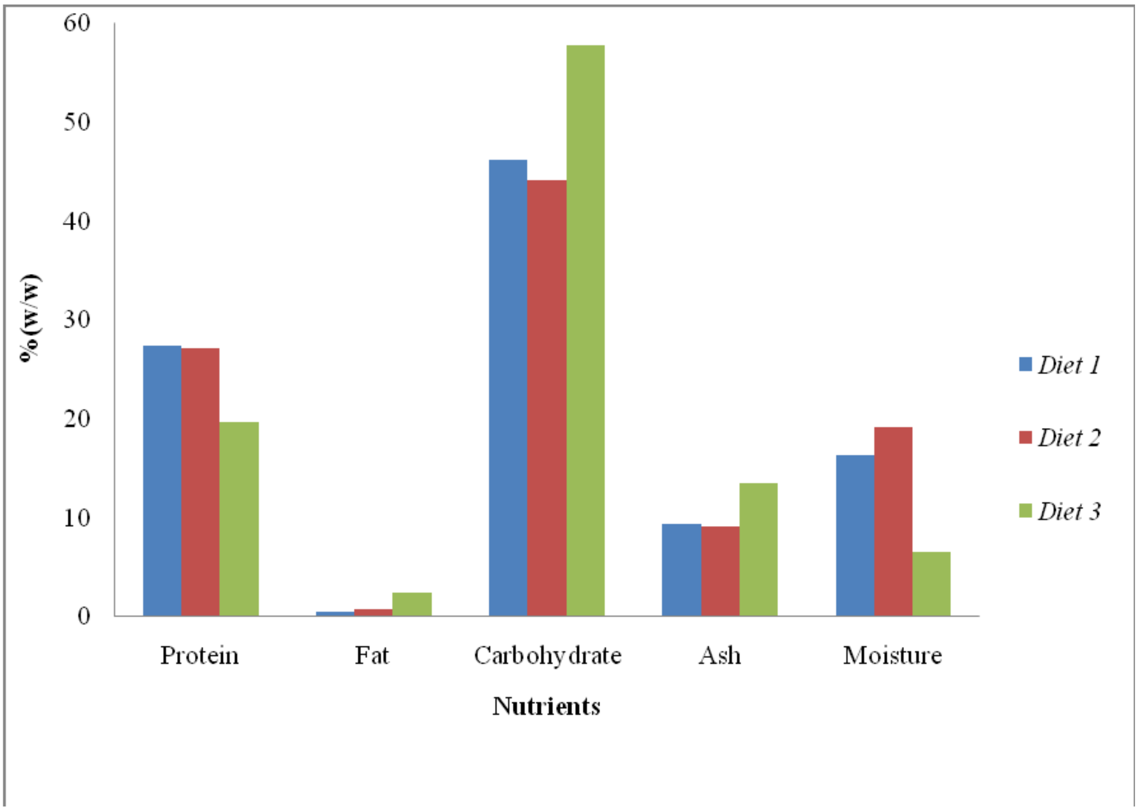

Figure 4.10: Proximate analyses of nutrients content in all three diets used in this study.

CHAPTER FIVE

DISCUSSION

5.1 Growth, survival and proximate analysis

Aquaculture feed ingredients tend to be mostly by-products of processing or milling industries consisting of natural products. In each formulation of these diets, ingredients are included and substitutions made within mixtures in accordance with market price, local availability and composition. The concept is to use available ingredients to provide the essential nutrients in the diets. Different proportions of cheaper ingredients can often be combined to achieve the nutrient balance of more expensive ones. However, it is also necessary to consider the factors such as the quality, palatability, functional properties of ingredients and content of anti-nutritional components which may affect the growth and health of the fishes.

The present study is a pioneer experiment of its kind which shows that *Pleurotus sajor-caju* waste stalks, incorporated with commercial diet is a good aquaculture feed enhancer for growth of the juvenile tilapia, *Oreochromis niloticus*. Supplemental diets will provide only portion of the nutritional demands of the fish, with the assumption that they will get most of the nutrients from the growing system (El-Sayed and Teshima, 1991). Supplemental diets are usually much less expensive than complete diets and usually high in carbohydrates. Some simple supplemental diets serve a dual purpose of fertilizing the pond as well as increasing productivity (De Silva and Perera, 1995). Considerable research has been conducted on complete diets and on fertilization programs for natural and man-made water bodies. Developments of supplemental diets are directed to provide required nutrients for growth (Olli *et al*., 1995).

Tilapia exhibit their best growth performances when they are fed a balanced diet that provides a proper mix of protein, carbohydrates, lipids, vitamins, mineral and fiber. Jauncey and Ross (1982), El-Sayed and Teshima (1991) and Stickney (1996) provide excellent reviews that examine the details of tilapia nutrition. The nutritional requirements are slightly different for each species and more importantly vary with life stage. Fry and fingerling fish require a diet higher in protein, lipids, vitamins and minerals and lower in carbohydrates as they are developing muscle, internal organs and bone with rapid growth. Sub-adult fishes need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth. Adult fishes need even less protein; however the amino acids that make up that protein need to be available in certain ratios. Feed formulators will adjust protein sources to fit the desired pattern of amino acids through the growth cycle. Brood fish may require elevated protein and fat levels to increase reproductive efficiency (Santiago *et al*., 1985; Chang *et al*., 1988).

Apart from that, decline in fish growth performance and feed utilization with increasing levels of stocking densities has been observed in several studies (Vijayan and Leatherland, 1988; Suresh and Lin, 1992). Similarly, an increase in net yields with increasing levels of stocking densities has also been recorded (Honer *et al*., 1987; Suresh and Lin 1992). Vijayan and Leatherland (1988) have also observed a similar reduction in feed consumption with increasing fish stocking densities and suggested that this may lead to growth depression. It has been observed that animals under stress need to spend more energy for homeostatic processes (Schereck, 1982). The decreased feed utilization, therefore, may be an indicator of the higher level of stress encountered by the fish at higher densities. This present study is carried out with 20 tilapias per tank which shows increasing value in overall growth performance with stocking density of 20 fishes. This growth level may be enhanced by reducing the stocking density either to 10 or 15 fishes per tank. Wee and Tuan (1988), reported *O. niloticus* with different crude protein diets had a significant effect on specific growth rate (SGR) varied from 1.25-1.53% per day. In this study SGR was considerably higher than the results by Wee and Tuan's (1988). The tilapia species is suggested to have a fast growth performance with optimum utilization of natural and supplemental feed as in the present study.

Effect of formulated diet compared with commercial feed on performance of growth in length and specific growth rate in this study was observed for 8 weeks of feeding trial. The fish were collected from local source and fed with Diet 1, Diet 2 and Diet 3. The proximate analyses of the diets are reflected in Figure 4.10. This fishes, placed in fish tanks were observed for 8 weeks, showed significant differences in growth performance. Overall growth performance of fish in length over week in Diet 1 and Diet 2 is better than Diet 3. This is illustrated by the fact that content of protein in both Diet 1 and Diet 2 is better compared to Diet 3. When absolute values were considered for growth performance, between Diet 2 and Diet 3 shows not much different. Jauncey (1982) and De Silva and Perera (1995) reported that decreased growth performance is dependent on quality and quantity of diet.

Dietary protein levels of all three diets in the present study significantly affected the growth of *O. niloticus*. This study shows, *O. niloticus* grew best when fed with Diet 1 which consists of 27.45% protein, 0.55% fat, 46.25% carbohydrate, 9.45% ash and 16.3% moisture. Furthermore, fishes fed with Diet 1 which obtained a higher specific growth rate give the optimal growth performance in length. Previous studies show that inadequate dietary protein levels results in lower growth as well as lower protein and energy utilization (Tibaldi *et al*., 1996; Hernandez *et al*., 2001). In general, protein has been considered the fundamental unit for growth (De Silva and Gunasekara, 1991; El-Sayed and Teshima, 1992). The growth performance of *O. niloticus* fed with 19.65% of protein (Diet 3) and 27.2% (Diet 2) of protein were lower compared to fishes fed with 27.45% (Diet 1). The optimal protein levels varied according to fish species and also for the same species in different studies (Nankervis *et al*., 2000; Morris, 2001). These traits may be due to the variation of other aspects besides fish species such as experimental dietary protein level. The different design of dietary nutrient levels affects the estimation of nutrient requirements (Coloso *et al*., 1988; Li and Lovell, 1992). Therefore, estimation of optimal dietary protein levels is important in designing dietary levels in fish. Previously, Shearer (2000) and Bell (1998) used different dietary protein levels to produce optimal growth. Relationship between dietary protein and food consumption was affected by a complex interaction of a number of factors, which include activities similar to the present study. Relationship between dietary protein and feed consumption of fish are revealed by the better growth performance. Growth is affected by protein and energy levels; less space and limited movement of fish (Teshima *et al*., 1985 and Wang *et al*., 1985a, b).

In present study, the total length gain of juveniles fed with Diet 1 and Diet 2 for eight weeks was 4.40 ± 0.03cm and 4.26 ± 0.04cm respectively; while for juveniles fed with Diet 3 was 4.21 ± 0.01cm. Previous studies on daily length gain reported a slightly lower value (0.06cm) in length of *O. niloticus* by Cavalheiro *et al*. (2007) where the fish were fed diets with 10%, 20% and 30% fish residue silage, while (0.006–0.012cm) average daily gain in length for tilapia fed with by-product of industrial gelatine. However, 0.14cm gain per day was reported for the same species during a period of 10 weeks by feeding sorghum. Possibly, the inconsistency in the results is caused by different ambient conditions during the study, including the presence of sorghum.

During the 8 weeks (55 days) feeding trial, mortality rate was observed with various types of diet, including the commercial diet. The survival of juveniles ranges from 72 to 85%, with 85.18 ± 0.85% for Diet 1, 72.22 ± 1.11% for Diet 2 and 79.63 ± 0.64% for Diet 3 (Figure 4.1). Diana *et al*. (1994) reported a survival of 48.2–87.2% with supplemental feed with inorganic fertilizer as compared to the present study, whereby in Diet 1 there was significant different in survival with 85.18±0.85%. In addition survival of tilapia fish was significantly higher in the group of fishes fed with Diet 1 as compared to the Diet 3. It may be due to the content of β-glucans in *P. sajor-caju* itself (Synytsya, *et al.,* 2009). Previous studies on the use of β -glucans in fish diets were aimed at improving the immune response and resistance to diseases. Since the duration of these studies were short, the effects of these compounds on growth performance were not determined. The negative effects of high doses and prolonged feeding of β-glucan on fish immune responses and disease resistance have been reported by Anderson (1992) and Couso *et al*., (2003). Previous studies have proven that, prolonged feeding or high doses of β-glucan causes increases susceptibility of Atlantic salmon (Robertsen *et al*., 1990), rainbow trout (Jeney *et al.,* 1998), and Gilthead Seabream (Couso *et al*., 2003) to bacterial infection. The type of β-glucan used may also be a contributing factor and the effective concentration of dietary β-glucan varied with the source (Raa, 1996).

Feeding rates will vary with tilapia fish sizes and water temperature. An appropriate amount of feeding rate is measured by the average of body weight gain (%).The daily feed ration must be adjusted to compensate the growth. Growth of tilapia fish was significantly higher in the group of fish fed with Diet 1 and Diet 2 compared to Diet 3.

Many researchers have worked on the biological activities and medicinal value of oyster mushroom. However, there were few cited works on the uses of oyster mushroom by-products as supplements in animal feeds. Song *et al*., (2007) performed a study on the effects of fermented oyster mushroom (*P. ostreats*) by-product supplementation on growth performance, blood parameters and meat quality in finishing Berkshire pigs. The growth performances of Berkshire pigs are improved by addition of fermented oyster mushroom by-product during the end of their studies.

5.2 Water quality

The water quality in the experimental tanks was maintained throughout the feeding period and only slight fluctuations in parameters such as dissolved oxygen (DO), temperature and pH occurred for each diet. Water temperature, dissolved oxygen concentration (DO), and photoperiod are potent influences on feed consumption, metabolic rate and energy expenditure, thus, on growth of poikilothermic vertebrates, including fish (Elliott, 1982 with Salmonids; Bhikajee and Gobin, 1998 with hybrid red tilapia).

The DO concentration was measured and maintained within the total mean of 5.58 ± 0.42ppm, because the tanks were continuously aerated. The tolerance of DO concentrations varies depending on the species of fish. In general, most species perform well at 4mg/L and may survive for extended periods at 3mg/L (Stickney, 2000). For those *O. niloticus* reared under different culture systems, oxygen concentration of 3mg/L and above have been recommended (Coche, 1982; Ross and Ross 1983).The DO concentration in fish tanks fed with Diet 1 was at mean of 5.81 ± 0.13ppm which showed higher O_2 accumulation followed by Diet 2 and Diet 3. As the water temperature increase, the tilapia needs protein for growth, reproduction and to repair the damaged tissue and injuries. An increase in DO concentration, up to certain limits, results in enhanced growth of fish (Brett and Groves, 1979; Cuenco *et al*., 1985; Neill and Bryan, 1991).

The range of pH level of water in this present study was slightly alkaline, which fell within the total mean of 7.31 ± 0.06 for all three diets. In general, the nitrogenous waste was excreted from fish as well as from unconsumed food. These toxic and unconsumed foods as well as decomposition process contributed to the slight alkalinity in the fish water. Therefore, this present study initially shows slight fluctuation in pH level; however, after the population of fish stabilized, the pH began to balance steadily at a slower rate.

Fish excrete ammonia as their principal nitrogenous waste. This can represent a serious constraint to successful aquaculture practices. Intensively cultured fish stock when fed with protein-rich feeds, excrete high concentrations of total ammonia-nitrogen (TAN) into the water (Buttner *et al.,* 1993). NH_3-N exists in ionized (NH_4^{+}) and unionized (NH_3) forms in the water and is a highly toxic metabolic waste of fish. The proportion of NH_3-N to ionized form depends upon the pH and temperature of the water.

Present study shows stable pH and temperature, therefore ammonia concentrations do not increase drastically. This was supported by Lawson, (1995), whereby the higher the pH and temperature levels; the higher is the percentage of toxic unionized ammonia concentration. Baras *et al*, (2001) reported that the standard rearing temperatures were in the range of 27–28°C. In the present study, temperature shows significant differences at $p<0.05$ with mean range of 26.3 ± 0.00 to 26.57 ± 0.12°C.

Even though the temperature of water was measured every morning, the difference in timing when each measurement was taken could explain the variation in temperature of fish tank water. The weather condition throughout the experimental period as well as the position of the tanks can also influence the water tank temperature. For example, the weather in the morning which was cool and humid will give different readings compared to the hot weather.

Most studies provided evidence that water temperatures also governed the phenotypic sex of *Oreochromis* spp. A vast majority of experiments demonstrated that higher temperatures favoured the production of (almost) monosex male progenies of *O. niloticus*: (Baroiller *et al*., 1995 1996a, b); *O. aureus*: (Desprez and Melard, 1998; Baras *et al*., 2000.), even when 100% XX progenies were evaluated in *O. niloticus* (Baroiller *et al*., 1996a). There has been more recent evidence that high temperatures could feminise some genetic males of *O. niloticus* (Abucay *et al*., 1999).

During this present study, significant values of ammonia and nitrite ($p<0.05$) were monitored and NH_3-N values increased significantly as the fish density and feed input increased and mean of NH_3-N concentrations ranges from 2.18 ± 0.07 to 5.95 ± 0.19mg/L. The higher value was traced during the beginning of the study especially on week 1 but was reduced in the following weeks. Ammonia concentration in Diet 1 was higher as compared to Diet 2 and Diet 3. It could be due to the higher protein content of *P. sajor-caju* itself (Garcha *et al.,* 1993). Protein level in these feeding trial was given in Figure 4.10. Significant differences of ammonia concentration in this present study seemed not to cause any harmful effect and this was proven by the higher survival at the end of study.

Nitrite (NO_2) is a naturally occurring intermediate product in two bacteria-mediated process which involved transformations of nitrogen in water and soils (Mevel and Chamroux, 1981). Nitrite accumulates in aquaculture systems and can be toxic to aquatic animals (Huertas *et al*., 2002; Svobodova *et al*., 2005). Results of present study on nitrite shows very low concentration of nitrite accumulated in water after the feeding trial with formulated feed as well as commercial feed, which was in the range of 0.02 ± 0.002 to 0.03 ± 0.00mg/L. At the beginning, the range of nitrite concentration in water fed with *P. sajor-caju* formulated diets was slightly higher than the control diet. As nitrite is an intermediate of both nitrification and denitrification, it can accumulate in aerobic as well as anoxic environments. Nitrite can be produced from ammonium, through nitrification, and from nitrate, through a number of nitrate reductive pathways. For instance, in fast flowing aerobic small streams, ammonia oxidation via nitrification is mainly responsible for elevated nitrite concentrations, whereas in slow flowing conditions nitrite concentrations are more attributed to anaerobic nitrate-reducing processes (Kelso *et al*., 1997). From present study it can be assumed that nitrite accumulation was due to slightly higher level of ammonia concentration in water with a range of 2.18 ± 0.07 to 5.95 ± 0.19mg/L but not in harmful range. Nitrite accumulation is a common trait of dissimilatory nitrate reduction to ammonia due to either inhibitory effect of nitrate on the nitrite reductase or repression of this enzyme. Nitrite accumulates especially when high concentrations of nitrates are present (Kelso *et al*., 1997). In the present study, nitrate concentration was in the range of 0.22 ± 0.005 to 0.22 ± 0.03mg/L which was not significant ($P>0.05$). However, this does not give any complication during this present study. The minor accumulation of nitrite in this present study may be due to the presence of a little amount of nitrate (Kelso *et al*., 1997).

The range of sensitivity to environmental nitrite was very wide among fishes depending on the test conditions and organismal traits (Lewis and Morris, 1986). Table 5.1, shows previous studies that were carried out to evaluate the toxicity of nitrite in many fishes but only few were done with *O. niloticus*. Wang *et al*., (2006), explained about the 96-h LC50 of nitrite in tilapia, *O. niloticus*, was 28.18 and 44.67mg/L in a semi-static environment with chloride at 35.0 and 70.0mg/L respectively. The 96-h median lethal concentration of nitrite to small tilapia (4.4 ± 1.5g) was up to 81mg/L as reported by Atwood *et al*., (2001). The occurrence of high concentrations of nitrite is an important water quality concern as it is highly toxic to human, fauna and flora. Moreover, passage of nitrite into the bloodstream results in the irreversible conversion of haemoglobin to methaemoglobin, thus compromising the oxygen-binding capacity (Van Leeuwen, 2000), causing respiratory deficiencies in aquatic animals and human beings (Bradberry *et al*., 1994). Besides human, nitrite is capable of inducing methaemoglobinemia in a wide range of species, i.e., cattle, sheep, swine, dogs, guinea pigs, rats, chickens and turkeys. In rats, chronic nitrite exposure causes pathological changes in a variety of tissues, alterations in motor activity and brain electrical activity and alters gastric mucosal absorption (Brunning-Fann & Kaneene 1993).

Table 5.1: Toxicity of nitrite to aquatic fauna

Species	Test	Nitrite mg N L−1	Reference
Gilthead seabream (12-day old larvae	LC50 24 h	607	Parra and Yufera (1999)
Australian crayfish	LC50 24 h	42.9	Meade and Watts (1995)
Australian crayfish	LC50 48 h	37.1	Meade and Watts (1995)
Australian crayfish	LC50 96 h	25.9	Meade and Watts (1995)
European eel	LC50 96 h	144	Kamstra *et al*., (1996)

Tiger prawn	LC50 96 h	14	Chen and Chin (1988)
Juvenile grass carp	LC50 96 h	10.6	Alcaraz and Espina (1997)
Nile tilapia	LC50 96 h	8–81	Atwood *et al*.,(2001)

*LC50: Concentration at which 50% mortality is observed.

5.3 Feed quality

Replacement of fish meal by cheaper ingredients of either animal or vegetable origin in aquatic animal feed is necessary because of the rising cost and uncertain availability of fish meal (Kaushik, 1990; Higgs *et al*., 1995). While partial replacement of dietary fish meal protein with plant protein has been successfully accomplished in a number of Teleost fishes (Tacon, 1993), only a few reports have appeared on the utilization of plant protein as a sole protein source in fish diets (Smith, 1977; Gomes *et al*., 1995; Kaushik *et al*., 1995). Present study is also an example of partial replacement of dietary meal by using *P. sajor-caju* waste stalks. It is important to evaluate the nutritional value of alternative ingredients and formulate diets based on a mixture of such ingredients, which can replace fish meal in the diet of fish. Knowledge of the digestibility of these various ingredients is a basic requirement for formulating diets (Cho and Kaushik, 1990). Digestibility studies on tilapia species have been confined to investigate the ingredients of feeds of mixed origin. Few studies have been undertaken to evaluate the apparent digestibility coefficients of feed ingredients for tilapia (Sintayehu *et al*., 1996; Degani *et al*., 1997; Fagbenro, 1998). Nutrition essentials to fish are the same as those required by most other animals. These include water, proteins (amino acids), lipids (fats, oils, fatty acids) carbohydrates (sugar, starch) vitamins and mineral (Julie-Anne and Frank, 2003). The general proportions of various nutrients in a standard fish diet are given in Table 5.2.

Table 5.2: General amounts of nutrients incorporated into diets for growing fish (Julie-Anne and Frank, 2003).

Nutrients	**Amounts (percent by dry weight)**
Proteins: Including the 10 essential amino acids, lysine, phenylalanine, arginine, valine, leucine, isoleucine, methionine, threonine, tryptophan and histidine	32 – 45%
Fat: Used as a source of energy and polyunsaturated fatty acids. In general, freshwater fish require fatty acids of the linolenic (w-3) and linoleic (w-6) series. Saltwater and coldwater fish require greater amounts of EPA and DHA (w-3)	4 – 28% Should contain at least 1 – 2% of the w-6 or w-3 essential fatty acids series.
Carbohydrates: These are inexpensive sources of energy and binding agent. No essential requirements have been identified. These are poorly digested when fed raw. Highest digestibility is achieved when cooked. Major carbohydrates are starch, cellulose and pectin	10 – 30%
Minerals: Some 20 inorganic mineral elements, including calcium, phosphorus, magnesium, iron, copper, manganese, zinc, iodine and selenium.	1.0 – 2.5% Fed as multi-mineral premix
Vitamins: These are inorganic substances required in trace amounts that can be divided into fat-soluble (vitamins A,D,E and K) and water-soluble (vitamins C and the B-complex [thiamine, riboflavin, pyridoxine, panthothenic acid, cyanocobalamin, niacin, biotin, folic acid, choline and myoinositol])	1.0 – 2.5% Fed primarily as a multi-vitamin premix. Vitamin C and choline are added separately from the premix because of their chemical instability.

In this present study, pellet preparations were done with bovine based gelatine as a binding agent which is known to be an important composition in fish diets to provide shape and consistency to the pellet and reduce nutrients from dissolving into the water (Francis-Floyd and Reed, 1994). In earlier days, beef heart was been used as a source of protein and an effective binder in home-made animal feeds. Carbohydrates in the form of starch, cellulose, pectin and other polysaccharides extracts or derivates from animals (gelatine), plants (gum Arabic, locust bean), and seaweeds (agar, carageenin, and other alginates) are also commonly used binding agents in feed preparations. Apart from fish, squid and brine shrimp meal, algae, zooplanktons in live or dried forms are other suitable feedstuffs commonly used in the enhancement of fish diets (Julie-Anne and Frank, 2003).

5.4 Recommendations for further studies

Further investigations can be carried out in this study by adjusting the composition of *P. sajor caju* to evaluate the effects survival and growth by rearing the *O. niloticus* in concrete tanks with large amounts of fish population. Feed conversion ratio (FCR) and protein efficiency ratio (PER) need to be study to evaluate the efficiency of the tilapia feed. This present study can also be used to observe the effect of reproduction in *O. niloticus*. Due to the observation of higher survival in this present study, the effects on the role of stress and immune resistance in tilapia by feeding *P. sajor caju* incorporated diets with varying concentrations can be explored further.

CHAPTER SIX

CONCLUSION

As a conclusion, this study has revealed the usefulness of *Pleurotus sajor-caju* waste stalks (powdered) formulated pellets in the development of growth and survival of juvenile tilapia (*Oreochromis niloticus*), as a feed enhancer as well as in improved good water quality.

In terms of water quality parameter, feeding on Diet 1 provides a proper environment condition with good dissolved oxygen (DO) for tilapia followed by Diet 2. The least DO exhibited for fish fed on Diet 3 showed that the use of powdered *P. sajor-caju* waste stalks in formulated pellets can be an added advantage for fish in maintaining oxygen concentration with proper aeration for their survival. Besides that, pH level also showed improvement after using powdered *P. sajor-caju* waste stalks as a supplement. However, in terms of ammonia and nitrite concentration, they did not manage to perform better than the commercial diet. This could be due to improper pelletizing (home-made pellet) compared to Diet 3 which was manufactured industrially. Apart from that, prolonged accumulation of excess feeds and debris will cause changes in water quality which leads to cloudiness or turbidity. *P. sajor-caju* as a feed supplement can help to reduce the contents of nitrate compared to commercial diet.

Overall, utilization of Diet 1 gave a remarkable improvement on tilapias growth and survival followed by Diet 2 and Diet 3. Supplementation of diets with powdered *P. sajor-caju* waste stalks enhances the survival of tilapia. β-glucans present in *P. sajor-ca-*

ju may have played an important role to enhance the immune response and resistance to disease. It also helped in growth performance while maintaining good survival. Generally, a proper maintenance task by changing water on a daily routine and providing good aeration also leads to a better water quality as well as growth performance and survival.