Chapter 2

2.0 LITERATURE REVIEW

2.1 Feed Formulation

The development of new species-specific diet formulation could support and also sustain the aquaculture industry as it expands to satisfy increasing demand for affordable, safe and high quality flesh product. Thus, the process needs the appropriate feed ingredients to produce and meet the minimum requirement of nutrients for cultured fish. A nutritionally balanced and palatable diet is the criteria that should be given attention to in the formulation of diets from its various ingredients in adequate amount. In aquaculture practice, feed cost has become a major problem in both intensive and semi-intensive fish culture and therefore some consideration needs to be given to address this issue. In supplying adequate nutrition for various aquaculture species, bioavailability of nutrients, diet acceptability (palatability), feed processing, storage methods and chemical contamination can give impact on quality of diet and hence affect the performance of fish and production respectively. Based on nature of culture practice to satisfy the formulation, aquaculture system requires all the nutritional requirements to be fulfilled whereby in semi-intensive fish culture the formulated feed must supply supplementary nutrition used to fortify the naturally available diet.

2.1.1 Factors to be considered in Feed Formulation

Many factors need to take into account to formulate the diet which will provide the optimal growth performance of the cultured fish and yet remain cost-effective. Some factors are outlined in Table 2.1:

2.1.1.1 Nutritional requirements

The most apparent differences in nutritional requirements are seen in the essential amino acid content and protein dietary requirements (NRC, 1993). The essential amino acid requirements tend to play a vital role in its transformation into body muscle of fish. It is important that the protein sources selected meet minimum requirement to maintain optimum growth performances and to avoid it from succumbing to disease.

Table 2.1: General amount of nutrient incorporated into diets for growing fish (Royes and Chapman, 2003)

Nutrient	Requirement (Percentage by dry diet)
Protein: (10 essential amino acids): Lysine, phenylalanine, arginine, valine, leucine, isoleucine, methionine, threonine, tryptophan, histidine.	32 - 45%
Fat: Used as a source of energy and polyunsaturated fatty acid. In general, freshwater fish require fatty acid of linolenic (omega-3) and linoleic (omega-6) series.	4 – 28% (should contain at least 1-2% of the polyunsaturated *n*-6 or *n*-3 essential fatty acid series)
Carbohydrates: These are inexpensive source of energy and are binding agent properties. 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 - 20%
Minerals: Some 20 inorganic mineral elements including calcium, phosphorus, magnesium, iron, copper, manganese, zinc, iodine and selenium.	1.0 - 2.5% fed as a multi mineral premix.
Vitamins: These are inorganic substances required in trace amounts that can be divided into fat-soluble (Vitamin A, D, E and K) and water-soluble (Vitamin C and the B-complex.	1.0 - 2.5% fed primarily as a multi vitamin premix.

2.1.1.2 Composition of ingredients

The most comprehensive information on feedstuff composition is provided in the United State - Canada tables of feed composition (NRC, 1993). The compositions of raw ingredients are known to be different based on region and ways of processing as well as storage. The feed ingredients should be analysed in actual condition prior to feed formulation. Feedstuffs also need to be screened first for the presence any enzyme inhibitors or anti nutritional factors present in the ingredients.

2.1.1.3 Digestibility and nutrient availability

The knowledge of the digestibility of the individual nutrients of all the ingredients is important and need to be identified before feed formulation. Ingredient digestibility can be defined as the measurement of proportion of the energy and nutrients, which an animal can absorb within the diet during the digestive process (Glencross *et al.,* 2007). However, general digestibility values for most common used ingredients for the familiar consumption of cultured species are presently available and often used widely in feed formulation. Only the unconventional ingredients need to undergo digestibility test. Cho *et al.* (1974) introduced the estimation of the digestibility of an ingredient and it has become standard procedure to use 7:3 ratio of reference diet to ingredients. Austreng (1978) introduced the use of chromic oxide as a digestion indicator and confirmed the suitability of this procedure for measuring the digestibility of fish towards diets given.

Apart from straight forward digestibility considerations, the improvement of feed digestibility needs to take into consideration because it would reduce waste outputs excreted as faeces that could deteriorate water quality. Bureau and Cho (1999) stated that research has been concentrated on using highly digestible diets to reduce faeces output. For preliminary formulations, New (1987) recommends the use of digestible energy values given in Table 2.2.

Table 2.2: Digestible energy value for fish feeds

Nutrient	GE[1] (kj/g)	DE (estimated kj/g)
Carbohydrates		
Non Legumes	17.15	12.55
Legumes	17.15	8.37
Proteins		
Plants	23.01	15.90
Animals	23.01	17.78
Fats	38.07	33.47

[1]GE = amount of heat released when the substance is completely oxidized in a bomb calorimeter at 25 - 30 atmospheres at oxygen. This also referred to as physiological fuel value.
GE = gross energy; DE = digestible energy

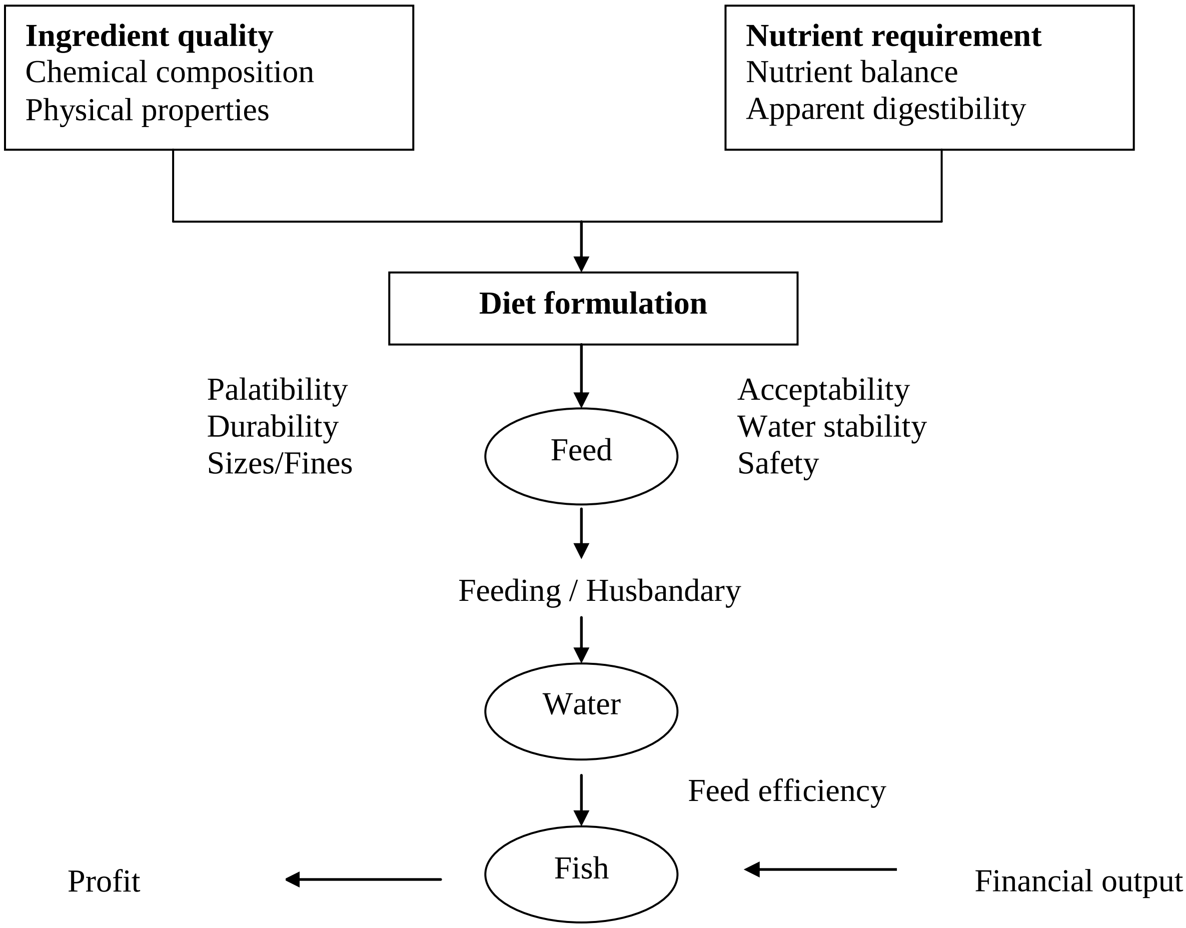

Figure 2.1: Fish nutrition relationship in aquaculture (Cho *et al.*, 1985)

2.2 Feed Processing

Feed formulation is followed by processing and manufacturing. The processing method which include sourcing, mixing, pelleting, drying and storing is very important to ensure the bioavailability of nutrients, feed acceptability, palatability and durability which often have profound effect on performance of fish (Gabriel *et al.*, 2007). This can be done by selecting the cheap source of ingredients but rich in nutrients for fish growth and optimum performances. Effective substitution can be done when the knowledge of nutrient availability of the substitute is well known and identified. There are three methods of manufacturing the fish feeds: steam pelleting, extrusion and dravo processing (Cho *et al*., 1985). These procedures shared similar methods as follows:

2.2.1 Sourcing the Ingredients

This method involves the purchase of high quality ingredients since the quality of ingredients determines the quality of feedstuffs and cost of feeds. Purchasing the raw ingredients is the common issue among the feed producers. If this situation continues without any alternative from locally available sources the aquaculture sector will be in trouble for consuming high price feed. Feed producers should buy the available ingredients at the cheapest price during the harvest time.

2.2.2 Grinding of Feed Ingredients

This is normally done using a hammer mill. The structure of ingredients whether it is coarse or fine has a significant effect on both the physical properties and nutritional value of a finish product (Igbinosun, 1991). A major function in feed manufacturing is grinding or particle size reduction. Grinding generally improves feed digestibility, mixing together of various ingredients, reduce feed breakage and increase feed acceptability and ingestion by fish (Igbinosun, 1991).

2.2.3 Weighing and Mixing

Weighing must be accurate in order to ensure the feed ingredients are in their correct proportions as formulated in the diet. The estimated value for each chemical composition on each diet could be verified after analysis is done. After weighing the feed ingredients are properly mixed in homogenous form manually to ensure proper mixing. This is the most important and difficult part of feed manufacturing, as the feed manufacturer must ensure that the feed ingredients are blended together.

2.2.4 Pelleting of Feed

Pelleting is described as the agglomeration (process molding into mass) of small particles into large particle by using the mechanical process combined with moisture, heat and pressure (Falk, 1985). It converts the homogenous blend of dry ingredients into durable forms that makes its physical characteristics suitable for feeding purpose. Pelleted feeds have advantages of less feed wastage, uniform feed intake and destruction of growth inhibitors.

2.2.5 Drying of Feed

Drying is done to remove moisture in the feed after pelleting process. Drying enhances the durability of the feeds for the proper storage. The method of drying depends on the ingredients used, processing method and scale of production.

2.3 Significant Milestones of Fish Meal Replacement Research

Table 2.3: Significant time line of fish meal replacement

Year	Author(s)	Significant Findings
1990	Shiau *et al.*	Either defatted soybean meal or full-fat soybean meal can be used to replace 30% fish meal protein in a diet for *Oreochromis niloticus* x *O. aureus* fingerling hybrids when the dietary protein level is low (24%).
1996	Gaber	Nile tilapia (*Oreochromis niloticus*) fed on diet with 40% combined poultry byproduct and feather meal had maximum growth rates, feed conversion (1.71) and body lipid content (6.7%).
1997	Olvera-Novoa *et al*.	Highest growth rate and feed utilization were observed with 20 – 30% replacement of fish meal with cowpea (*Vigna unguiculata*) protein concentrate, while protein efficiency was best at 40% inclusion level.
1997	El-Saidy and Gaber	Optimum growth performance and desired meat quality of Nile tilapia fry can be attained by replacing fish meal with SBM (soy bean meal) up to 33% supplemented with 1% DL-methionine.
1998	El-Sayed	Growth of fish fed on SM (shrimp meal), PBM (poultry by-product) and MBM (meat bone meal) was not significantly different from those fed on the FM-based diet, feed conversion and protein efficiency ratios were significantly reduced. Further reduction in fish performance was noticed when BM (blood meal) or BM + MBM replaced FM in the control diet.
1999	Belal and Al-Dosari	Weight gain was reduced when fish were fed diets with higher levels of salicornia meal, and growth was slowest for fish fed diets formulated entirely from salicornia meal. Concluded that salicornia meal can replace up to 40% of the fish meal in *O. niloticus* feeds without affecting growth or body composition.
1999	Fontainhas-Fernandes *et al*.	Significant differences for weight gain among fish fed diets; Diet 0, Diet 33, Diet 66 and Diet 100 (containing only animal protein, 33, 66, and 100% of plant protein, respectively). Suggested also that extruded pea seed meal (92.6%), defatted soybean meal (94.4%), full-fat toasted soybean

		(90.0%) and micronized wheat (88.6 %) were the best vegetable proteins tested in the diet of Nile tilapia, *Oreochromis niloticus*.
2001	Ng *et al.*	Research was conducted on mealworms (*Tenebrio molitor*). Significantly, found whether used as such or transformed into a dry meal, were reported to be highly palatable to the African catfish. Catfish fed mealworm-based diets also tended to have significantly higher lipids in their carcass.
2002	Plascencia-Jatomea *et al.*	Shrimp head hydrolysate is a promising alternative protein source for tilapia feeding, improving growth ratio at dietary inclusion levels as high as 15%.
2003	Abdelghany	Generally, GFM (*Gambusia affinis* fish meal) exhibited good potential as a substitute for HFM (herring fish meal) in red tilapia diets with no adverse effects on growth, feed efficiency, body composition, blood parameters or apparent digestibility of dry matter, protein and gross energy compared with the HFM-based control diet. Growth performance of fish fed diets containing GFM at 25 or 50 % level of replacement showed the best result.
2003	Madu and Ufodike	The use of live maggots (*Musca domestica*) to supplement artificial feeds in catfish production farms is therefore highly recommended. It was shown that the greatest increase in body weight (99.7 g) of tilapia (*Oreochromis niloticus*) fry was achieved with Diet 4 (maggots + artificial feed) followed by Diet 1, where maggots were fed exclusively (77.5 g).
2008	Sogbesan and Ugwumba	The utilization of termite (*Macrotermes subhyalinus*) as dietary protein source in the diets of *Heterobranchus longifilis* fingerlings.
2008	Zerai *et al.*	50% of the fish meal protein in a typical commercial diet could be replaced with brewer's waste with no adverse effect on growth and feed utilization for tilapia.
2008	Salem *et al.*	The final body weight, live weight gain, daily gain, specific growth rate, feed conversion ratio, protein efficiency ratio and protein productive value was insignificantly differences between the fish fed diets containing 33.33 or 66.66% of SWPM (silkworm pupae meal) and the control diet.
2009	Pratumsri *et al.*	25% of Eri silkworm (*Philosamia ricini*,

		Boisd) protein hydrolysate substitution for fish meal protein in catfish diet containing the 30% crude protein has growth, survival rate including feed efficacy equal to the group of fish obtained diet containing protein from fish meal ($p>0.05$).
2010	Perschbacher *et al.*	Spirulina-fed fish contained significantly ($P<0.01$) greater linolenic, docosapentaenoic, eicosatrienoic, and eicosatetraenoic (arachidonic) fatty acids. Total polyunsaturated fatty acids (PUFA) did not differ, however highly unsaturated fatty acids (HUFA) levels were approximately 50% higher.
2010	Gumus *et al.*	Sand smelt (*Antherina boyeri*) meal can replace up to 75% of the fish meal in diets for Nile tilapia fry.
2011	Sealey *et al.*	Enriched black soldier (*Hermetia illucens*) larvae can be used to replace up to 50% of fish meal portion without significantly affecting growth and the sensory quality of rainbow trout fillet.

2.4 Tilapia Culture

Tilapia is a generic term which is used to refer to a group of commercially important food fish belonging to the family Cichlidae. Tilapia has been raised for human consumption for a long time suggesting that the Nile tilapia, *Oreochromis niloticus* has been cultured for more than 3000 years (Maar *et al*., 1966). Three genera popularly cultured were *Oreochromis*, *Tilapia* and *Sarotherodon*. The Nile tilapia belongs to the genus *Oreochromis*.

More than 100 tilapia species have been identified (Balarin, 1979). Currently, tilapia culture is widely practiced in many tropical and subtropical regions of the world. Presently, more than 22 tilapia species are being cultured worldwide. However, the Nile tilapia (*Oreochromis niloticus*), *Mozambique* tilapia (*O. mossambicus*), blue tilapia (*O. aureus*), *O.hornorum*, *O. galilaneus*, *Tilapia zilli* and *T. rendalii* are the most commercially cultured tilapia species. Tilapia species are used in commercial farming systems in almost 100 countries and are developed to be one of the most important fish for aquaculture of this century (Fitzsimmons, 2000). In addition, research on nutrition and culture systems, along with market development and processing advances have led to rapid expansion of the industry since the mid 1980.

Generally, red tilapia cultured in Asian regions originated from hybridization of two main species: *Oreochromis niloticus* and *Orechromis mossambicus* (Hamzah *et al.*, 2008). Red tilapia (*Oreochromis spp*.) was first introduced into Malaysia in the early 80's from Taiwan. In the mid 80's, red tilapia (*Oreochromis spp.*), a hybrid species with fast growth rate and more striking color began to be cultured (Lamin, 2010). This hybrid can also adapt to a wide range of farming production systems in Malaysia.

2.4.1 Tilapia Production

Production of tilapia has a wide distribution with 72% are raised in Asia particularly in China and Southeast Asia, 19% in Africa and 9% in America. Tilapia is the second largest group of farmed finfish species after carps (FAO, 2012). World tilapia production has been increasing dramatically with output more than doubling from 1.5 million metric tonnes in 2002 to 3.2 million metric tonnes in 2010 (Fitzsimmons *et al.,* 2010). About 2.3 million metric tonnes or 99% of farmed tilapia were produced in developing countries in 2006 with Africa producing about 13% of this amount. Egypt is the second biggest top producer (202 606 metric tonnes) after China, the world's top producer. In Malaysia, about 21 266 metric tonnes of tilapia was produced in 2002, with a value of RM 101.86 million. This fact represents 46% of the total production of fresh water aquaculture in Malaysia. Under the Third National Agricultural Policy (NAP3), tilapia is determined as the main species cultured besides giant freshwater prawns, sea bass and tiger prawns. The production target set for tilapia is 150 000 metric tonnes per year or 75% of the total freshwater fish production by the year 2010. But till 2010, this target has not been achieved as tilapia production was about 29 013 metric tonnes with value of RM 192.99 million.

The remarkable success of tilapia as a farmed fish can be attributed mainly to its desirable qualities as a food fish such as white flesh, natural taste and firm texture, which has made it gain acceptance (Shiau, 2002). They are also easily cultured fish in term of fast growth performance, disease resistance and easily to reproduce in captivity and able to tolerate a wide range of environmental conditions (Shiau, 2002).

Suitable ranges of water quality parameters for tilapia to survive are shown in Table 2.3. They consume food produced in culture system naturally as well as manufactured feed composed of plant ingredients (Shiau, 2002; Suresh 2003). Based on tilapia ability to face extreme environmental condition, Pullin (1984) referred to tilapia as an ‘aquatic chicken’, an animal that can be farmed easily and economically and with the same broad market appeal as the chicken.

Table 2.4: Water quality parameters for tilapia

Water temperature	Tolerance range[1]	Desirable range[2]
Temperature,	12.0 – 35.0	26 – 32
pH	5.0 – 10	6.5 – 8.5
Salinity, ppt	3.0 – 25.0	0 – 20
Dissolved oxygen, mg/L	2.0 – 8.0	>3.0 – 5.0
Ammonia, mg/L	0.6 – 2.0	<1.0
Nitrate, mg/L	0.0 – 3.0	-
Nitrite, mg/L	0.1 – 0.2	-
Alkalinity, mg/L	>20	>20
Hardness, mg/L	>20	<50

[1]Adapted from (Hussain, 2004; Swann, 2007); [2] (Suresh, 2003).

2.4.2 Nutrient Requirements of Tilapia

Tilapia are very good aquaculture species because they are omnivorous feeding at low trophic level. They consume on a variety of food materials including both plants and animals. They are able to produce high protein fillet from less refined protein sources thus making them ecologically attractive as an animal protein for human consumption (Jauncey, 1998). The genus *Oreochromis* generally feed on algae, aquatic plants, small invertebrates and bacterial films. Individual species may have preferences between these materials (Pompa and Masser, 1999).

Oreochromis are able to utilize all kinds of food and therefore are considered as opportunistic species. This provides an advantage for farmers because this fish can be either reared in extensive system depending on natural productivity of water body or intensive situations where low cost feed is offered (Fitzsimmons, 1997). In Malaysia, most commonly tilapia culture, 64% used earthen pond (Ng *et al.,* 2009).

The best growth performance is exhibited when tilapia are fed a balanced diet that provides a proper amount of protein, lipid, carbohydrates, vitamins, minerals and fiber. Nutritional requirements of fish differ according to fish species and more importantly vary with life stages. According to Fitzsimmons (1997), fry and fingerlings require diets with higher protein, lipid, vitamins and minerals and lower carbohydrates as they are developing the muscles, bone and internal organs with rapid growth. The protein requirement for juvenile tilapia is reported to range between 30 – 56% (Jauncey, 1998; Suresh, 2003). The protein requirement of fish decreases with the age and optimum dietary requirement for tilapia is summarized in Table 2.5.

Table 2.5: Dietary requirements of tilapia

Approximate wet body weight (g)	Optimum dietary protein content (%)
Fry to 0.5	30 – 46, recommended 40 – 45
0.5 to 10.0	30 – 40, recommended 30 – 35
10.0 to 30.0	recommended 25 – 30
30.0 to market size	recommended 25 – 30

(Jauncey, 1998)

Tilapia also requires essential amino acids that must be supplied from its diets because of their disability to synthesis them. The quantitative requirements for these amino acids for growth of juvenile tilapia have been determined by Santiago and Lovell (1988) and are presented in Table 2.6.

Table 2.6: Essential amino acid requirements of tilapia (*Oreochromis niloticus*)

Amino Acids	Requirements (% of dietary protein)
Arginine	4.20
Histidine	1.72
Isoleucine	3.11
Leucine	3.39
Lysine	5.12
Methionine	2.68
Phenylalanine	3.75
Threonine	3.75
Tryptophan	1.00
Valine	2.80

(Santiago and Lovell, 1988; NRC, 1993).

Basically, the recommended dietary lipid level for tilapia ranges from 5% to 12% (Suresh, 2003). Dietary lipid contains both omega-3 and omega-6 fatty acid each representing 1% of the diet, although some reports suggested that fish grows well with higher proportions of these fatty acids (Teshima *et al.*, 1982, Table 2.7). Jauncey (2000) found that to get maximum protein utilization, dietary fat concentration should be between 8 to 12% for 25 g of tilapia and 6 to 8% for larger fish. Older fish seems to require higher fiber content, a maximum of 8 - 10% (Jauncey, 1998) than younger ones at about 6 – 8% (Fitzsimmons, 1997) Carbohydrates usually represent less than 25% of the diet (Shiau, 1997). Tilapia appears to have the ability to utilize carbohydrate level up to 30% in the diet (Anderson *et al.,* 1984).

Table 2.7: Essential fatty acid requirements of *Oreochromis niloticus*

Fatty Acid type	Level	References
18:2n-6	0.5 – 1.0%	Teshima *et al.* (1982)
20:4n-6	1.0%	Takeuchi *et al.* (1983)

Minerals and vitamins are crucial for good and balanced nutrition and a lot of research has been conducted to determine these requirements (El-Sayed and Teshima, 1991; Jauncey and Ross, 1982; Watanabe *et al*., 1997). Excessive supplies of a certain mineral can lead to deformities and in some extreme cases can cause toxicity to fish.

Feeding rate in practical feeding can be classified into two major options. Feeding to satiation and feeding with restricted ration (Suresh, 2003). Best growth is normally achieved by feeding to satiation. But, satiation level is not an economical approach to feeding practice because the food conversion through this method is very low. Besides, it is difficult to determine the satiation level of the fish as food consumption may not be fully maximized. This may cause overfeeding and leading to water quality deterioration. As a result, restricted ration is recommended for feeding fish (Suresh, 2003). Some recommendation of feeding rate is given in Table 2.8.

Table 2.8: Feeding allowances and frequencies for feeding of various tilapias at 28 °C

Sizes (g)	Daily Feeding Rate (% of body weight gain)	Times Fed Daily
0.1 – 1	30 – 10	8
1 – 5	10 – 6	6
5 – 20	6 – 4	4
20 – 100	4 – 3	4 – 3
Larger than 100	3	3

Jauncey (1982); NRC (1993); Suresh (2003)

Measuring food consumption in water is difficult. Food fed to fish apparently can be ignored or delivered at inappropriate time. To overcome this, several methods are perfected over the years. Some commonly used techniques in investigating the feed intake include x - radiography, self feeding devices, direct observation using chemical markers and stomach chemical analysis (Jobling *et al*., 2001).

2.5 Fish Meal in Aquaculture Diets

Feeding cost represents about 80% of the total cost of tilapia in pond culture (Hishamunda *et al.*, 2009). The shortage in world production of fish meal (FM), which is the main conventional protein source coupled with its increased demand in feeds for livestock and poultry is likely to reduce dependence on it as a single protein source in aquafeeds (El-Sayed, 1999). FM is considered the most desirable animal protein ingredient in aquafeeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential fatty acid, DHA and EPA (Hardy and Tacon, 2002). Dietary protein is actually the most crucial nutrient considered when formulating a fish feed to avoid any deficiency that may distort the fish growth and loss of weight. Hence, the selection of dietary protein in the right amount in terms of quality and quantity is a major consideration for obtaining high income fish production.

FM has been used in aquafeeds for carnivorous and omnivorous species to sustain the metabolic activity and supply all adequate energy requirements for subsequent fish growth. FM carries large quantities of energy per unit weight and is an excellent source of protein, lipids (oils), amino acid composition (particularly lysine and methionine), mineral and vitamins and has been recognized by the aquaculture industry as a high-quality, very digestible feed ingredient that is efficiently viable for making quality diet (Alvarez *et al.*, 2007).

Available FM can be distributed into two types; white fish meal produced from non-oily whole fish, post-filleting residues, and brown fish meal made from oily whole fish from a large proportion of the oil that has been extracted (Jauncey, 1998). Currently, fish meal is widely distributed and is utilized as feeds for domestic livestock such as poultry and aquaculture especially for carnivorous species and also as feed attractant for omnivorous species (FAO, 2007).

Crude protein and ash contents of fish meal may vary from 500 to 720 g/kg and 100 to 200 g/kg respectively depending on type of fish, source and way of processing (Drew *et al*., 2007). The fat content of fish meal is species-specific and is normally extracted from the fish. However fish meal from oily fish species may contain up to 9 % oil (De Boer and Bickel, 1988). The oil in fish meal is rich in PUFA, mostly from the omega-3 family (Hertrampf and Piedad-Pascual, 2000). Fish meal also has high ash content especially when made mainly from fish bones. Generally, the higher the ash content of fish meal, the higher the calcium, phosphorus and magnesium content (De Boer and Bickel, 1988). However, in some cases high ash content will cause problem in feed formulation and feeding management as this undesirable ash will result in nutrient leaching. Mainly due to its desirable nutritional value, fish meal has traditionally been used as a major protein source in aquafeeds (Sargent and Tacon, 1999).

Recently, there were reports on fish meal that has been contaminated by Persistent Organic Pollutants (POP) with a potential negative implication on fish, fish final products at harvestable points and consequently to public health. In addition, a slight decline in consumer demand arose due to this problem. The POPs include dioxins and furans, dioxin-like-poly-chlorinated biphenyls (PCBs) and others. Reports on the level of contaminants which are acceptable as regulated by EU (European Union) and WHO (World Health Organization) as well as their implication on human health are available (Hendricks, 2002; Mozaffarian and Rimm, 2006).

2.5.1 Current Production of Fish Meal

Global fish meal production was estimated at 5.52 million metric tonnes in 2003 (Tacon *et al*., 2006). Low production of fish meal was usually caused by the effect of El Nino events on catches of Peruvian anchovy (Tacon *et al*., 2006). El Nino events occured in the rich fishing grounds of Peru by changing the ocean water temperatures. Water temperatures increase lead to migration of Peruvian anchovies to cooler deeper areas where they become difficult and unavailable to fishing boats. Peruvian anchovies have contributed over 25% of the global production (Hardy, 2006). The El Nino is a periodical phenomenon and hence will have an impact on fisheries in the future. As fish meal demonstrates a dramatic decline of global production which leads to short supply, it will cause an increase in fish meal prices. As consequences of El Nino phenomenon, fish meal has experienced a series of peaks and drastic drops in the last few decades, going from 12.5 million tonnes in 1994 to 4.2 million tonnes in 2010 (FAO, 2012).

Global aquafeed production in 2003 was estimated at approximately 19.5 million metric tonnes and according to Barlow (2000), production is expected to increase over 37 million metric tonnes by the end of the decade (2010), with an increase of 17.5 million metric tonnes (Hardy, 2006). According to Pike and Barlow (2003), estimated fish meal use in aquafeeds was 2.22 million metric tonnes and has increased dramatically. Naylor *et al*. (2000) came up with the opinion that considering the volumes of fish meal and fish oil used in aquafeeds, especially for carnivorous species, the culture of these species should be considered as a net fish consumer rather than producer and this practice has raised concerns about the long-term sustainability of these industries. Tacon and Forster (2000) predicted that fish meal use in aquafeeds will decrease from 2.2 million metric tonnes in 2000 to 1.6 million metric tonnes in

2010. Their prediction was based on the assumption that prices of fish meal will increase at the same time that market prices for farmed fish and shrimp decrease, forcing the fish feed industry to replace portions of fish meal in aquafeeds with less expensive ingredients.

Nowadays, aquaculture still uses fish meal as the main protein source for carnivorous species as the production from global fisheries remain static. This could be risky and harmful for the viability and profitability of this industry (Sargent and Tacon, 1999). Although there are some efforts made on reducing the dependence of this sole protein source in fish diets, the progress is slow.

Hence, there is a need to search for other protein alternatives to fish meal so as to reduce the dependence on fish meal in aquafeeds. New (2003) suggested that positive results with alternative proteins in lower use-levels of fish meal incorporated within aquafeeds will result in potential performance that would be required to produce quality aquafeeds in 2010. Indeed, the need for reducing the fish meal share in aquafeeds has been paid attention to in numerous reports and scientific papers and it presents a considerable challenge for the future development of aquaculture (Sargent and Tacon, 1999; Delgado *et al.*, 2003).

2.6 Alternative Protein Source to Fish Meal

Feed for juvenile fish often requires good quality and high dietary protein level inclusion, which may demand even higher dietary inclusion levels of fish meal up to 70% used (Tacon, 1981). According to Tacon *et al.* (2006), the aquafeed sector consumed about 52.6% of total global production of fish meal with a value of 2.7% for tilapia feed. Therefore, it is necessary to find alternative protein sources to develop suitable diets for tilapia, especially for the juvenile stage in order to increase aquaculture production.

Nile tilapia (*Oreochromis niloticus*) feeds efficiently on natural fauna and flora and can utilize supplementary feed materials to achieve rapid growth and weight gain increment. For tilapia feeds, the most typical protein sources examined have included poultry by-product (Gaber, 1996), cottonseed meal, sunflower meal (El-Saidy and Gaber, 2003), gammarid meal and crayfish exoskeleton meal (Koprucu and Ozdemir, 2005). More recent, researchers have replaced fish meal with peanut leaf meal in tilapia diet (Garduno-Lugo and Olvera-Novoa, 2008).

For an alternative protein source to replace fish meal effectively, certain prerequisites must be satisfied. This protein source must be competitive in price context compared to fish meal on a unit protein basis and their inclusion shall not negatively affect fish performance in terms of production characteristics such as weight gain, animal health and welfare or final product quality. They also must be sustainable (Hardy and Tacon, 2002). Finally, this potential alternative protein must not contribute to environmental waste, especially with respect to nitrogen and phosphorus discharge (Cho and Bureau, 1997).

Although, soybean meal represents one of the most widely used alternative protein sources utilized by nutritionists due to its global distribution, relatively low cost, good digestibility, it also contains a variety of anti nutritional factors that negatively affect growth performance of cultured fish (Francis *et al*., 2001). Nevertheless, some of the limitation such as the deficiency in essential amino acids such as methionine and lysine need to be supplied from other sources or through dietary supplementation.

Nowadays insects have become a more promising alternative protein source and a relatively new approach in fish nutrition due to the low success in fish meal replacement from animal or plant origin. Banjo *et al.* (2006) listed 17 species of edible insets representing nine families for their nutrient composition. This view clearly defined that insect could be an important source of protein and has high digestibility (Ramos-Elorduy *et al.*, 1997). Among the researchers, Spinneli (1979) cultivated and tested the use of housefly (*Musca domestica*) meal in the diet of *Salmo gairdineri* and *O. niloticus*. This is the same as tadpole meal as discussed by Ayinla *et al*. (1992) to sustain the production. Bondari and Sheppard (1987) mentioned that insects in various developmental stages have been used to feed fish and farm animals. Nandeesha *et al*. (2000) reported that growth and organoleptic quality are not affected when common carp are fed on nondefatted silkworm pupae, a major byproduct of the sericulture industry in India. Ng *et al.* (2001) has recently demonstrated that *T. molitor* larval meal is highly palatable to African catfish (*Clarias gariepinus*) and could substitute up to 40% of the fish meal component without reducing growth performance. Dried ground soldier fly larvae has also been successfully fed to chickens with no adverse effects on their growth performance and feed conversion (Hale, 1973). In recent years, there has been some new approach to substitute fish meal with housefly maggot meal in tilapia and African catfish diets (Ajani *et al*., 2004; Fasakin *et al*., 2003). Housefly can be easily produced but in view of the fact that maggot contained 90% moisture, it will require a large quantity of it to produce a sufficient meal to sustain a meaningful fish feed production

2.6.1 *Zophobas morio* meal

Super worms, *Zophobas morio* are the larval stages of one of the many darkling beetles and looked similar to meal worms, *Tenebrio molitor* physically but are slightly different in terms of nutritional elements and physical size (Figure 2.2). Supplementation with calcium is necessary if they are to be used as staple food item fed to animal to increase the nutrition content inside their body. *Z. morio* belongs to the family Tenebrionidae, a member of the same family of mealworm (*Tenebrio molitor*). Super worms are one of the common feeder insects used in reptile pet supplementary diet (Finke and Winn, 2004). Super worms are indigenous to the tropical region of Central and South America. The super worms nowadays are traditionally accepted as human food in many countries. They are nutritious, being a good source of protein, mineral, lipid and fatty acid and provide adequate energy (Bukkens, 1997). Super worms grow to approximately up to 2 inches long. They are naturally large and not treated with hormones to delay pupation taking more time for growth unlike the meal worm. *Z. morio* is rich in protein and fat content and provides sufficient quantities of essential amino acids (Finke, 2002). It also contains a variety of carbohydrates, vitamins, phosphorus and many minerals (Finke and Winn, 2004).

According to Patrick (1953), plant and animal proteins are usually low in lysine and methionine which are critical to fish diet. The use of insect such as super worm as alternative protein source in replacing fish meal is a promising option due to its essential amino acids which are comparable to fish meal and is also a sustainable resource. Economically, the super worm can consume a variety of by-products and converts all the waste feedstuff into nutritious feedstuff ready to be fed to animal for inclusion into fish diet. Thus, the environment can be kept clean and with zero waste production. This can be done by using gut loading method, which increase the insect nutrient content due to the food retained in their gastrointestinal tract (Allen and

Oftedal, 1989).

Neptune Industries Inc. has tried to develop fish diet research in response to protein source depletion. This research is focused on development of a sustainable, high protein meal derived from the commercial mass production of select insect species. The company has completed a process patent on the production protocol for a product called Ento-Protein™. Ento-Protein™ is a high quality dry protein meal created from commercially raised and processed insects. Through a cooperative research effort with Mississippi State University (MSU), Neptune is in the beginning stages of assessing Ento-Protein's™ commercial feasibility. The research was completed after feeding juvenile hybrid striped bass with encouraging result obtained including feed acceptability and flavor testing. The result summarized that the fish fed the Ento-Protein™ based diet had a sweeter, milder taste than those fed with standard fishmeal diets over the fish fed to control fishmeal diet (Papadoyianis, 2008). Table 2.9 revealed the comparison of the nutritional characteristics of selected insects with common proteins.

Table 2.9 Comparison of the nutritional characteristics of selected insects with common protein (as % of sample)

	Fishmeal*	Ento-protein™**	Soybean meal	Poultry meal
Crude Protein	**62-67**	**41.58 - 62.47**	47	67
Fat	**8-12**	**20.21 – 51.48**	1.56	10.87
Ash	**16-21**	**2.41 – 9.03**	5.80	13.98
Omega 6	**0.89**	**3.90 – 10.74**	0.40	2.00
Omega 3	**2.02**	**0.15 - 0.39**	0.05	0.10
Limiting Amino Acid (%)				
Methionine	**1.75**	**0.55 – 1.02**	0.68	0.86 – 1.03
Lysine	**4.88**	**2.01 – 3.60**	3.03	2.65 – 2.81
Arginine	**4.24**	**1.94 - 3.68**	3.51	2.28 – 3.69

*Menhaden analysis from Eurofin Scentific
**Initial analysis of 4 selected species from Eurofin Scientific
Papadoyianis, (2008)

Figure 2.2: A plate of super worm, *Zophobas morio* before laboratory analysis (length ± 12 cm)

2.6.2 *Pleurotus sajor caju*

The current research on prebiotics with fish is very limited although several preliminary studies have been conducted. Gatlin *et al.* (2006) tested a commercial prebiotic, GroBiotic-A and three other prebiotics; mannanoligosaccharides (MOS), galactooligosaccharides (GOS) and FOS and found that a significant increase in protein and organic matter digestibility in fish diet supplemented with prebiotic.

For a long time, the most common method dealing with the occurrence of bacterial infections in aquaculture is the administration of antibiotics. However, aquaculture still faces serious problems and it seems that many farms are unable to afford the antibiotics due to it expensive price (Yousefian and Amiri, 2009). Therefore, a prebiotic is introduced to overcome this problems hoping that growth rate will be greatly improved as well as strengthening the immune system by modifying the gastrointestinal (GI) tract microbial community (Gibson, 1999). Mahious and Ollevier (2005) and Fooks *et al.* (1999) suggested that any foodstuff that reaches the colon, including non-digestible carbohydrates can be a prebiotic candidate. This prebiotic action beneficially affects the host by selectively stimulating the growth and thus improves host health (Gibson and Roberfroid, 1995). Recent development in the search for prebiotics found that mushrooms seems to be the most potential candidate for prebiotics due to its carbohydrates content such as chitin and hemicelluloses, β (beta) and α (alfa) glucans, mannans, xylans and galactans. Prebiotics which is as non-digestible is to ensure that can withstand the digestive process and thus stimulate the beneficial bacteria effectively (Gibson and Collins, 1999; Macfarlane *et al.,* 2008).

Pleurotus sajor caju (Figure 2.3), an edible mushroom is normally called oyster mushroom, “hiratake”, “shimeji” or “houbitake” (Mizuno and Zhuan, 1995). Table 2.10 summarizes mushroom worldwide production as updated by Food and Agriculture Organization of the United States (2009). The productions of mushroom have increased, together with the waste products namely mushroom stalks after its harvest. This species usage is not only limited to food source but also as medicinal resources (Wasser, 2002). According to Mahajna *et al.* (2009), this fungi was classified as Basiodiomycota and received great attention because it contains various biologically active compounds such as polysaccharides, glycoproteins and antibiotics (Wasser, 2002). Moreover, mushrooms contain protein, essential amino acids needed for human diet and also low in fat content (Sadler, 2003). It contain significant amount of carbohydrates and fiber (Crisan and Sands, 1978; Chang and Buswell, 1996).

Developing new potential prebiotic from natural resources at affordable cost such as mushroom stalk should be considered. There are great advantages in incorporating mushrooms into fish diets as well as the human food as its polysaccharides are reported to exhibit immunomodulating properties, antitumor activities and anticancer activities. In addition, this prebiotic supplemented diet does not only provide essential nutrients to support growth and development of cultured organism, but also functions as health promoting and resistance to disease-causing agent (Gatlin, 2002).

Table 2.10: Worldwide mushroom production

Country	Production (tones)		Percentages (%)
	1997	2007	
China	562,194	1,605,000	65.0
United States	366,810	390,000	5.9
Canada	68,020	81,500	16.5
India	9,000	48,000	81.3
Indonesia	19,000	30,000	36.7
Islamic Republic of Iran	10,000	28,000	64.3
Republic of Iran	13,181	28,500	53.8
Vietnam	10,000	18,000	44.4
Thailand	9,000	10,000	10
Jordan	500	700	28.6
Kazakhtan	-	500	100

FAO (2009)

Figure 2.3: Mushroom (*Pleurotus sajor caju*) stalk before mashed into meal powder.

2.7 Limitations of the Utilization of Fish Meal Substitute

Any dietary change using alternative protein materials must ensure the growth and welfare of the cultured fish are not affected. The quality of the final product, including its highly beneficial properties for human health should be guaranteed.

2.7.1 Growth performance

Fish meal is a high quality protein source providing the essential amino acids (EAA) for optimum fish growth. Hence any reduction in the dietary levels of these main ingredients should take into account the potential losses in macro, micronutrients and energy (NRC, 1993).

Since fish can successfully convert efficiently the refined small protein source into their muscle carcass as protein composition, their essential amino acid content must be considered instead of protein content inside it. Essential amino acids are those that the fish cannot synthesis and must be provided into the diet to support maximum growth. Growth refers to increase in size, often measured as change in weight or in length.

Inadequate protein especially the essential amino acids for instance, methionine and lysine that can be considered as a critical factor to growth can result in reduction in growth and loss of weight due to withdrawal of protein from less vital tissue to more vital tissue to maintain the functions. On the other hand, if excess protein is supplied, this will turn into growth reduction (Jauncey, 1982) and cause environmental pollution (Rennert, 1994). Hence, to minimize the reduction in growth rate, the potential of FM replacer should provide sufficient or comparable amount of EAA to avoid any sign of deficiency in growth (Poston *et al*., 1977).

2.7.2 Final Product Quality and Implication to Human Nutrition

Diet is known to influence the organoleptic quality of fish (Spinelli *et al.*, 1979). Therefore, the quality of feed may influence the final carcass composition and hence flesh quality of the final product. Thus, it is good that the feed is not only nutritionally adequate but also palatable. There are several reviews on the effects of diets in fish composition as a final quality product (Spinneli, 1979; Buckley and Groves, 1979; Shearer, 1994). According to Shearer (1994), there are two factors determining the final product of the fish species; the external and the internal ones. The internal factors are life cycle, sex and size. These factors will cover the majority of principle to determine the composition of fish. Redder flesh is desirable in shrimp and salmonids and is achieved by introducing carotenoids in the feeds. Therefore, feed development should aim to increase market acceptability and shelf life.

2.7.3 Feed Palatability and Acceptability

According to Houlihan *et al.* (2001) palatability of feed is a major factor that determines feed acceptance. Feed palatability is defined by Glencross *et al.* (2007) as acceptable to the taste or sufficiently agreeable in flavor to be eaten. Feed acceptance depends on a variety of chemical, nutritional and physical characteristics, all of which can be influenced by the choice of feed ingredients and processing conditions used in the feed manufacturing (Jobling *et al.,* 2001). The ability of fish to ingest and detect the feed can be influenced by physical and chemical properties, pellet density (sinking rate), size, color and texture (hardness) and the chemical composition of feed which will depend on the ingredients used (Jobling *et al.,* 2001). It is known that insect larvae consist of high lipid content mainly saturated fatty acids. Ogunji *et al.* (2006) observed that incorporating high level of maggot meal in the tilapia diet led to unacceptable and poor growth performances. This is actually attributed to the unpalatability of the feed.

2.7.4 Chitin Content

The cuticle of insects is composed of chitin matrix with cuticular protein, lipids and other compounds (Kramer *et al*., 1995; Nation, 2002). Chitin, an unbranched polymer of *N*-acetylglucosamine is a primary component of invertebrate of exoskeleton and indigestible to many fish species. Chitin also could be categorized as fiber constituent that is non toxic but as this compound increases in its content it would consequently result in a decrease in the total dry matter and nutrient digestibility of the diet resulting in poor performance of final product of target fish cultured (De Silva and Anderson, 1995). Absence of chitinase enzyme in the fish may result in poor digestibility of the nutrients. In addition, Buddington (1980) reported that *O. niloticus* has no ability to digest chitin.