TOTAL SULFUR AMINO ACID REQUIREMENT AND ITS APPLICATION TO PRACTICAL DIETS FOR JUVENILE TILAPIA (*Oreochromis* spp.)

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TOTAL SULFUR AMINO ACID REQUIREMENT AND ITS APPLICATION TO PRACTICAL DIETS FOR JUVENILE TILAPIA (*Oreochromis* spp.)

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TO PRACTICAL DIETS FOR JUVENILE TILAPIA (*Oreochromis* spp.)

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DISSERTATION ABSTRACT

TOTAL SULFUR AMINO ACID REQUIREMENT AND ITS APPLICATION

TO PRACTICAL DIETS FOR JUVENILE TILAPIA (*Oreochromis* spp.)

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Tilapia have become one of the most important fish species for aquaculture and play an increasing role in the international aquatic food trade. Therefore, the development of cost-effective feeds using inexpensive and locally available plant and animal protein sources will contribute to sustainable aquaculture development for the future. Three separate studies, each contained two feeding experiments, were conducted to evaluate practical applications of various protein sources in grow-out diets, to determine total sulfur amino acid (TSAA) requirement and replacement of cystine for methionine in semi-purified diets and to determine and confirm methionine requirement in practical diets for juvenile tilapia (*Oreochromis* spp.).

Results of the first study showed that dehulled solvent-extracted soybean meal (DSESM) and expeller pressed soybean meal (EPSM) could totally replace fish meal's inclusion rate in commercial diets for juvenile tilapia (*Oreochromis* spp.) and methionine did not appear to be limiting in practical diets using cottonseed meal (CSM), meat and bone meal (MBM) and DSESM as primary protein sources.

To further optimize practical diets with respect to TSAA, the second study was conducted to more precisely determine TSAA requirement and replacement value of cystine for methionine of juvenile Nile tilapia in semi-purified diets. Broken-line regression analysis on weight gain data indicated that the TSAA requirement of juvenile Nile tilapia was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% of methionine requirement based on an equimolar sulfur basis. This means methionine requirement of juvenile Nile tilapia was 0.43% of the diet or 1.54% of dietary protein.

It is important to utilize methionine requirement value determined by semi-purified diets in the formulation of practical diets to determine and confirm the practical application of methionine requirement data. Hence, the last study was conducted to determine and confirm methionine requirement in practical diets for juvenile Nile tilapia (*O. niloticus*). Results of the last study indicated that methionine requirement of juvenile Nile tilapia was 0.49% of the diet or 1.75% of dietary protein. Furthermore, methionine appeared to be limiting when a portion of DSESM was replaced by gelatin and methionine supplementation did not have positive effects on weight gain, survival or feed efficiency ratio (FER) of juvenile Nile tilapia fed diets using CSM and DSESM as protein sources.

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CHAPTER I

INTRODUCTION

Status of world fisheries and aquaculture

Capture fisheries and aquaculture supplied the world with about 106 million tonnes of food fish in 2004, providing an apparent per capita supply of 16.6 kg (live weight equivalent), which is the highest on record. Of this total, aquaculture accounted for 43 percent. Overall, fish provided more than 2.6 billion people with at least 20 percent of their average per capita animal protein intake (FAO, 2007).

Aquaculture continues to grow more rapidly than all other animal food-producing sectors, with an average annual growth rate for the world of 8.8 percent per year since 1970, compared with only 1.2 percent for capture fisheries and 2.8 percent for terrestrial farmed meat production systems over the same period. Aquaculture production in 2004 was reported to be 45.5 million metric tonnes (MT) with a value of US$ 63.3 billion or, if aquatic plants are included, 59.4 million MT with a value of US$ 70.3 billion. Production from aquaculture has greatly outpaced population growth, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.1 kg in 2004, representing an average annual growth rate of 7.1 percent (FAO, 2007).

An overview of tilapia fisheries and culture

Tilapia are freshwater fish belonging to the family Cichlidae. They are native to Africa, but were introduced into many tropical, subtropical and temperate regions of the world during the second half of the 20^{th} century (Pillay, 1990). Tilapia are currently known as “aquatic chicken” due to their fast growth, adaptability to a wide range of environmental conditions, disease resistance, high flesh quality, ability to grow and reproduce in captivity and feed on low trophic levels. Therefore, these fish have become excellent species for aquaculture, especially in tropical and subtropical regions. Tilapia culture is believed to start more than 4,000 years ago, but very little information is available on their culture during those ancient times. The first trials of tilapia culture were recorded in Kenya in the 1920s (El-Sayed, 2006). Since then, tilapia culture has been established in many tropical and subtropical regions, and even in areas beyond their native ranges, where they have been introduced for various purposes. As a result, considerable attention has been paid to tilapia culture during the last three decades. Tilapia is currently cultured in more than 100 countries worldwide (El-Sayed, 2006).

Global landing of tilapia from capture fisheries increased progressively during the 1950s to the 1980s. During the 1990s and early 2000s, the landings were almost stable, fluctuating around 585,000-600,000 MT per year. Africa is by far the most important tilapia producer from capture fisheries, where it contributed about 70% of global landing in 2002, followed by Asia (18%), North America (9%) and South America (3%). Therefore, it is no surprise that, among the world’s top ten tilapia producers from capture fisheries, six are African countries (Egypt, Uganda, Tanzania, Kenya, Mali and Malawi). The top ten producers included three Asian countries (Thailand, Philippines and Sri Lanka) and one North American country (Mexico) (El-Sayed, 2006).

Tilapia culture has witnessed a huge expansion during the past decade. The production of farmed tilapia has increased more than 390%, from 383,654 MT in 1990 to 1,505,804 MT in 2002, representing 2.28% and 2.93% of total aquaculture production, respectively. The average annual growth of tilapia production during this period approached 12.2% (El-Sayed, 2006). In 2004, world production of farmed tilapia exceeded 2,002,087 metric tons, trailed behind that of the carps and exceeded that of the salmonids. Tilapia have been domesticated more quickly and to a greater extent than any other group of fish. The rapid improvements in domestication and wider consumption patterns may mean that tilapia may eventually overtake the carps to become the most important farmed fish. Tilapia have already become one of the most important farm-raised fish and have an increasing role in the international seafood trade (Fitzsimmons, 2006). There are about ten species of tilapia have been used for aquaculture. Nile tilapia (*O. niloticus*) is, by far, the most important farmed tilapia species in the world. It represented more than 80% of total tilapia production during 1970-2002. Nile tilapia also ranked sixth in terms of global farmed fish production in 2002, after silver carp, grass carp, common carp, crucian carp and big head carp (El-Sayed, 2006).

Traditionally, tilapia have been cultured in extensive systems. As the industry expands and the technology development continues, traditional extensive culture of tilapia is being replaced by semi-intensive and intensive production systems. In semi-intensive farming systems, supplemental feeds that consist of locally available, low-cost single feedstuffs such as rice bran, copra meal, coffee pulp, brewery by-products and/or their combination are generally used as supplements to natural food (Lim, 1989). As stocking rate increases, the contribution of natural food decreases and more nutritionally complete feeds are needed. In intensive culture systems such as in ponds, raceways, cages and tanks, feed is the most expensive item, often ranging from 30 to 60 percent of the total variable expenses, depending on the intensity of the culture operation (Lim and Webster, 2006). Therefore, the development of cost-effective feeds using cheap and locally available plant and animal protein sources has a great contribution to its sustainable aquaculture development in the future. In order to achieve this goal, the understanding of protein and essential amino acid requirements of juvenile tilapia is very important.

Protein requirements

Protein is the principal organic constituent of animal tissue and is the most expensive component in fish diets. It is made up of single amino acids, linked together by peptide bonds. There are 18 amino acids that can be found in most protein sources, although protein can contain 22 to 26 amino acids. Amino acids that cannot be synthesized by the animals or are not synthesized in sufficient amounts to meet physiological requirements and must be supplied in the diets are referred to as essential or indispensable amino acids. For those that can be synthesized in adequate quantity are termed non-essential or dispensable amino acids (Lim and Webster, 2006). Although the non-essential amino acids can be adequately synthesized by fish, their presences in the diets have nutritional significance because the need for fish to synthesize them is reduced. Two special examples of sparing action are the conversion of methionine to cystine and phenylalanine to tyrosine. These non-essential amino acids (cystine and tyrosine) can only be synthesized from essential amino acid precursors (methionine and phenylalanine) (NRC, 1983). Since body protein is constantly undergoing two major processes: protein synthesis (anabolism) and protein breakdown (catabolism), animals, including tilapia, need a continuous supply of protein throughout life for maintenance, growth, and other physiological functions. Inadequate intake of protein will result in retardation or cessation of growth, or loss of weight due to the withdrawal of protein from less vital tissues to maintain the function of more vital ones. If too much protein is supplied, however, one part will be used to synthesize new tissue and the remainder will be converted to energy (NRC, 1983). Tilapia, like other fish, do not have a true protein requirement, but need a well-balanced mixture of indispensable and dispensable amino acids (Shiau, 2002).

Protein requirements at different life stages of various tilapia species have been studied extensively. These values have generally been determined by measuring growth response of tilapia fed test diets containing graded levels of protein. Generally, semi-purified diets containing casein, casein/gelatin mixtures or casein/crystalline amino acid mixtures as protein sources or practical diets formulated using conventional feedstuffs such as fish meal (FM) or soybean meal as protein sources, have been used to determine protein requirements.

Protein requirement of tilapia is depended on many factors such as species, size, protein source and quality, non-protein energy level in the test diets, feeding rate, water quality variables (temperature, dissolved oxygen, salinity), the presence and density of natural food (NRC, 1993). For example, casein was traditionally used as the only dietary protein source in many studies. It contains adequate levels of most essential amino acids

(EAA) but is deficient in arginine. When casein is used as a sole protein source, higher levels of protein are required to compensate for the arginine deficiency. Since the amino acid profile is imbalanced, the observed protein requirement for tilapia would be lower if arginine is supplemented to the diet (El-Sayed, 1989). Due to faster growth rates, smaller fish often have higher protein requirements than bigger ones. Insufficient non-protein energy in the diet will also lead to a higher dietary protein requirements because fish will utilize part of the protein as energy to meet their metabolic energy needs. Water quality variables such as temperature and dissolved oxygen (DO) have considerable effects on metabolic rate of fish and thus their dietary protein requirements. A higher dietary protein level is required at optimum water temperature and DO for growth than at lower temperature or DO. Tilapia are very efficient in utilizing natural food. At low stocking densities in earthen ponds, they obtain significant amount of protein from natural food and therefore lower protein level is required (Lim and Webster, 2006). Dietary protein requirements of some commercially important tilapia species vary from 25 to 56% diet (Table 1).

Qualitative and quantitative amino acid requirements

Qualitative requirements for ten essential amino acids (EAA) (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) have been demonstrated in all fish species examined so far (NRC, 1993). Growth studies have been used to determine qualitative amino acid requirements of following fish species: channel catfish (Dupree and Halver, 1970); common carp (Nose et al., 1974); Japanese eel (Arai et al., 1972); chinook salmon (Halver et al., 1957); sockeye salmon (Halver and

Table 1: Dietary protein requirements of some commercially important tilapia species.

Species	Protein source	Size (g)	Protein requirement (%)	Reference
O. niloticus	Fish meal	0.012	45	El-Sayed and Teshima, 1992.
	Casein, gelatin	0.56	35	Teshima et al., 1985
	Casein	3.5	30	Wang et al., 1985.
		9.0	25	
	Fish meal, soybean meal	Broodstock	40	El-Sayed et al., 2003.
	Fish meal	Broodstock	45	Siddiqui et al., 1998.
O. mossambicus	Fish meal	Fry	50	Jauncey and Ross, 1982.
		0.5-1.0	40	
		6.0-30.0	30-35	
O. aureus	Soybean meal or fish meal	0.3-0.5	36	Davis and Stickney, 1978.
	Casein, albumin	Fry-2.5	56	Winfree and Stickney, 1981.
		2.5-7.5	34	
Tilapia zillii	Casein	1.3-3.5	35	Mazid et al., 1979.
	Casein, gelatin	1.4	35	El-Sayed, 1987.

Shanks, 1960); red sea bream (Yone, 1976) and redbelly tilapia (Mazid et al., 1978). According to Mazid et al. (1978), redbelly tilapia required the same ten EAA as other fish species.

Amino acid requirements of animal species have close relationship to whole body, muscle or egg amino acid profiles. In channel catfish, EAA requirements and their patterns of the whole body have closer correlation than that of both muscle and egg (Wilson and Poe, 1985). Cowey and Tacon (1983) found a good correlation between amino acid requirements and the whole body amino acid profile. However, Ketola (1982) recommended that the EAA profile of fish egg is a good reference for feed formulation due to its close correlation to EAA requirements. Therefore, the basic approach used to determine quantitative amino acid requirements is the utilization of crystalline amino acids or mixtures of crystalline amino acids and purified (gelatin and casein) or conventional protein sources to simulate amino acid profile of a particular protein such as whole hen's egg (Mertz, 1972), whole body or muscle or egg, or to meet all amino acid requirements of the particular animal (Baker, 1977), except for the test amino acids.

Quantitative amino acid requirements of various fish species have been studied using practical, purified or semi-purified diets. In all cases a diet must be nutritionally complete with the exception of the amino acid of interest. Quite often the basal diet is then modified to increase the levels of the amino acid of interest to produce a dose response. In practical test diets, conventional protein sources such as soybean meal, fish meal and peanut meal are used to formulate a basal diet which is deficient in the target amino acid. The remaining diets are formulated by adding graded levels of a purified amino acid to a basal diet or using a complimentary protein source rich in the specific amino acid of interest. The advantages of using conventional protein sources as compared to that of crystalline amino acids are superior growth rate and better palatability. However, the use of conventional protein sources requires that the amino acid composition and digestibility values for the test diets be determined. Furthermore, the requirements of some amino acids cannot be determined using intact protein sources as a basal diet deficient only in the amino acid of interest cannot be developed.

The same procedure has been used when utilizing purified and semi-purified test diets. In purified test diets, crystalline amino acids serve as the only nitrogen sources. Woodham and Deans (1975) and Wilson (1985) made a comparison between the uses of crystalline amino acids and conventional protein sources and stated that purified diets using only crystalline amino acids eliminate the necessity of amino acid profile analyses but they are expensive and poorly utilized by some fish species. For example, purified diets were used to determine amino acid requirements of common carp (Nose, 1979). However, growth rate was inferior as compared to that of fish fed diets formulated from intact protein. Leaching may be another problem associated with purified diets. Semi-purified diets generally refer to the use of a purified intact protein sources such as gelatin and casein in combination with a mixture of crystalline amino acids. They often take advantages of both practical and purified diets and thus, have usually been used to quantify amino acid requirements in many aquatic animal species.

Quantitative requirements of all essential amino acids have been determined for a limited number of juvenile fish species such as rainbow trout (*Oncorhynchus mykiss*) (Ogino, 1980); Indian carp (*Catla catla*) (Ravi and Devaraj, 1991); chinook salmon (*Oncorhynchus tshawytscha*) (Halver et al., 1958; Halver et al., 1959; Halver, 1965; Delong et al., 1962; Chance et al., 1964; Klein and Halver, 1970); channel catfish (*Ictalurus punctatus*) (Harding et al., 1977; Wilson et al., 1977; Wilson et al., 1978; Wilson et al., 1980; Robinson et al., 1980a; Robinson et al., 1980b; Robinson et al., 1981); Japanese eel (*Anguilla japonica*) (Nose, 1979), common carp (*Cyprinus carpio*) (Nose, 1979) and white sturgeon (*Acipenser transmotanus*) (Ng and Hung, 1995). Although tilapia have already become one of the most important farm-raised fish in the world, specific EAA requirements of most farmed tilapia species have not been determined (El-Sayed, 2006). There are relatively few studies designed to determine EAA requirements of tilapia. The quantitative requirements of ten essential amino acids have been determined for Mozambique tilapia (*O. mossambicus*) (Jauncey et al., 1983) and Nile tilapia (*O. niloticus*) (Santiago and Lovell, 1988) employing different methods. Jauncey et al. (1983) used whole body and muscle amino acid profile methods (Cowey and Tacon, 1983) to formulate test diets. Santiago and Lovell (1988) made basal diet from gelatin and casein and supplemented with crystalline L- amino acids to provide amino acid profile similar to that of 28% whole egg protein except for the test amino acids. Quantitative essential amino acid requirements of *O. niloticus* and *O. mossambicus* juveniles are shown in Table 2. The values determined by Jauncey et al. (1983) for *O. mossambicus* are lower than those of *O. niloticus* as reported by Santiago and Lovell (1988), except for leucine. Among all of the essential amino acids required by fish in general and tilapia in particular, methionine is often one of the most limiting EAA in feeds. Hence, the determination of methionine or total sulfur amino acid (TSAA, consists of methionine and

Table 2: Quantitative essential amino acid requirements of *O. niloticus* and *O. mossambicus* juveniles.

Amino acid	Requirements (Percent of dietary protein)	
	O. niloticus[a]	*O. mossambicus*[b]
Arginine	4.20	2.82
Histidine	1.72	1.05
Isoleucine	3.11	2.01
Leucine	3.39	3.40
Lysine	5.12	3.78
Methionine	2.68[c]	0.99
Phenylalanine	3.75[d]	2.50
Threonine	3.75	2.93
Tryptophan	1.00	0.43
Valine	2.80	2.20

[a]Santiago and Lovell, 1988; [b]Jauncey et al., 1983; [c]In the presence of cystine at 0.54% of dietary protein; [d]In the presence of tyrosine at 1.79% of dietary protein.

cystine) requirements of juvenile tilapia is critical to the production of cost-effective feeds.

Total sulfur amino acid requirements

Methionine has three major metabolic functions: as an essential amino acid for protein synthesis; as a sulfur source for synthesis of other sulfur-containing biochemicals; and as a methyl donor for methylation reactions (Mehler, 1986). Total sulfur amino acid requirements, expressing as percent of dietary protein, have been determined for various fish species such as Mozambique tilapia (3.2%: Jackson and Capper, 1982), Indian major carp (3.23%: Murthy and Varghese, 1998), common carp (3.1%: Nose, 1979), Japanese flounder (3.1%: Alam et al., 2001), red drum (3.03%: Moon and Gatlin, 1991); rainbow trout (2.9%: Kim et al., 1992); chinook salmon (5.0%: Halver et al., 1959) and channel catfish (2.34%: Harding et al., 1977).

Total sulfur amino acid requirement of tilapia can be met by methionine alone or a proper mixture of methionine and cystine (Shiau, 2002). Since cystine can only be synthesized metabolically from methionine precursor, its presence in the diets can spare a portion of methionine requirement for maximum growth. Therefore, the determination of replacement value of cystine (a non-essential amino acid) for methionine in aquatic animal species used for aquaculture is also important since we can minimize the incorporated level of methionine in practical diets without reducing growth and thus, minimize feed cost. To date, the replacement values of cystine for methionine, based on an equimolar sulfur basis, have been determined for following fish species: chinook salmon (50%: NRC, 1973); channel catfish (60%: Harding et al., 1977); red drum (40%: Moon and Gatlin, 1991);

rainbow trout (40%: Rumsey et al., 1983) and blue tilapia (44%: Liou, 1989).

Similar to other EAA, TSAA requirement of tilapia have been determined by several investigators. However, the reports covered a wide range of values. For example, Santiago and Lovell (1988) determined that TSAA requirement of Nile tilapia fry was 0.9% of the diet (consisted of 0.75% methionine and 0.15% cystine) or 3.22% of dietary protein while Kasper et al. (2000) concluded that this requirement for the same species was only 0.5% of the diet or 1.56% of dietary protein.

Tilapia is one of the most popular cultured fish species in the world and feed cost must be reduced as much as possible by using inexpensive and locally available plant and animal protein sources. Quite often, diets formulated from these protein sources are limited in methionine. Consequently we must precisely determine TSAA requirement and sparing effects of cystine for methionine to allow the use of ingredients that have low levels of methionine.

Statistical methods for estimation of nutrient requirements from growth studies

Several statistical and mathematical methods have been used to estimate nutrient requirements of fish from growth data. Generally, when replicated groups of fish fed test diets containing graded levels of a certain nutrient such as an essential amino acid, the growth increases linearly as concentration of this amino acid in the diets increases. However, the growth plateaus or even decreases as its concentration continues to increase after the requirement has been met. The approach to the asymtote is considered the break point or the requirement. The actual break point has been determined by mathematical or statistical model that best fit growth data. Robbins et al. (1979) used broken line and sigmoidal curve methods to fit nine sets of data and observed that the sigmoidal curve fitted all of them while broken line method fitted six data sets. However, when a set of data fitted both of them, the estimated requirements were almost identical. Zeitoun et al. (1976) compared the protein requirement of rainbow trout estimated by multiple comparison, polynomial and broken line analyses and found that polynomial regression analysis was more accurate than other methods. Baker (1986) suggested a minimum of four levels (preferably six or nine levels) of the nutrient under study is required in order to fit statistical or mathematical models, thus facilitating objective estimation of nutrient requirement.

Since researchers favored different methods to fit growth data, there is no generally accepted method for estimating nutrient requirement values. Baker (1986), however, found that broken line method clearly described an objective break point since it selected the requirement value representing the average group of animals in the population. Therefore, this method appears to be the most acceptable one for determination of nutrient requirements.

Overview of studies on evaluation of alternative plant and animal protein sources to replace fish meal in practical diets for tilapia

The nutritional value of protein sources, commonly referred to as protein quality, is determined based on the EAA profile and their digestibility or bioavailability. A protein source with EAA profile that closely matches EAA requirements of fish is likely to have high nutritional value (Lim and Webster, 2006). As protein is the most expensive components of aquatic feeds, the utilization must be optimized and the cost minimized. The development of commercial feeds for aquaculture has been traditionally based on the use of fish meal as the main protein source. This is due to its high protein content and balanced EAA profile. Fish meal is also an excellent source of essential fatty acids, digestible energy, minerals and vitamins. Because of its nutritive value, it is no surprise that fish meal is the most expensive protein source in animal feeds (Tacon, 1993).

The production of fish meal based on captured fisheries is at or beyond sustainable limits. The limited supply coupled with an increasing demand from the animal feed industry, results in a high price for this ingredient. Given the escalating cost of fish meal, it is critical that all animal production systems reduce their reliance on fish meal. This is particularly important in feeds for aquatic species as they often contain high levels of fish meal (El-Sayed, 1999). Furthermore, there are growing environmental concerns with regards to the use of wild fish to produce fish meal. Hence, there is interest in replacing fish meal with less expensive protein sources. Replacement of fish meal in practical diets without reducing the performance would result in a more profitable production of tilapia. Many studies have been conducted to evaluate the replacement of fish meal by the low-cost, locally available plant and animal protein sources in practical diets for tilapia.

A number of conventional and unconventional plant protein sources have been evaluated to replace fish meal in tilapia diets. Soybean meal (SBM), because of its availability, consistent quality, high protein content with good amino acid profile and low cost, is the most studied plant feedstuff in aquaculture diets (Lim and Dominy, 1989). However, it is considered limiting in methionine and contains some anti-nutrients such as trypsin inhibitor, hemagglutinin and anti-vitamins (Tacon, 1993). Davis and Stickney (1978) evaluated the interaction between dietary protein levels and the ratios of FM and SBM in practical diets for juvenile blue tilapia (*Oreochromis aureus*). At low-protein diets (15%), SBM-based diet could not meet the EAA requirements and thus inferior growth rate was obtained as compared to a FM-based diet. However, when the protein level was increased to 36%, a SBM-based diet was adequate and comparable to the FM-based diet. Conversely, Shiau et al. (1989) reported that, at 32% protein diet, replacing FM with 30% SBM significantly reduced growth and feed efficiency of hybrid tilapia (*O.niloticus* x *O.aureus*) due to poor amino acid balance and the presence of trypsin inhibitor. However, the authors reported that, at 24% protein diet, SBM could replace up to 67% FM. Viola and Arieli (1983) reported that SBM could replace up to 50% of FM in a 25% protein diet without any amino acid supplementation. Supplementation of 0.8% D,L-methionine to a test diet in which 75% of brown fish meal was replaced by SBM improved the growth of Nile tilapia to a level comparable to that obtained from FM diet (Tacon et al., 1983). Viola et al. (1988) found no negative effect of FM replacement on hybrid tilapia growth if 3% dicalcium phosphate was supplemented to the diet, while Wu et al. (2004) found that growth of hybrid tilapia (*O.niloticus* x *O.aureus*) offered diets devoid of FM was significantly lower ($P<0.05$) than those offered diets containing FM due to poor palatability. Supplementation of encapsulated methionine to non-FM diets did not improve growth (Wu et al., 2004) . A test diet in which 50% FM was replaced by SBM resulted in growth reduction of *O. mossambicus* (Jackson et al., 1982). This growth reduction was attributed to the deficiency of methionine and the presence of antinutritional factors such as trypsin inhibitors and hemagglutinin.

Contradictory results on the use of SBM as FM replacement in tilapia diets could be due to the quality and processing of the SBM, the strain or size of fish used, or environmental factors during study periods (El-Sayed, 1999). Wassef et al. (1988) reported that defattening of SBM helped to reduce the activity of protease inhibitors. Heat application to SBM also helped to rupture cellulose membrane and therefore made the cell content more available (Tacon and Jackson, 1985). Therefore, it is important that scientists report tilapia species, strain, size as well as SBM sources and environmental conditions in FM replacement studies.

Cottonseed meal (CSM) is an important protein source for terrestrial animals. However, its utilization in fish feeds is limited, mainly due to the presence of gossypol and low available lysine (Lim and Webster, 2006). Prepress solvent extracted CSM containing 300 mg gossypol/kg has been shown to be a good protein source for *O. mossambicus*. The fish offered a diet in which 100% FM was replaced by CSM grew at similar rate as the FM control diet (Jackson et al., 1982). Viola and Zohar (1984) found that low gossypol CSM could be incorporated at the same level as SBM in diets of marketable-size hybrid tilapia (*O.niloticus* x *O.aureus*) cultured in earthen ponds. A replacement of one-third of SBM by 19% CSM and lysine supplementation had no negative effect on growth of juvenile Nile tilapia. However, increasing CSM level to 38% or higher adversely affected weight gain and hematological parameters (Lim et al., 2002). Juvenile Nile tilapia fed a diet containing 19.4% cottonseed cake showed a reduction in growth as compared to those fed FM diet (Ofojekwu and Ejike, 1984). El-Sayed (1990) observed reductions of 24 and 35% in weight gain and feed efficiency, respectively, in juvenile Nile tilapia fed a diet containing 65% CSM as compared to those fed the FM-based diet. Varying results obtained from these studies may be due to the variation of free gossypol levels in different sources of CSM and the potential toxic effects of gossypol on a certain tilapia species. Although some fish species can tolerate relatively high dietary levels of CSM, its use is probably continue to be limited to about 10-15% of the diet (Li and Robinson, 2006).

Animal by-product meals such as meat and bone meal (MBM) have also been evaluated as a substitute for FM in practical diets for tilapia. Wu et al. (1999) found that the inclusion of 6% MBM as a total replacement of menhaden FM had no negative effect on growth of Nile tilapia. El-Sayed (1998) obtained a similar growth rate of Nile tilapia fed diet containing 40% of MBM as replacement of 30% FM. Tacon et al. (1983) found that hexane-extracted MBM supplemented with methionine could successfully replace up to 50% of FM protein in 45% protein diets fed to Nile tilapia fry. Davies et al. (1989) conducted a feeding trial on *O. mossambicus* fry and observed that optimum MBM/blood meal (BM) ratios could replace up to 75% FM. Furthermore, diets containing MBM or high MBM/BM ratio (3:1) were superior to FM even at 100% replacement level. Red tilapia and Nile tilapia have been reported to efficiently utilize MBM as a sole protein source in their diets without reduction in growth rate and feed efficiency (Mansour, 1998; El-Sayed, 1998).

Results of studies mentioned above indicated that it is possible to eliminate FM from tilapia diets with the utilization of SBM. If organic SBM such as organic expeller pressed SBM (Professional Proteins Ltd, Washington, USA) has reasonable price as compared to regular SBM, it may be more profitable to produce organic tilapia since they often have higher prices than non-organic ones. To date, the demand for organic products from aquaculture has been increasing due to the growing awareness concerning environmental pollution and the safety of aquatic products for human consumption, as well as the state of global fishery resources and long-term sustainability of current aquatic food production systems (Tacon and Brister, 2002). Although no statistical data are available concerning the global production of certified organic aquaculture products, it is estimated that total production in the year 2000 was only about 5,000 MT, primarily from European countries. This modest quantity represents about 0.01% of total global aquaculture production or about 0.25% of European aquaculture production. Based on current estimates of certified organic aquaculture production and an anticipated annual growth rate of 30% from 2001 to 2010, 20% from 2011 to 2020 and 10% from 2021 to 2030, it is estimated that production will increase 240-fold, from 5,000 MT to 1.2 million MT by 2030. Such a production of certified organic aquatic products would be equivalent to 0.6% of the total estimated aquaculture production in 2030 (Yussefi, 2004). Since tilapia is one of the most popular aquaculture species in the world, attempts to produce them organically would have a great contribution toward sustainable aquaculture development in the future.

In general, the development of practical diets using non-FM protein sources for tilapia has potentially reduced feed cost as well as allowed the development of organic products to satisfy the growing demand of this market. These shifts will also increase stability and availability of the feeds for the commercial culture of tilapia. Therefore, research related to limiting nutrients such as the total sulfur amino acid requirement and replacement value of cystine for methionine in both semi-purified and practical diets, and evaluation of alternative plant and animal protein sources to replace FM in practical diets, should be pursued to support sustainable development of global tilapia culture industry.

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CHAPTER II

EVALUATION OF ALTERNATIVE PROTEIN SOURCES TO REPLACE FISH MEAL IN PRACTICAL DIETS FOR JUVENILE TILAPIA (*Oreochromis* spp.)

Abstract

Two feeding experiments were conducted to evaluate if methionine is limiting in practical grow-out diets for tilapia (*Oreochromis* spp.). Four diets containing 32% protein and 5% lipid were designed to compare the use of diets high in dehulled solvent-extracted soybean meal (DSESM) and expeller pressed soybean meal (EPSM) as compared to a diet containing 6% fish meal (FM). Tilapia (4.78 ± 0.07 g, mean ± SD) were randomly stocked into twelve 600-L flow-through tanks at 20 fish per tank. After six weeks, there were no notable trends or statistically significant differences (P>0.05) in final mean weight, survival rate and feed conversion ratio (FCR) among the treatments. Since results of this study showed that DSESM could totally replace fish meal in practical diets for juvenile tilapia, a second batch of diets were formulated using typical levels of cottonseed meal (CSM), DSESM and meat and bone meal (MBM) to evaluate whether methionine could be limiting. Two basal diet formulations were tested either without or with methionine supplement (0.06g/100g diet). The first diet contained 15% CSM, 27% DSESM and 10% MBM and the second diet contained 15% CSM and 37% DSESM. These diets contained 28% protein and

5% lipid. Tilapia (3.90 ± 0.05 g) were randomly stocked into twelve 60-L glass aquaria of a recirculation system at 18 fish per aquarium for five weeks and then moved to the 600-L flow-through tanks for five more weeks. After ten weeks, there was no statistically significant differences ($P>0.05$) in final mean weight, survival rate and FCR among the four treatments. Results of the present study demonstrated that DSESM and EPSM could totally replace FM's inclusion rate in commercial diets for juvenile tilapia and methionine did not appear to be limiting in practical diets using typical levels of CSM, DSESM and MBM as primary protein sources.

Introduction

Tilapia are freshwater fish belonging to the family Cichlidae. They are native to Africa, but were introduced into many tropical, subtropical and temperate regions of the world during the second half of the 20th century (Pillay, 1990). Tilapia are known as "aquatic chicken" due to their fast growth, good quality flesh, disease resistance, adaptability to a wide range of environmental conditions, ability to grow and reproduce in captivity and feed on low trophic levels. Therefore, they have become an excellent choice for aquaculture, especially in tropical and subtropical environments (El-Sayed, 2006).

Due to the importance of this species, it is critical that feeds are both economically and environmentally sustainable. Protein is one of the most expensive components of aquaculture diets. Animal protein sources, especially fish meal, have relatively high cost, limited supply and variable quality. The development of commercial aquatic feeds has been traditionally based on fish meal as the main protein source due to its high protein content and balanced essential amino acid profile. Fish meal is also an excellent source of essential fatty acids, digestible energy, minerals and vitamins. Therefore, it is no surprise that fish meal is the most expensive protein source in animal and aquaculture feeds (Tacon, 1993). However, the limited supply of fish meal, coupled with its increased demand in feeds for livestock and poultry is likely to reduce the dependence on fish meal in aquatic feeds (El-Sayed, 1999). Furthermore, there are growing environmental concerns on the use of wild fish to produce fish meal. Hence there is interest in replacing fish meal with less expensive protein sources. Many studies have been conducted to evaluate the replacement of fish meal with the low-cost, locally available plant and animal protein sources in practical diets for tilapia (Novoa et al., 1997; El-Sayed, 1998; Fasakin et al., 1999; Abdelghany, 2003; El-Saidy and Gaber, 2003; Richter et al., 2003; El-Saidy and Gaber, 2004; Fasakin et al., 2005; Gaber, 2006; Borgeson, 2006). Replacement of fish meal in practical diets without reducing the performance would result in a more profitable production of tilapia. Therefore, this aspect should be studied further in order to widen the list of appropriate plant and animal protein sources to replace fish meal.

The development of practical diets using non-fish meal protein sources for tilapia has the potential to reduce feed cost as well as allow the development of organic products to satisfy the growing demand of this market. Generally, plant derived protein sources are deficient in one or more essential nutrients and most contain some anti-nutritional factors. The major deficiency in soybean meal as compared to fish meal is its content of essential amino acid (EAA), particularly a low methionine level. Quite often it is felt that in practical diet formulations for juvenile tilapia, the total sulfur amino acid level (TSAA, consists of methionine and cystine) is limiting. To date, the requirement for TSAA of juvenile tilapia has been determined by several investigators. However, the reports covered a wide range of values. For example, Santiago and Lovell (1988) reported that TSAA requirement in semi-purified diet of Nile tilapia (*Oreochromis niloticus*) fry was 0.9% of the diet (consisted of 0.75% methionine and 0.15% cystine) or 3.22% of dietary protein while Kasper et al. (2000) concluded that this requirement was only 0.5% of the diet or 1.56% of dietary protein for the same species. Such great variations in results by various investigators suggested that methionine might not be limiting in feeds that are devoid of FM and chances of producing cost-effective tilapia feeds without fish meal are possible.

Consequently the objectives of the present study were to evaluate the possibility of using DSESM and EPSM to totally replace fish meal in practical diets for juvenile tilapia, and to determine whether non-fish meal practical diets using CSM, DSESM and MBM (which are low in methionine) as primary protein sources result in diets that are methionine limited.

Materials and Methods

Experimental fish, *ingredients and diets*

The present study was conducted at the E. W. Shell Fisheries Center in Auburn, Alabama. Red tilapia (*O.* spp., Santa-Fe strain) and Nile tilapia (*O. niloticus*, Ivory Coast strain) spawned at the Center were used in experiments 1 and 2, respectively. Swim-up fry were collected from earthen ponds, stocked into 45-L aquaria of an indoor recirculation system and fed with the methyltestosterone-treated feed (Rangen Inc., Buhl, Idaho, USA) for four weeks, then with a commercial fry feed (AquaMax, St. Louis, Missouri, USA) until the beginning of each experiment. All test ingredients were bought from commercial sources. Experimental diets were made and stored at the Fish Nutrition Processing Lab, E. W. Shell Fisheries Center. Feed ingredients were ground, weighed and then homogenized in a Hobart mixer (Hobart Inc., Troy, Ohio, USA) for 20 minutes, then hot water were added to produce a mash appropriate for extruding. All diets were then extruded using a 3-mm die, dried at 35 C in a forced-air oven for 24 h and stored at -20 C until fed. Test diets were sent to New Jersey Feed Laboratory, Inc., Trenton, New Jersey, USA for analysis of methionine and cystine contents.

Experiment 1

The first experiment was designed to compare the use of DSESM and EPSM as primary protein sources in practical diets for tilapia as compared to a diet containing fish meal. Four diets were formulated to contain 32% protein and 5% lipid. Three of these diets contained no fish meal and the fourth, the control diet, contained 6% fish meal, the level commonly used in commercial feed for juvenile tilapia (Table 1). The first two diets contained DSESM as the sole protein source with and without the addition of 0.05% methionine (diet 1 and 2, Table 1). The third diet was formulated using EPSM and was supplemented with 0.05% methionine. Methionine level was equal to 0.58% in all diets except in the diet that did not contain either fish meal or methionine supplements. In this diet (diet 2), methionine level was 0.53% of the diet. Tilapia (4.78 ± 0.07 g) were randomly stocked into twelve circular polyurethane 600-L flow-through tanks at 20 fish per tank. Dissolved oxygen and temperature were measured once a day using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA) while pH, total ammonia-nitrogen (TAN) and nitrite-nitrogen (nitrite-N) levels were monitored twice per week. The pH was measured by an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA). Total ammonia-nitrogen and nitrite-N were measured using methods described by Solorzano, 1969 and Parsons et al., 1985, respectively. Each of the four diets was assigned to three randomly chosen tanks. Fish were offered experimental diets at a daily ration of 5-6% body weight (BW), divided into two equal feedings at 8-9 AM and 4-5 PM. They were weighed weekly to adjust rations. Six weeks after the start of the experiment all fish were harvested, counted and group weighed.

Table 1: Composition of four practical diets fed to tilapia in the first experiment.

Ingredient (g/100g)	Diet			
	1 (DSESM + Met)	2 (DSESM)	3 (EPSM + Met)	4 (FM)
Fish meal[a]	0.00	0.00	0.00	6.00
DSESM[b]	63.80	63.80	0.00	56.00
EPSM[c]	0.00	0.00	68.10	0.00
Organic soybean oil[d]	4.10	4.10	0.00	3.56
Wheat starch[e]	6.30	6.35	6.25	9.24
Whole wheat[e]	20.00	20.00	20.00	20.00
Trace mineral premix[f]	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free)[g]	2.00	2.00	2.00	2.00
Stay C (25% Vitamin C activity)[h]	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic[e]	3.15	3.15	3.00	2.60
L-Methionine[e]	0.05	0.00	0.05	0.00
Methionine (% diet)	0.58	0.53	0.58	0.58
Cystine (% diet)	0.48	0.48	0.43	0.46

[a] Special Select™, Omega Protein Inc., Hammond, Louisiana, USA

[b] Southern Sates Cooperative Inc., Richmond, Virginia, USA.

[c] Organic soybean meal, Professional Proteins Ltd, Washington, USA.

[d] Clarkson Grain Co., Inc., Cerro Gordo, Illinois, USA

[e] MP Biochemicals Inc., Solon, Ohio, USA.

[f] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.

[g] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; alpha-cellulose, 955.589.

[h] Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

Experiment 2

Since levels of methionine used in the first experiment did not appear to affect growth, a second experiment was conducted to evaluate whether methionine is limited in diets using CSM, DSESM and MBM as primary protein sources. Four diets were formulated to contain 28% protein and 5% lipid (Table 2). The first diet contained 15% CSM, 27% DSESM and 10% MBM. The second diet was the same as the first diet but was supplemented with 0.06% methionine. The other two diets contained 15% CSM, 37% DSESM and without and with 0.06% methionine supplementation. Eighteen tilapia (3.90 ± 0.05 g) were randomly stocked into each of the twelve 60-L aquaria of an indoor recirculation system for the first five weeks. In this system, water temperature was maintained around 28 C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using airstones in each aquarium and the sump connected to a regenerative blower. Photoperiod was set at 14 h light and 10 h dark. Diets were offered to fish in three randomly selected aquaria at 5-6% BW daily, divided into two equal feedings at 8-9 AM and 4-5 PM. Fish were weighed weekly and ration adjusted accordingly. Five weeks after the beginning of the experiment, fish from aquaria were relocated into 600-L flow-through tanks described in the first experiment and fed the same diets for five more weeks. Dissolved oxygen, temperature, pH, TAN and nitrite-N in both recirculation and flow-through tank systems were monitored using the same procedures as described in the first experiment. At the end of the 10th week from the start of the experiment, fish were harvested, counted and group weighed.

Table 2: Composition of four practical diets fed to tilapia in the second experiment.

Ingredient (g/100g)	Diet			
	1 (MBM)	2 (MBM + Met)	3 (No MBM)	4 (No MBM + Met)
Cottonseed meal[a]	15.00	15.00	15.00	15.00
DSESM[b]	27.00	27.00	37.00	37.00
Meat and bone meal[c]	10.00	10.00	0.00	0.00
Menhaden fish oil[d]	3.25	3.25	4.20	4.20
Wheat starch[e]	33.15	33.09	30.40	30.34
Whole wheat[e]	9.00	9.00	9.00	9.00
Trace mineral premix[f]	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free)[g]	1.00	1.00	1.00	1.00
Stay C (25% vitamin C activity)[h]	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic[e]	1.00	1.00	2.80	2.80
L-Methionine[e]	0.00	0.06	0.00	0.06
Methionine (% diet)	0.46	0.52	0.49	0.55
Cystine (% diet)	0.49	0.49	0.51	0.51

[a] Faithway Feed Co. Inc., Guntersville, Alabama, USA.
[b] Southern Sates Cooperative Inc., Richmond, Virginia, USA.
[c] Griffin Industries, Inc., Cold Spring, Kentucky, USA.
[d] Omega Protein Inc., Reedville, Virginia, USA.
[e] MP Biochemicals Inc., Solon, Ohio, USA.
[f] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.

[g] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; alpha-cellulose, 955.589.

[h] Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

Statistical analysis.

Statistical analyses were performed using SAS (version 9.1, SAS Institute, Cary, North Carolina). Data from both experiments were analyzed using one-way analysis of variance to determine if there were significant differences ($P \leq 0.05$) in growth and survival. Student-Newman-Keuls multiple comparison test (Steel & Torrie, 1980) were utilized to determine differences among treatment means.

Results

Experiment 1

The mean (± SD) of water quality variables of the first experiment were as follow: Dissolved oxygen, 6.74 ± 0.43 mg/L; water temperature, 27.0 ± 0.9 C; TAN, 0.157 ± 0.074 mg/L; nitrite-N, 0.033 ± 0.013 mg/L; pH, 7.9 ± 0.1. These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal, 1988, Watanabe et al., 1993, El-Shafai et al., 2004, Wangead et al., 1988). There were no significant differences in final mean weight, survival and FCR among four treatments of the first experiment (Table 3). Tilapia offered feeds with or without FM grew at similar rates. There were also no effects on final mean weight, survival and FCR due to substitution of DSESM with EPSM. Methionine supplementation to diet containing DSESM as the primary protein source (Diet 1) had no effects on final mean weight, survival and FCR (Table 3).

Table 3: Initial weight, final weight, survival and feed conversion ratio (FCR) of tilapia fed four different practical diets in the first experiment[1].

Diet	Initial Wt (g)	Final Wt (g)	Survival (%)	FCR[2]
1 (DSESM + Met)	4.77 ± 0.10	25.64 ± 8.43[a]	100.0 ± 0.0[a]	1.23 ± 0.03[a]
2 (DSESM)	4.83 ± 0.06	23.89 ± 0.36[a]	100.0 ± 0.0[a]	1.33 ± 0.01[a]
3 (EPSM + Met)	4.76 ± 0.04	25.75 ± 2.78[a]	100.0 ± 0.0[a]	1.22 ± 0.11[a]
4 (Fish meal)	4.77 ± 0.03	26.59 ± 2.29[a]	96.7 ± 4.7[a]	1.28 ± 0.16[a]
PSE[3]		1.30	1.67	0.07
P value		0.54	0.44	0.65

[1] Values reported are mean ± standard deviation of three replicates. Means in the same column with the same superscript are not significantly different (P>0.05).

[2]FCR = Dry weight of feed offered/wet weight gain.

[3]PSE = Pooled standard error.

Experiment 2

The mean (± SD) of water quality variables over the first five weeks of the second experiment were as follow: Dissolved oxygen, 6.83 ± 0.47 mg/L; water temperature, 28.5 ± 1.2 C; TAN, 0.125 ± 0.050 mg/L; nitrite-N, 0.094 ± 0.049 mg/L; pH, 8.2 ± 0.2. During the last five weeks, these values were: Dissolved oxygen, 6.65 ± 0.50 mg/L; water temperature, 27.2 ± 1.1 C; TAN, 0.256 ± 0.176 mg/L; nitrite, 0.042 ± 0.043 mg/L; pH, 7.8 ± 0.2. These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal, 1988, Watanabe et al., 1993, El-Shafai et al., 2004, Wangead et al., 1988).

At the termination of the second experiment, final mean weights of tilapia varied from 49.46 to 55.50 g. High survival rates were obtained in all treatments, from 98.1 to 100.0%. Feed conversion ratios were from 1.26 to 1.35. There were no significant differences in final mean weight, survival and FCR among treatments of the second experiment. Tilapia offered feeds with or without MBM grew at similar rates. There was also no effect of methionine supplementation on growth, survival and FCR (Table 4).

Discussion

Soybean meal (SBM) is the best plant protein source in terms of protein content and essential amino acid profile. However, it is potentially limiting in sulfur-containing amino acids (methionine and cystine) and contains some anti-nutrient substances such as trypsin inhibitor, hemagglutinin and anti-vitamins (Tacon, 1993). Researchers have evaluated SBM as replacements of FM in tilapia diets and obtained varying results. Davis and Stickney (1978) reported that the utilization of SBM as primary protein source in diets containing 15%

Table 4: Initial weight, final weight, survival and feed conversion ratio (FCR) of tilapia fed four different practical diets in the second experiment[1].

Diet	Initial Wt (g)	Final Wt (g)	Survival (%)	FCR[2]
1 (MBM)	3.88 ± 0.06	49.46 ± 3.18[a]	98.1 ± 2.6[a]	1.35 ± 0.03[a]
2 (MBM + Met)	3.89 ± 0.06	51.68 ± 2.84[a]	100.0 ± 0.0[a]	1.27 ± 0.06[a]
3 (No MBM)	3.92 ± 0.01	54.99 ± 2.38[a]	98.1 ± 2.6[a]	1.28 ± 0.06[a]
4 (No MBM + Met)	3.89 ± 0.03	55.50 ± 4.97[a]	98.1 ± 2.6[a]	1.26 ± 0.09[a]
PSE[3]		2.46	1.73	0.05
P value		0.33	0.80	0.65

[1] Values reported are mean ± standard deviation of three replicates. Means in the same column with the same superscript are not significantly different (P>0.05).

[2]FCR = Dry weight of feed offered/wet weight gain..

[3]PSE = Pooled standard error.

protein reduced growth of blue tilapia while SBM could totally replace FM if protein level was 36% because the essential amino acid profile at this protein level was above the requirement. Conversely, Shiau et al. (1989) reported that at a 32% protein diet, replacing FM with 30% SBM significantly reduced growth and feed efficiency of hybrid tilapia (*O.niloticus* x *O.aureus*) due to poor amino acid balance and the presence of trypsin inhibitor. However, the authors reported that at 24% protein diet, SBM could replace up to 67% FM. Viola et al. (1988) found no effect of FM replacement by SBM on hybrid tilapia growth if 3% dicalcium phosphate was supplemented to the diet. Wu et al. (2004) found that growth of hybrid tilapia (*O.niloticus* x *O.aureus*) offered diets devoid of FM was significantly lower ($P<0.05$) than those fed diets containing FM due to poor palatability. They also observed that supplementation of encapsulated methionine into non-FM diets did not improve growth.

Contradictory results of FM replacement in tilapia diets could be due to the quality and processing of the SBM, species, strain or size of fish used, or environmental factors during study periods (El-Sayed, 1999). Wassef et al. (1988) reported that defattening of SBM helped to reduce the activity of protease inhibitors. Heat application to SBM also helped to rupture cell membrane and therefore made the cell content more available (Tacon and Jackson, 1985). Therefore, it is important that scientists report tilapia species, strain and size used in FM replacement experiments as well as SBM sources and environmental conditions during the study.

The results of the first experiment confirmed that DSESM and EPSM could totally replace FM in practical diets for juvenile tilapia and that methionine supplementation to non-

FM diets had no positive effects on final mean weight, survival and FCR. This may be due to TSAA levels of non-FM diets already met or exceeded the requirement of juvenile tilapia. Total sulfur amino acid and methionine levels of a non-FM diet without methionine supplementation (diet 2) were 1.01% and 0.53% of the diet, or 3.16% and 1.66% of dietary protein, respectively. On a sulfur molar basis, dietary cystine could spare up to 50% of the TSAA requirement of Mozambique tilapia (*O. mossambicus*) (Jauncey and Ross, 1982). As red tilapia used in the first experiment are of the same genus as Mozambique tilapia, it is reasonable to assume that the replacement value of cystine for methionine was also around 50%. In this experiment, cystine levels of the test diets were almost the same as those of methionine, indicating that methionine:cystine ratio was about 1:1. The combined levels of methionine and cystine (TSAA) of all test diets were at or above 1.01% of the diet, a level that is higher than the requirement levels reported by Santiago and Lovell (1988) and Kasper et al. (2000). Since TSAA levels of all test diets already met or exceeded the requirement, supplementation of methionine had no positive effect on growth, survival and FCR.

The results of the first experiment demonstrated that methionine requirement of juvenile tilapia could be met by SBM-based diets without supplementation of crystalline methionine. No palatability problem was observed in fish fed non-FM diets during this experiment. Thus, it appears that tilapia could utilize all-SBM diets containing 32% protein without the reduction in final mean weight, survival and FCR. Furthermore, there were no negative effects of using EPSM, an organic protein source, rather than DSESM in SBM-based diets for juvenile tilapia. Therefore, it is possible to rear tilapia using organic EPSM as the primary protein source without growth reduction and requiring methionine supplementation. To date, the demand for organic products from aquaculture has been increasing due to the growing awareness concerning environmental pollution and the safety of aquatic products for human consumption, as well as the state of global fishery resources and long-term sustainability of current aquatic food production systems (Tacon and Brister, 2002). Aquatic products produced organically often have higher prices than non-organic ones and tilapia is not an exemption. If organic protein source such as EPSM has reasonable price as compared to DSESM, it may be more profitable to produce organic tilapia. Since tilapia is one of the most popular aquaculture species in the world, attempts to produce them organically would have a great contribution toward sustainable aquaculture development in the future.

Cottonseed meal is an important protein source for terrestrial animals. However, its utilization in fish feeds is limited, mainly due to the presence of gossypol and low available lysine (Lim and Webster, 2006). Viola and Zohar (1984) found that low gossypol CSM could be incorporated at the same level as SBM in diets of marketable-size hybrid tilapia (*O.niloticus* x *O.aureus*) cultured in earthen ponds. A replacement of one-third of SBM by 19% CSM and lysine supplementation had no negative effect on growth of juvenile Nile tilapia. However, increasing CSM level to 38% or higher adversely affected weight gain and hematological parameters (Lim et al., 2002). Generally, CSM has been used at relatively low levels in aquatic animal diets, partially because its safe level appears to differ for different fish species. Although some fish species can tolerate relatively high dietary levels of CSM, its use is probably continue to be limited to about 10-15% of the diet due to gossypol toxicity (Li and Robinson, 2006). In the second experiment, CSM was incorporated at a level of 15%

of the diet to minimize negative effect of gossypol on growth and health of tilapia.

Meat and bone meal, an animal by-product meal, is a good protein source and has also been evaluated as a substitute for FM in practical diets for tilapia. Wu et al. (1999) found that the inclusion of 6% MBM as a total replacement of menhaden FM had no negative effect on growth of Nile tilapia. El-Sayed (1998) obtained a similar growth rate when Nile tilapia were fed diet containing 40% of MBM as replacement of 30% FM. Tacon et al. (1983) found that hexane-extracted MBM supplemented with methionine could successfully replace up to 50% of FM protein in a 45% protein diet fed to Nile tilapia fry. Meat and bone meal was added to test diets of the second experiment at 10% of the diet. Both CSM and MBM were used in addition to DSESM to investigate whether methionine could be limiting.

Results of the second experiment showed that methionine was not limiting in practical diets utilizing CSM, MBM and DSESM as primary protein sources. Tilapia diets could also be formulated without animal protein source such as MBM with no reduction in final mean weight, survival and FCR. Fish fed diets containing 15% CSM and 37% DSESM had the same performances as fish offered diets containing 15% CSM, 10% MBM and 27% DSESM (Table 4). In this experiment, cystine levels in all test diets were about the same as those of methionine and the TSAA levels were at or above 0.95% of the diet or 3.39% of dietary protein. Since TSAA levels of these diets had met the requirement of juvenile Nile tilapia as reported by Santiago and Lovell (1988) and Kasper et al. (2000), methionine supplementation had no positive effects on final mean weight, survival and FCR. These results demonstrated that adequate methionine and TSAA levels in practical diets for juvenile

Nile tilapia could be achieved without methionine supplementation and it is feasible to formulate diets containing 15% CSM and 10% MBM, which are cheap protein sources, to reduce feed cost.

Conclusions

The results of the present study demonstrated that DSESM and EPSM could totally replace FM's inclusion rate in commercial diets for juvenile tilapia and methionine did not appear to be limiting in practical diets using typical levels of CSM, DSESM and MBM as primary protein sources.

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CHAPTER III

TOTAL SULFUR AMINO ACID REQUIREMENT AND REPLACEMENT VALUE OF CYSTINE FOR METHIONINE IN SEMI-PURIFIED DIETS OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*)

Abstract

Two feeding experiments were conducted to determine the total sulfur amino acid (TSAA, methionine and cystine) requirement and replacement value of cystine for methionine of juvenile Nile tilapia (*O. niloticus*). Semi-purified diets used in both experiments contained 351 kcal gross energy and 28 g protein per 100 g diet from casein, gelatin and crystalline amino acids. The basal diet of the first experiment contained 0.31% methionine and 0.04% cystine. L-methionine was added to the seven remaining diets at 0.1% increment to produce methionine levels ranging from 0.31% to 1.01% of the diet. Each diet was fed to four replicate groups of juvenile Nile tilapia (1.28 g mean weight) in a recirculation system for eight weeks. Broken-line regression analysis of weight gain data indicated that the TSAA requirement of juvenile Nile tilapia was 0.85% of the diet or 3.04% of dietary protein. In the second experiment, TSAA level was set at 95% of the requirement value determined in the first experiment. Seven diets were made with different ratios of L-methionine and L-cystine (20:80, 30:70, 40:60, 50:50, 60:40, 70:30 and 80:20, based on an

equimolar sulfur basis). Each diet was also fed to four replicate groups of juvenile Nile tilapia (4.14 g mean weight) in a recirculation system for eight weeks. Regression analysis of weight gain data using broken-line model indicated that cystine (on a molar sulfur basis) could replace up to 49% of methionine requirement in semi-purified diets for juvenile Nile tilapia.

Introduction

Tilapia is one of the most important farm-raised fish and has an increasing role in the international aquatic food trade. In 2004, world production of farmed tilapia exceeded 2,002,087 metric tonnes, trailing behind carps but exceeded that of the salmonids. The rapid improvements in domestication and wider consumption patterns may mean that tilapia may eventually overtake the carps to become the most important farmed fish (Fitzsimmons, 2006). There are about ten species of tilapia that have been used for aquaculture. Nile tilapia (*O. niloticus*) is, by far, the most important farmed tilapia species in the world. It represented more than 80% of total tilapia production during 1970-2002. Nile tilapia also ranked sixth in terms of global farmed fish production in 2002, after silver carp, grass carp, common carp, crucian carp and big head carp (El-Sayed, 2006).

In intensive culture systems, nutritionally complete feed has been used and comprised a major proportion of tilapia production cost. Hence, the development of cost-effective feeds is critical to economic success. As protein is the most costly component of complete feeds, the utilization of cheap and locally available plant and animal protein sources would reduce feed costs. Quite often, these ingredients are limited in total sulfur amino acid (TSAA), especially methionine. Thus, to maximize their utilization, the understanding of the TSAA requirement of juvenile Nile tilapia is very important. Generally, the TSAA requirement could be met by either methionine alone or the proper mixture of methionine and cystine (Shiau, 2002). Since cystine can only be synthesized from a methionine precursor, its presence in the diets reduces methionine requirement and finally, feed cost. Up to date, several studies have been conducted to determine the TSAA requirement of juvenile Nile tilapia. However, there are considerable differences in reported values. Santiago and Lovell (1988) determined that TSAA requirement of Nile tilapia fry was 0.9% of the diet (consisted of 0.75% methionine and 0.15% cystine) or 3.22% of dietary protein while Kasper et al. (2000) concluded that the requirement of these amino acids for the same species was only 0.5% of the diet or 1.56% of dietary protein. If we are to maximize the use of low-cost protein sources, we must better determine the TSAA requirement and replacement value of cystine for methionine for juvenile Nile tilapia. This serves as the objective of the present study.

Materials and Methods

Experimental fish

The present study was conducted at the E. W. Shell Fisheries Center in Auburn, Alabama. In the first experiment, Genetically Male Nile tilapia fry were obtained from Til-Tech Aquafarm, Robert, Louisiana, USA and raised in a recirculation system for four weeks using a commercial fry feed (AquaMax, St. Louis, Missouri, USA) before stocking. The second experiment utilized Nile tilapia fry (*O. niloticus*, Ivory Coast strain) spawned at E. W. Shell Fisheries Center, collected as swim-up fry from earthen ponds. The fry were stocked into 45-L aquaria of an indoor recirculation system and fed with methyltestosterone-treated feed (Rangen Inc., Buhl, Idaho, USA) for four weeks, then with a commercial fry feed (AquaMax, St. Louis, Missouri, USA) until the beginning of this experiment. All juvenile were acclimated to a basal diet for one week before starting each experiment.

Experimental diets

Semi-purified test diets of both experiments were formulated to contain 351 kcal gross energy and 28 g protein per 100 g diet. Gelatin, casein and crystalline amino acids served as protein sources. Choline chloride was added to all test diets to meet the requirement as determined by Kasper et al.(2000) so that methionine would not be used as a methyl donor. In order to enhance palatability, pH of all diets was adjusted to 7.0 with a solution of 6N sodium hydroxide (NaOH) (MP Biochemicals Inc., Solon, Ohio, USA) based on the method reported by Wilson et al. (1977). A 5-g portion of the diet was homogenized with 50 mL of distilled water and pH of the supernatant was determined. Sodium hydroxide solution was then added to this supernatant to establish a pH value of 7.0. Feed ingredients were weighed and homogenized in a Hobart mixer (Hobart Inc., Troy, Ohio, USA) for 20 minutes. After mixing, hot water and NaOH (6N) were added to produce a mash appropriate for extruding. All diets were then extruded using a 3-mm die, dried at 35 C in a forced-air oven for 24 h and stored at -20 C until fed.

Experiment 1

The first experiment was designed to determine the TSAA requirement of juvenile Nile tilapia. Eight diets were formulated to contain 11.36 g protein per 100 g diet originated from gelatin and casein and the rest came from crystalline amino acids. Protein level of these diets (28%) was slightly lower than the reported requirement for juvenile Nile tilapia (Wang et al., 1985; Abdelghani, 2000) for maximum utilization of protein for growth. The basal diet (Table 1) contained 0.31% methionine and 0.04% cystine. L-methionine was added to the

Table 1: Composition of the basal diet for determining TSAA requirement (Experiment 1).

Ingredient	(g/100 g)
Gelatin[a]	2.00
Casein (vitamin-free)[a]	11.00
Amino acid mixture[b]	15.40
L-Methionine[a]	0.00
Glutamic acid[a]	1.24
Corn starch[a]	46.96
Menhaden fish oil[c]	5.00
Trace mineral premix[d]	0.50
Vitamin premix (Choline and vitamin C free)[e]	1.00
Calcium phosphate, dibasic[a]	4.00
Choline chloride[a]	0.30
Stay C (25% vitamin C activity)[f]	0.10
Cellulose[a]	12.50

[a] MP Biochemicals Inc., Solon, Ohio, USA.

[b] Tables 2 and 3.

[c] Omega Protein Inc., Reedville, Virginia, USA.

[d] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.

[e] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; alpha-cellulose, 955.589.

[f] Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

seven remaining diets at 0.1% increment to provide methionine levels ranging from 0.31% to 1.01% of the diet. Glutamic acid was used as a replacement for methionine to keep protein level in all diets constant. Other essential amino acids were added to reach 105% of the requirements as determined by Santiago and Lovell (1988) to ensure that they are not limited. Amino acid profile of the basal diet is presented in Table 2. Non-essential amino acids were included to simulate the amino acid content of a 28% whole egg protein (Table 3).

Experiment 2

The second experiment was conducted to determine the replacement value of cystine for methionine for juvenile Nile tilapia. Total sulfur amino acid level of test diets was set at 95% of the requirement determined in the first experiment. Seven semi-purified diets (28% protein) were formulated to contain different methionine:cystine ratios (20:80, 30:70; 40:60; 50:50; 60:40, 70:30 and 80:20, based on an equimolar sulfur basis) (Table 4). These diets contained 9.17 g protein per 100 g diet originated from gelatin and casein and the rest came from crystalline amino acids. Glutamic acid was used as a replacement for methionine and/or cystine to keep protein level in all diets constant. The inclusions of essential and non-essential amino acids into each diet were followed the same procedure described in the first experiment (Tables 5 and 6).

Experimental procedures

Juvenile Nile tilapia averaging 1.28 g and 4.14 g were used for the first and second experiments, respectively. Fish were randomly stocked into 45-L aquaria at 15 fish per

Table 2: Amino acid composition (g/100 g) of the basal diet calculated based on reported values for determining TSAA requirement (Experiment 1).

Amino acid	Gelatin and casein	Crystalline amino acid[a]	Total
Methionine	0.31	-	0.31
Cystine	0.04	-	0.04
Arginine	0.51	0.73	1.24
Histidine	0.30	0.20	0.50
Isoleucine	0.58	0.34	0.92
Leucine	0.99	0.01	1.00
Lysine	0.83	0.67	1.50
Phenylalanine	0.53	0.57	1.10
Tyrosine	0.52	0.76	1.28
Threonine	0.46	0.65	1.11
Tryptophan	0.13	0.16	0.29
Valine	0.78	0.04	0.82

[a] MP Biochemicals Inc., Solon, Ohio, USA.

Table 3: Composition of non-essential amino acid premix (g/100 g) of the basal diet for determining TSAA requirement (Experiment 1).

Amino acid[a]	(g/100 g)
Aspartic acid	2.62
Serine	1.93
Proline	0.94
Glutamic acid	3.42
Glycine	0.91
Alanine	1.45

[a] MP Biochemicals Inc., Solon, Ohio, USA.

Table 4: Composition of the basal diet for determining the replacement value of cystine for methionine (Experiment 2).

Ingredient	(g/100 g)
Gelatin[a]	11.00
Casein (vitamin-free)[a]	3.00
Amino acid mixture[b]	15.14
L-Methionine[a]	0.00
Cystine[a]	0.50
Glutamic acid[a]	0.10
Corn starch[a]	46.96
Menhaden fish oil[c]	5.00
Trace mineral premix[d]	0.50
Vitamin premix (Choline and vitamin C free)[e]	1.00
Calcium phosphate, dibasic[a]	4.25
Choline chloride[a]	0.30
Stay C (25% vitamin C activity)[f]	0.10
Cellulose[a]	12.15

[a] MP Biochemicals Inc., Solon, Ohio, USA.

[b] Tables 5 and 6.

[c] Omega Protein Inc., Reedville, Virginia, USA.

[d] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.

[e] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; alpha-cellulose, 955.589.

[f] Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

Table 5: Amino acid composition (g/100 g) of the basal diet calculated based on reported values for determining the replacement value of cystine for methionine (Experiment 2).

Amino acid	Gelatin and casein	Crystalline amino acid[a]	Total
Methionine	0.16	-	0.16
Cystine	0.02	0.50	0.52
Arginine	0.87	0.37	1.24
Histidine	0.16	0.34	0.50
Isoleucine	0.30	0.62	0.92
Leucine	0.56	0.44	1.00
Lysine	0.60	0.90	1.50
Phenylalanine	0.32	0.78	1.10
Tyrosine	0.19	1.09	1.28
Threonine	0.31	0.80	1.11
Tryptophan	0.04	0.25	0.29
Valine	0.43	0.39	0.82

[a] MP Biochemicals Inc., Solon, Ohio, USA.

Table 6: Composition of non-essential amino acid premix (g/100 g) of the basal diet for determining the replacement value of cystine for methionine (Experiment 2).

Amino acid[a]	(g/100 g)
Aspartic acid	2.13
Serine	1.57
Proline	0.76
Glutamic acid	2.78
Glycine	0.74
Alanine	1.18

[a] MP Biochemicals Inc., Solon, Ohio, USA.

aquarium equipped with an indoor recirculating system. There were four replicates per treatment. In this system, water temperature was maintained at around 28 C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using airstones in each aquarium and the sump connected to a regenerative air blower. Dissolved oxygen and temperature were measured once a day using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA) while pH, TAN and nitrite-N were measured twice per week. pH was measured by an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA). Total ammonia-nitrogen and nitrite-N were measured using the methods described by Solorzano, 1969 and Parsons et al., 1985, respectively. Photoperiod was set at 14 h light and 10 h dark. Diets were offered to fish at 3-7% BW daily, according to fish size and divided into two equal feedings at 8-9 AM and 4-5 PM. Tilapia were weighed weekly and feed rations adjusted accordingly. Both experiments were conducted for eight weeks. At the end of each experiment, fish were counted and group weighed to determine weight gain and survival.

Statistical analysis.

Statistical analyses were performed using SAS (version 9.1, SAS Institute, Cary, North Carolina). Data from both experiments were analyzed using one-way analysis of variance to determine if significant differences ($P \leq 0.05$) exist in weight gain and survival. Student-Newman-Keuls multiple comparison test (Steel & Torrie, 1980) were utilized to determine differences among treatment means. Regression analysis of weight gain using broken-line model (Robbins, 1986) was used to determine the TSAA requirement and replacement level of cystine for methionine.

Results

Experiment 1

The mean (± SD) of water quality variables of the first experiment were as follow: Dissolved oxygen, 6.15 ± 0.31 mg/L; water temperature, 30.2 ± 1.2 C; TAN, 0.140 ± 0.149 mg/L; nitrite-N, 0.017 ± 0.008 mg/L; pH, 8.4 ± 0.1. These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal, 1988; Watanabe et al., 1993; El-Shafai et al., 2004; Wangead et al., 1988).

No significant differences were observed among the survival of juvenile Nile tilapia of all treatments (63.3% to 83.3%) (Table 7). Although there were no significant differences in survival rate among treatments, the values were lower at methionine levels of 0.31, 0.41 and 1.01% of the diet as compared to other levels. As dietary methionine levels increased from 0.31% to 0.81% of the diet (from diet 1 to diet 6), weight gain of juvenile Nile tilapia significantly increased. However, methionine at levels higher than 0.81% of the diet did not improve weight gain (diets 7 and 8) (Table 7). There were no significant differences in weight gain among the first five treatments. Weight gain of these treatments was significantly lower than that of the last three treatments. Broken-line regression analysis of weight gain indicated that the TSAA requirement was 0.85% of the diet or 3.04% of dietary protein.

Table 7: Weight gain and survival of juvenile Nile tilapia fed graded levels of methionine in the first experiment. Values are means of four replicates. Means with the same superscript in the same column are not significantly different (P>0.05).

TSAA level (% of diet)	Weight gain (%)[1]	Survival (%)
0.35	346.5[c]	63.3[a]
0.45	347.7[c]	66.7[a]
0.55	405.6[c]	83.3[a]
0.65	427.4[c]	75.0[a]
0.75	459.3[bc]	75.0[a]
0.85	551.3[ab]	76.7[a]
0.95	615.5[a]	70.0[a]
1.05	563.7[ab]	68.3[a]
PSE	28.7	5.19
P value	0.0001	0.21

[1]: Weight gain (%) = [(Final wt. - Initial wt.)/Initial wt.] x 100.

Experiment 2

The mean (± SD) of water quality variables of the second experiment were as follow: Dissolved oxygen, 6.34 ± 0.40 mg/L; water temperature, 27.8 ± 0.7 C; TAN, 0.198 ± 0.082 mg/L; nitrite-N, 0.085 ± 0.061 mg/L; pH, 7.4 ± 0.4. These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal, 1988; Watanabe et al., 1993; El-Shafai et al., 2004; Wangead et al., 1988).

There were no significant differences in survival rate among treatments. The survivals of all treatments were above 90%. Weight gain increased significantly as methionine:cystine ratio increased from 20:80 to 50:50. However, no further increase of weight gain was obtained at methionine:cystine ratio above 50:50 (Table 8). Regression analysis of weight gain using broken-line model indicated that cystine could replace up to 49% of methionine requirement in a semi-purified diet for juvenile Nile tilapia.

Discussion

Since all of juvenile tilapia were acclimated to basal diet for one week before the initiation of each experiment, no palatability problem was observed in either experiment. Water quality variables were at or near optimal ranges for maximum growth of juvenile tilapia. Methionine is an indispensable amino acid that plays an important role on growth performance, reproduction and physiological functions of animal species, including tilapia. Poor growth and survival are common signs of methionine deficiency. In certain species of fish, a deficiency of methionine leads to pathologies because it is not only incorporated into proteins but also used for the synthesis of other essential compounds (NRC, 1993). In

Table 8: Weight gain and survival of juvenile Nile tilapia fed different ratios of methionine and cystine in the second experiment. Values are means of four replicates. Means with the same superscript in the same column are not significantly different (P>0.05).

Met:Cys ratio (equimolar sulfur basis)	Weight gain (%)[1]	Survival (%)
20:80	194.5[d]	98.3[a]
30:70	289.9[c]	91.7[a]
40:60	369.9[b]	96.7[a]
50:50	451.2[a]	95.0[a]
60:40	458.6[a]	95.0[a]
70:30	469.0[a]	96.7[a]
80:20	451.7[a]	90.0[a]
PSE	14.8	3.1
P value	0.0001	0.50

[1]: Weight gain (%) = [(Final wt. - Initial wt.)/Initial wt.] x 100.

rainbow trout, cataract formation has been associated with methionine deficiency (Walton et al., 1982). Diets which contain crystalline methionine at levels above the requirement may lead to inferior growth rate and low survival. When methionine requirement has been met, increasing levels of free methionine supplementation to diets containing groundnut, soya, fish meal and crystalline amino acids as protein sources resulted in a significant reduction in specific growth rate of Mozambique tilapia *(Sarotherodon mossambicus*) (Jackson and Capper, 1982). Smaller tilapia have higher dietary protein requirement than larger ones due to higher specific growth rate. Dietary protein requirement of 0.8 g Nile tilapia (*O. niloticus*) was 40% as compared to 30% for 30 g fish (Siddiqui et al., 1988). Jauncey and Ross (1982) reported that protein requirements of *O. mossambicus* fry, 0.5-1.0 g and 6-30 g were 50, 40 and 30-35% of the diet, respectively. Since methionine requirement is often expressed as percentage of dietary protein, higher dietary protein requirement in small fish equals to higher dietary methionine requirement. Therefore, deficient or excessive levels of methionine in the diets may have more negative effects in terms of growth and survival on the small ones than the elders. The utilization of small tilapia (1.28 g mean weight) in the first experiment may contribute to the lower survivals of fish in diets containing 0.31, 0.41 and 1.01% methionine. Since bigger tilapia (4.14 g mean weight) were used in the second experiment, higher survival rates were obtained.

The TSAA requirement of 3.04% of dietary protein determined in the first experiment for juvenile Nile tilapia was similar to that of Mossambique tilapia (3.2%: Jackson and Capper, 1982), Indian major carp (3.23%: Murthy and Varghese, 1998), common carp (3.1%: Nose, 1979), Japanese flounder (3.1%: Alam et al., 2001), red drum

(3.03%: Moon and Gatlin, 1991) and rainbow trout (2.9%: Kim et al., 1992). This requirement was lower than that of chinook salmon (5.0%: Halver et al., 1959) and higher than that of channel catfish (2.34%: Harding et al., 1977). There are many factors affecting the TSAA requirement of fish such as size, water temperature, genetics, feeding rate, energy concentration and other diet factors, and method of data analysis (Lovell, 1989).

Methionine is an indispensable amino acid that is required by all animals including fish (Ketola, 1982). It has three major metabolic functions: as an essential amino acid for protein synthesis; as a sulfur source for synthesis of other sulfur-containing biochemicals; and as a methyl donor for methylation reactions (Mehler, 1986). Since cystine, a non-essential amino acid, can only be synthesized metabolically from methionine, the requirement for sulfur-containing amino acids in tilapia can be met by either methionine alone or the proper mixture of methionine and cystine. Therefore, the presence of cystine in the feed could spare a portion of methionine, leading to a reduction in its requirement. In the second experiment, weight gain of juvenile Nile tilapia increased significantly as methionine:cystine ratio increased from 20:80 to 50:50. However, no further increase of weight gain was observed as methionine: cystine ratio increased to 60:40 or higher. Broken-line regression analysis of weight gain indicated that cystine could replace up to 49% of methionine requirement. The replacement value of cystine for methionine determined for juvenile Nile tilapia in this study was similar to that of chinook salmon (50%: National Research Council, 1973). However, it was lower than that of channel catfish (60%: Harding et al., 1977) and higher than that of red drum (40%: Moon and Gatlin, 1991), rainbow trout (40%: Rumsey et al., 1983) and blue tilapia (44%: Liou, 1989).

Conclusions

The results of this study indicated that the TSAA requirement of juvenile Nile tilapia determined by semi-purified diets was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% of methionine requirement based on an equimolar sulfur basis.

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CHAPTER IV

METHIONINE REQUIREMENT IN PRACTICAL DIETS OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*)

Abstract

Two feeding experiments were conducted to determine and confirm methionine requirement in practical diets of juvenile Nile tilapia (*O. niloticus*). Test diets used in both experiments contained 414 kcal gross energy, 28 g protein and 5 g lipid per100 g diet. In the first experiment, seven diets were made using CSM, DSESM and gelatin as intact protein sources. Methionine was added to five of these diets at 0.03% or 0.06% increments to produce methionine levels ranging from 0.33% to 0.57% of the diet. Cystine level of all test diets was 0.45% of the diet. For essential amino acids other than methionine which have lower levels than those reported by Santiago and Lovell (1988), crystalline forms were added (except diet 1) to reach 110% requirement in order to ensure they are not limited. Each diet was fed to four replicate groups of male juvenile Nile tilapia (5.62 g mean weight) in a recirculation system for eight weeks. Broken-line regression analysis of weight gain indicated that methionine requirement was 0.49% of the diet or 1.75% of dietary protein. The second experiment was designed based on methionine requirement determined in the first experiment and also contained seven test diets. The first six diets contained CSM and

DSESM as protein sources. Methionine was added to five of these diets at an increasing rate of 0.06% to produce methionine levels ranging from 0.49% to 0.79% of the diet. Cystine level of these diets was 0.51% of the diet. In the last diet (Diet 7), a portion of DSESM was replaced by gelatin to reduce methionine level to 0.33% of the diet in order to test whether methionine is limited. Cystine level of this diet was 0.45% of the diet. Each diet was also fed to four replicate groups of male juvenile Nile tilapia (2.32 g mean weight) in a recirculation system for nine weeks. At the termination of the second experiment, there were no significant differences in terms of weight gain, survival and feed efficiency ratio (FER) among the first six diets. However, weight gain and FER of fish fed these diets were significantly better than those fed diet 7, confirming the methionine requirement value as has been determined in the first experiment.

Introduction

The main constraint in formulating nutritionally balanced diets for tilapia cultured in intensive and semi-intensive systems are the paucity of nutritional knowledge (Jackson and Capper, 1982). Traditionally, fish meal has been used as the main protein source in aquatic feed due to its high protein content and balanced EAA profile. However, the production of fish meal based on captured fisheries is at or beyond sustainable limits. The limited supply coupled with an increasing demand from the animal feed industry result in a high price of this ingredient. Given the escalating cost of fish meal, it is critical that all animal production systems reduce their reliance on fish meal. This is particularly true in case of feeds for aquatic species as they often contain high levels of fish meal (El-Sayed, 1999). Therefore, many studies have been conducted to totally or partially replace fish meal with cheap and locally available protein sources. The inclusions of non-fish meal protein sources into tilapia feeds have allowed the production of cost-effective feeds. This makes tilapia culture more competitive in the global market.

To formulate least-cost feeds, nutrient requirements of tilapia must be known, especially those that are limiting in low-cost feed ingredients. Quite often, methionine is one of the most limiting essential amino acids in non-fish meal protein sources commonly used in tilapia feeds. Thus, to maximize the utilization of cheap plant and animal protein sources, especially those that have low methionine levels, the determination of methionine requirement in semi-purified diets and its application to practical diets are important for the development of least-cost diet for tilapia. It is also critical to investigate the effects of free methionine supplementation to practical diets which are deficient in methionine. Generally, feeds produced by supplementation of free methionine to methionine-deficient protein sources are often cheaper than those formulated from ingredients with high levels of methionine such as fish meal. If Nile tilapia could utilize free methionine efficiently, there would be a great potential to produce cost-effective feeds using non-fish meal protein sources. Previous study using semi-purified diets showed that the TSAA requirement of juvenile Nile tilapia was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% methionine requirement based on an equimolar sulfur basis. This means methionine requirement of juvenile Nile tilapia fed semi-purified diets was 0.43% of the diet or 1.54% of dietary protein in the presence of cystine at 0.42% of the diet or 1.50% of dietary protein.

Since semi-purified test diets utilized purified protein sources (gelatin and casein) in which the nutrients are highly digestible, the TSAA requirement value determined using these diets is often lower than that using practical diets. It is also critical to determine the TSAA requirement in practical diets before the application of this value to formulate commercial tilapia feeds. Therefore, the objectives of this study were to determine and confirm methionine requirement in practical diets for juvenile Nile tilapia based on the value obtained from semi-purified diets.

Materials and Methods

Experimental fish and diet preparation

Two feeding experiments were conducted at the E. W. Shell Fisheries Center in Auburn, Alabama. Both experiments utilized Nile tilapia fry (*O. niloticus*, Ivory Coast strain) spawned at this Center, collected as swim-up fry from earthen ponds. Fry were stocked into 45-L aquaria of an indoor recirculation system and fed with methyltestosterone-treated feed (Rangen Inc., Buhl, Idaho, USA) for four weeks, then with a commercial fry feed (AquaMax, St. Louis, Missouri, USA) until the beginning of each experiment. Experimental diets were made and stored at the Fish Nutrition Processing Lab, E. W. Shell Fisheries Center. Feed ingredients were ground, weighed and then homogenized in a Hobart mixer (Hobart Inc., Troy, Ohio, USA) for 20 minutes. After mixing, hot water was added to produce a mash appropriate for extruding. All diets were then extruded using a 3-mm die, dried at 35 C in a forced-air oven for 24 h and stored at -20 C until fed. Diet 1 (first experiment) and diet 7 (second experiment) were sent to New Jersey Feed Laboratory, Inc., Trenton, New Jersey, USA for amino acid profile (Table 1) and methionine and cystine analyses, respectively.

Experiment 1

The first experiment was designed to determine the methionine requirement in practical diets of juvenile Nile tilapia. Seven test diets were formulated using CSM, DSESM and gelatin as intact protein sources. Methionine was added to diets 3-7 at 0.03% or 0.06% increments to provide methionine levels ranging from 0.33% to 0.57% of the diet. For other dietary essential amino acids which have lower levels than those reported by Santiago and Lovell (1988), crystalline forms were added (except diet 1) to reach 110% requirement to ensure they are not limited (Table 2). Each diet was fed to four replicate groups of juvenile Nile tilapia (5.62 g mean weight) in a recirculation system for eight weeks.

Table 1: Amino acid profile of the basal diet in the first experiment.

Amino acid	Percent of sample
Methionine	0.33
Cystine	0.45
Lysine	1.31
Phenylalanine	0.99
Leucine	1.52
Isoleucine	0.81
Threonine	0.82
Valine	0.86
Histidine	0.47
Arginine	2.22
Glycine	3.49
Aspartic acid	2.90
Serine	1.30
Glutamic acid	4.99
Proline	1.64
Hydroxyproline	0.96
Alanine	1.70
Tyrosine	0.49

Table 2: Composition of seven practical diets fed to tilapia in the first experiment.

Ingredient (g/100g)	Diet						
	1	2	3	4	5	6	7
Cottonseed meal[a]	15.00	15.00	15.00	15.00	15.00	15.00	15.00
DSESM[b]	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Gelatin[c]	10.00	9.20	9.20	9.20	9.20	9.20	9.20
Menhaden fish oil[d]	3.25	3.25	3.25	3.25	3.25	3.25	3.25
Wheat starch[c]	44.25	44.23	44.20	44.17	44.11	44.05	43.99
Trace mineral premix[e]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free)[f]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay C (25% vit. C activity)[g]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic[c]	2.90	2.90	2.90	2.90	2.90	2.90	2.90
L-Histidine[c]	0.000	0.032	0.032	0.032	0.032	0.032	0.032
L-Isoleucine[c]	0.000	0.117	0.117	0.117	0.117	0.117	0.117
L-Lysine[c]	0.000	0.185	0.185	0.185	0.185	0.185	0.185
L-Phenylalanine[c]	0.000	0.058	0.058	0.058	0.058	0.058	0.058
L-Threonine[c]	0.000	0.360	0.360	0.360	0.360	0.360	0.360
L-Tryptophan[c]	0.000	0.070	0.070	0.070	0.070	0.070	0.070
L-Methionine[c]	0.00	0.00	0.03	0.06	0.12	0.18	0.24
Methionine (% diet)	0.33	0.33	0.36	0.39	0.45	0.51	0.57
Cystine (% diet)	0.45	0.45	0.45	0.45	0.45	0.45	0.45

[a] Faithway Feed Co. Inc., Guntersville, Alabama, USA.
[b] Southern Sates Cooperative Inc., Richmond, Virginia, USA.
[c] MP Biochemicals Inc., Solon, Ohio, USA.
[d] Omega Protein Inc., Reedville, Virginia, USA.
[e] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.
[f] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; alpha-cellulose, 955.589.
[g] Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

Experiment 2

The second experiment was designed to confirm methionine requirement in practical diets for juvenile Nile tilapia. This experiment also consisted of seven test diets. The first six diets used CSM and DSESM as protein sources. Methionine was added to five of these diets at 0.06% increment to produce methionine levels ranging from 0.49% to 0.79% of the diet. In the last diet (diet 7), a portion of DSESM was replaced by gelatin to reduce methionine level to 0.33% of the diet in order to test whether methionine is limited (Table 3). Each diet was also fed to four replicate groups of juvenile Nile tilapia (2.32 g mean weight) in a recirculation system for nine weeks.

Experimental procedures

Juvenile Nile tilapia were randomly stocked into 45-L aquaria of an 2,500-L indoor recirculation system at 15 fish (first experiment) and 12 fish (second experiment) per aquarium. There were four replicates per treatment. In this system, water temperature was maintained at around 28 C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using airstones in each aquarium and the sump connected to a regenerative air blower. Dissolved oxygen and water temperature were measured once a day using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA) while pH, TAN and nitrite-N were measured twice per week. pH was measured by an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA). Total ammonia-nitrogen and nitrite-N were measured using the methods described by Solorzano, 1969 and Parsons et al., 1985, respectively.

Table 3: Composition of seven practical diets fed to tilapia in the second experiment.

Ingredient (g/100g)	Diet						
	1	2	3	4	5	6	7
Cottonseed meal[a]	15.0	15.0	15.0	15.0	15.0	15.0	15.0
DSESM[b]	37.0	37.0	37.0	37.0	37.0	37.0	21.0
Gelatin[c]	0.00	0.00	0.00	0.00	0.00	0.00	10.0
Menhaden fish oil[d]	3.25	3.25	3.25	3.25	3.25	3.25	3.25
Wheat starch[c]	31.3	31.2	31.2	31.1	31.1	31.0	37.3
Whole wheat[c]	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Trace mineral premix[e]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free)[f]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay C (25% vitamin C activity)[g]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic[c]	2.80	2.80	2.80	2.80	2.80	2.80	2.80
L-Methionine[c]	0.00	0.06	0.12	0.18	0.24	0.30	0.00
Methionine (% diet)	0.49	0.55	0.61	0.67	0.73	0.79	0.33
Cystine (% diet)	0.51	0.51	0.51	0.51	0.51	0.51	0.45

[a] Faithway Feed Co. Inc., Guntersville, Alabama, USA.
[b] Southern Sates Cooperative Inc., Richmond, Virginia, USA.
[c] MP Biochemicals Inc., Solon, Ohio, USA.
[d] Omega Protein Inc., Reedville, Virginia, USA.
[e] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.
[f] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; alpha-cellulose, 955.589.
[g] Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

Photoperiod was set at 14 h light and 10 h dark. Diets were offered to fish at 4.5-6.0% BW daily, according to fish size and divided into two equal feedings at 8-9 AM and 4-5 PM. Fish were weighed weekly and feed rations adjusted accordingly. The first and second experiments were conducted for eight and nine weeks, respectively. At the end of each experiment, fish were counted and group weighed to determine weight gain, survival and FER.

Statistical analysis.

Statistical analyses were performed using SAS (version 9.1, SAS Institute, Cary, North Carolina). Data from both experiments were analyzed using one-way analysis of variance to determine if there were significant differences ($P \leq 0.05$) in weight gain, survival and FER. Student-Newman-Keuls multiple comparison test (Steel & Torrie, 1980) were utilized to determine differences among treatment means. Regression analysis of weight gain using broken-line model (Robbins, 1986) was used to determine methionine requirement in practical diets for juvenile Nile tilapia.

Results

Experiment 1

The mean (± SD) of water quality variables during the first experiment were as follow: Dissolved oxygen, 6.56 ± 0.27 mg/L; water temperature, 28.3 ± 0.4 C; TAN, 0.070 ± 0.062 mg/L; nitrite-N, 0.041 ± 0.028 mg/L; pH, 8.0 ± 0.2. These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal, 1988; Watanabe et al., 1993; El-Shafai et al., 2004; Wangead et al., 1988).

There were no significant differences in survival and feed efficiency ratio (FER) among treatments (Table 4). Juvenile tilapia offered diets with different methionine levels had the same survival and FER. The addition of crystalline essential amino acids (except methionine) to diet 2 had no positive effects on weight gain, survival and FER as compared to those of non-supplemented diet (Diet 1), indicating that they were not limited in test diets using CSM, DSESM and gelatin as intact protein sources. However, there were significant differences in terms of weight gain among treatments. As dietary methionine levels increased from 0.33% to 0.51% (Diet 1 to diet 6), weight gain of juvenile Nile tilapia increased significantly. However, higher dietary methionine level (0.57%) did not further increase weight gain (Diet 7). Weight gain became plateau at the last two diets (Diet 6 and 7). This means methionine levels of these two diets have met the requirement of juvenile Nile tilapia (Table 4). Broken-line regression analysis of weight gain indicated that methionine requirement of juvenile Nile tilapia was 0.49% of the diet or 1.75% of dietary protein.

Experiment 2

The means (± SD) of water quality variables during the second experiment were as follow: Dissolved oxygen, 6.56 ± 0.38 mg/L; water temperature, 28.5 ± 0.9 C; TAN, 0.061 ± 0.055 mg/L; nitrite-N, 0.014 ± 0.009 mg/L; pH, 8.1 ± 0.1. These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal, 1988; Watanabe et al., 1993; El-Shafai et al., 2004; Wangead et al., 1988).

Table 4: Weight gain, survival and feed efficiency ratio(FER) of juvenile Nile tilapia fed different test diets in the first experiment. Values are means of four replicates. Means with the same superscript in the same column are not significantly different (P>0.05).

Diet	Weight gain (%)[1]	Survival (%)	FER (%)[2]
1	474^{d}	98.3^{a}	64.1^{a}
2	488^{cd}	93.3^{a}	60.4^{a}
3	516^{bcd}	100.0^{a}	68.3^{a}
4	593^{abc}	85.0^{a}	63.0^{a}
5	626^{ab}	91.7^{a}	70.8^{a}
6	685^{a}	91.7^{a}	72.8^{a}
7	697^{a}	93.3^{a}	72.8^{a}
PSE	25.2	5.7	4.6
P value	<0.0001	0.62	0.34

[1]: Weight gain (%) = [(Final wt. - Initial wt.)/Initial wt.] x 100.

[2]: Feed efficiency ratio (%) = (Wet weight gain/dry weight feed offered) x 100.

There were no significant differences in survival rate among treatments. The survival in all treatments varied from 85 to 100%. However, there were significant differences in terms of weight gain and FER between the first six treatments and treatment 7 (Table 5). Fish offered diets 1 to 6 grew at about the same rate but faster and utilized feeds more efficiently than those offered diet 7. Weight gain and FER of juvenile Nile tilapia of treatments 1 to 6 varied from 1,126 - 1,264% and 79.4 - 85.0%, respectively, as compared to 804% and 63.6%, respectively for the group fed diet 7. Weight gain and FER of tilapia in the first six treatments were not significantly different. These data indicated that methionine level of diet 7 was deficient, but those in diets 1 to 6 were sufficient to meet the requirement as determined in the first experiment. When methionine requirement has been met, supplementation of crystalline methionine to diets 2 to 6 had no positive effect on weight gain, survival and FER.

Discussion

Weight gain, feed efficiency ratio, nitrogen retention, serum free amino acid level and amino acid oxidation are response criteria used to quantify amino acid requirements. Animals need a continuous supply of diets balanced in amino acids, especially the essential ones, for normal growth and development. When a certain amino acid in a diet is deficient, weight gain is reduced as its requirement has not been met. Weight gain appears to be a criterium most commonly used to determine EAA requirements of fish (Baker, 1977; Wilson, 1985; D' Mello, 1978). In this study, weight gain was also used as response criterium to determine methionine requirement in practical diets of juvenile Nile tilapia.

Table 5: Weight gain, survival and feed efficiency ratio (FER) of juvenile Nile tilapia fed different test diets in the second experiment. Values are means of four replicates. Means with the same superscript in the same column are not significantly different (P>0.05).

Diet	Weight gain (%)[1]	Survival (%)	FER (%)[2]
1	1,126[a]	95.9[a]	79.4[a]
2	1,130[a]	95.9[a]	81.2[a]
3	1,170[a]	89.6[a]	79.8[a]
4	1,208[a]	95.9[a]	83.2[a]
5	1,160[a]	95.9[a]	82.0[a]
6	1,264[a]	95.9[a]	85.0[a]
7	804[b]	91.7[a]	63.6[b]
PSE	<0.0001	0.73	<0.0001
P value	50.5	3.4	1.6

[1]: Weight gain (%) = [(Final wt. - Initial wt.)/Initial wt.] x 100.

[2]: Feed efficiency ratio (%) = (Wet weight gain/dry weight feed offered) x 100.

Cottonseed meal is a plant protein source that is relatively high in protein and generally less expensive on a per unit of protein basis than soybean meal. However, its use in aquatic feeds is limited due to the potential toxic effects of free gossypol and relatively low levels of lysine and methionine. Cottonseed meal has been used at relatively low levels in aquatic animal diets, partially because its safe level appears to differ for different fish species. Although some fish species can tolerate relatively high dietary levels of CSM, its use is probably continue to be limited to about 10-15% of the diet (Li and Robinson, 2006). A replacement of one-third of SBM by 19% CSM and lysine supplementation had no negative effect on growth of juvenile Nile tilapia. However, increasing CSM level to 38% or higher adversely affected weight gain and hematological parameters (Lim et al., 2002). Cottonseed meal was used in the present study in order to reduce methionine content of the basal diet and crystalline methionine was added to the remaining diets to create a dose-response experiment. However, its inclusion rate was limited to 15% of the diet to minimize negative effect of gossypol on growth and health of experimental fish.

The utilization of crystalline amino acids in practical diets of fish depends on the species ability to absorb and use such amino acids for protein synthesis as well as for other physiological functions. In tilapia, varying results have been obtained with crystalline amino acid supplementation. Weight gain of *O. niloticus* was improved when a 35% protein diet formulated from casein was supplemented with either arginine and lysine or tryptophan and methionine (Teshima et al., 1986). Growth and feed efficiency were not improved when either methionine and lysine or threonine were added to 24% protein diets containing soybean meal as the main protein source fed to hybrid tilapia (*O. niloticus* x *O. aureus*)

(Liou et al., 1986). Williams and Robinson (1988) found that peanut meal diets supplemented with lysine had no positive effect on growth of blue tilapia. The supplementation of crystalline amino acids to practical diets containing white fish meal, heat-treated solvent-extracted soybean meal and expeller groundnut cake as intact protein sources improved growth of Mozambique tilapia (*Sarotherodon mossambicus*) (Jackson and Capper, 1982). Tacon et al. (1983) reported that growth of Nile tilapia was improved to a level comparable to that obtained from a fish meal-based diet when 0.8% D,L-methionine was supplemented to a diet in which 75% of brown fish meal was replaced by soybean meal. In the first experiment, weight gain of juvenile Nile tilapia increased significantly as methionine levels increased through the supplementation of crystalline methionine, indicating that juvenile Nile tilapia could utilize crystalline methionine efficiently when methionine level in practical diets is deficient. Studies that showed no positive effect of crystalline amino acid supplementation to practical diets for tilapia (Liou et al., 1986; Williams and Robinson, 1988) may be because their levels in the feeds are sufficient to meet the requirements. Results of the second experiment supported this interpretation. Since methionine level in diet 1 of the second experiment has met the requirement as determined in the first experiment, supplementation of methionine to diets 2 to 6 had no positive effect on weight gain and FER. Our previous study (Chapter 2) also demonstrated that supplementation of methionine to practical diets containing DSESM and EPSM as protein sources, or a combination of DSESM, CSM and MBM, did not improve growth, survival and FCR of juvenile tilapia because methionine levels in non-supplemented diets have met the requirement. The test diets used in either experiment contained cystine at levels similar to those of methionine.

Since cystine could replace up to 49% of methionine requirement as determined in semi-purified diets (Chapter 3), the conversion of methionine to cystine was assumed not to occur.

When methionine requirement has been met, the increasing levels of free methionine supplementation to diets using groundnut, soya, fish meal and crystalline amino acids as protein sources resulted in a significant reduction in specific growth rate of Mozambique tilapia *(Sarotherodon mossambicus*) (Jackson and Capper, 1982). However, the results of the second experiment showed no adverse effects of excessive levels of methionine on weight gain, survival and FER of juvenile Nile tilapia (*O. niloticus*). The difference in the response of tilapia to excessive levels of methionine may be due to tilapia species used in feeding experiments. The results of the present experiments demonstrated that juvenile Nile tilapia could efficiently utilize crystalline methionine to meet dietary requirement and no negative effects in terms of weight gain, survival or FER were found in diets that had methionine levels above the requirement.

The previous study utilized semi-purified diets determined that the TSAA requirement of juvenile Nile tilapia was 0.85% of the diet and cystine could replace up to 49% of methionine requirement (Chapter 3). This means methionine requirement of juvenile Nile tilapia fed semi-purified diets was 0.43% of the diet or 1.54% of dietary protein. Since these diets utilized purified protein sources (gelatin and casein) in which the nutrients are highly digestible, the methionine requirement value determined is often lower than that of practical diets. The methionine requirement of 0.49% of the diet or 1.75% of dietary protein determined in this study was slightly higher than that obtained from semi-purified diets.

Conclusions

It is concluded from this study that methionine requirement of juvenile Nile tilapia fed practical diets using CSM, DSESM and gelatin as protein sources was 0.49% of the diet or 1.75% of dietary protein at cystine level of 0.45% of the diet. Supplementation of essential amino acids other than methionine to diets using CSM, DSESM and gelatin as protein sources did not show positive effects on weight gain, survival and FER of experimental fish, indicating that these amino acid contents were not limited in the test diets. Furthermore, the supplementation of methionine to test diets only improved growth of juvenile Nile tilapia when its level in the diets was lower than the requirement level.

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SUMMARY AND CONCLUSIONS

Tilapia is fast becoming one of the most important farmed fish species. Currently, annual aquaculture production of tilapia exceeds two million metric tonnes, with only carps culture surpassing this value. It is projected that tilapia aquaculture production in the year 2010 will be over three million metric tonnes. As the demand increases, intensive farming systems have become dominant. Therefore, nutritionally complete feeds are being used in ever increasing quantities. Since protein is one of the most expensive components of aquatic feeds, its utilization must be optimized and the cost minimized. Quite often, methionine is one of the most limiting amino acids in non-fish meal protein sources used to formulate commercial tilapia feeds. In order to maximize the utilization of these protein sources, methionine requirement must be precisely determined. The studies conducted through this dissertation tried to evaluate the possibility of using alternative plant and animal protein sources to replace fish meal in practical diets, as well as determine the TSAA requirement and replacement value of cystine for methionine in semi-purified diets and apply these data to practical diets for juvenile Nile tilapia.

Feed mill manufacturers and farmers would like to reduce costs and utilize inexpensive and locally available plant and animal protein sources but they are hesitant to totally remove fish meal due to possible nutrient deficiencies. Hence, we first evaluated practical applications of various protein sources such as dehulled solvent-extracted soybean meal (DSESM), expeller pressed soybean meal (EPSM), cottonseed meal (CSM) and meat and bone meal (MBM) in practical diets for tilapia (*Oreochromis* spp.). Results of these experiments indicated that DSESM and EPSM could totally replace FM's inclusion rate in commercial diets for juvenile tilapia and methionine did not appear to be limiting in practical diets using CSM, DSESM and MBM as primary protein sources. Although, methionine did not appear to be limiting in the mentioned practical diets, the precise determinations of TSAA requirement and replacement value of cystine for methionine of juvenile Nile tilapia are critical to continued improvements in formulating cost-effective feeds. There are several reports on the methionine requirement; however, there is considerable variation in the values making clear determination difficult. To refine the published values, we determined the TSAA requirement and replacement value of cystine for methionine using semi-purified diets. Broken-line regression analysis of weight gain indicated that TSAA requirement of juvenile Nile tilapia was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% of methionine requirement based on an equimolar sulfur basis.

The determinations of TSAA requirement and replacement value of cystine for methionine using semi-purified diets is critical but it is equally important to apply these values to practical diet formulations. Hence, two experiments were conducted to determine and confirm methionine requirement in practical diets of juvenile Nile tilapia. In the first experiment, CSM, DSESM and gelatin were utilized as intact protein sources and graded levels of methionine were added to five of these diets, producing methionine levels ranging from 0.33% to 0.57% of the diet. Broken-line regression analysis of weight gain revealed that methionine requirement of juvenile Nile tilapia was 0.49% of the diet or 1.75% of dietary protein, a value slightly higher than that obtained from semi-purified diets. The second experiment was designed to confirm methionine requirement in practical diets for the same species. The first six diets used CSM and DSESM as protein sources and methionine were added to the basal diet at 0.06% increment to produce methionine levels ranging from 0.49 to 0.79% of the diet. In the last diet (Diet 7), a portion of DSESM was replaced by gelatin to reduce methionine level to test whether methionine is limited. There were no significant differences in weight gain, survival and feed efficiency ratio (FER) of tilapia fed the first six diets. However, weight gain and FER of fish fed these diets were significantly better than those fed diet 7, confirming the methionine requirement determined in the first experiment.

Based on the results of these studies, it is concluded that DSESM and EPSM could totally replace FM's inclusion rate in commercial diets for juvenile tilapia and methionine did not appear to be limiting in practical diets using CSM, DSESM and MBM as primary protein sources. The TSAA requirement of juvenile Nile tilapia in semi-purified diets was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% of methionine requirement based on an equimolar sulfur basis. Moreover, methionine requirement of juvenile Nile tilapia in practical diets using CSM, DSESM and gelatin as protein sources was 0.49% of the diet or 1.75% of dietary protein. Methionine appeared to be limiting when a portion of DSESM was replaced by gelatin. The supplementation of methionine to the basal diet using CSM and DSESM as protein sources did not have positive effects on weight gain, survival and FER of juvenile Nile tilapia because its level has met the requirement.

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