

## Evaluation of Alternative Protein Sources to Replace Fish Meal in Practical Diets for Juvenile Tilapia, *Oreochromis* spp

TRI N. NGUYEN<sup>1</sup> AND D. ALLEN DAVIS<sup>2</sup>

*Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama 36849 USA*

I. PATRICK SAOUD

*Department of Biology, American University of Beirut, Bliss Street, Beirut Lebanon*

### 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) compared with a diet containing 6% fish meal (FM). Tilapia ( $4.78 \pm 0.07$  g, mean  $\pm$  SD) were randomly stocked into twelve 600-L flow-through tanks at 20 fish per tank. After 6 wk, 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. Because results of this study indicated that DSESM could totally replace FM in practical diets for juvenile tilapia, a second batch of diets were formulated using other protein sources. Typical levels of cottonseed meal (CSM), DSESM, and meat and bone meal (MBM) were used to evaluate whether methionine could be limiting. Two basal diet formulations were tested either without or with methionine supplement (0.06/100 g 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 ( $3.90 \pm 0.05$  g) were randomly stocked into twelve 60-L glass aquaria of a recirculation system at 18 fish per aquarium for 5 wk and then moved to the 600-L flow-through tanks for five more weeks. After 10 wk, there were no statistically significant differences ( $P > 0.05$ ) in final mean weight, survival rate, and FCR among the four treatments. Results of the present study indicated that DSESM and EPSM could totally replace FM's inclusion rate in commercial diets for juvenile tilapia. Furthermore, methionine did not appear to be limiting in practical diets using typical levels of CSM, DSESM, and MBM as primary protein sources.

Tilapia are known as "aquatic chicken" because of 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).

Because of 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 (FM), have relatively high cost, limited supply, and variable quality. The development of commercial aquatic feeds has been traditionally based on FM as the main protein source because of its high protein content and balanced essential amino acid (EAA) profile. FM is also an excellent source of essential fatty acids, digestible energy, minerals, and vitamins. Therefore, it is no surprise that FM is the most expensive protein source in animal and aquaculture feeds (Tacon 1993). However, the limited supply of FM, coupled with its increased demand in feeds for livestock and poultry, is likely to reduce the dependence on FM in aquatic feeds (El-Sayed 1999). Furthermore,

<sup>1</sup> Present address: Faculty of Fisheries, Nong Lam University, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam.

<sup>2</sup> Corresponding author.

there are growing environmental concerns on the use of wild fish to produce FM. Hence, there is interest in replacing FM with less expensive protein sources. Many studies have been conducted to evaluate the replacement of FM in practical diets for tilapia with cheap, locally available plant and animal protein sources (Novoa et al. 1997; El-Sayed 1998; Fasakin et al. 1999, 2005; Abdelghany 2003; El-Saidy and Gaber 2003; Richter et al. 2003; El-Saidy and Gaber 2004; Borgeson et al. 2006; Gaber 2006). Replacement of FM in practical diets without reducing the performance would result in more profitable production of tilapia. Therefore, this aspect should be studied further to expand the list of appropriate plant and animal protein sources to replace FM.

The development of practical diets using non-FM 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 (SBM) compared with FM is its content of 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 reported values varied greatly. For example, Santiago and Lovell (1988) reported that TSAA requirement in semipurified diet of Nile tilapia, *Oreochromis niloticus*, fry was 0.9% of the diet (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 FM are possible.

Consequently, the objectives of the present study were to evaluate the possibility of using dehulled solvent-extracted soybean meal

(DSESM) and expeller pressed soybean meal (EPSM) to totally replace FM in practical diets for juvenile tilapia and to determine whether non-FM practical diets using cottonseed meal (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, *Oreochromis* spp. (Santa-Fe strain), and Nile tilapia, *Oreochromis 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, ID, USA) for 4 wk and then with a commercial fry feed (AquaMax, St. Louis, MO, USA) until the beginning of each experiment. All test ingredients were bought from commercial sources. Feed ingredients were ground, weighed, and then homogenized in a Hobart mixer (Hobart Inc., Troy, OH, USA) for 20 min and then 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. 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 compared with a diet containing FM. Four diets were formulated to contain 32% protein and 5% lipid. Three of these diets contained no FM and the fourth, the control diet, contained 6% FM, 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 (Diets 1 and 2, Table 1). The third diet was

TABLE 1. *Composition of four practical diets fed to tilapia in the first experiment.*

Ingredient (g/100 g)	Diet			
	1 (DSESM + Met)	2 (DSESM)	3 (EPSM + Met)	4 (FM)
FM <sup>1</sup>	0.00	0.00	0.00	6.00
DSESM <sup>2</sup>	63.80	63.80	0.00	56.00
EPSM <sup>3</sup>	0.00	0.00	68.10	0.00
Organic soybean oil <sup>4</sup>	4.10	4.10	0.00	3.56
Wheat starch <sup>5</sup>	6.30	6.35	6.25	9.24
Whole wheat <sup>5</sup>	20.00	20.00	20.00	20.00
Trace mineral premix <sup>6</sup>	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free) <sup>7</sup>	2.00	2.00	2.00	2.00
Stay C (25% vitamin C activity) <sup>8</sup>	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic <sup>5</sup>	3.15	3.15	3.00	2.60
L-methionine <sup>5</sup>	0.05	0.00	0.05	0.00
Methionine (% of the diet) <sup>9</sup>	0.58	0.53	0.58	0.58
Cystine (% of the diet) <sup>9</sup>	0.48	0.48	0.43	0.46

FM = fish meal; DSESM = dehulled solvent-extracted soybean meal; EPSM = expeller pressed soybean meal.

<sup>1</sup> Special Select™; Omega Protein Inc., Hammond, LA, USA.

<sup>2</sup> Southern Sates Cooperative Inc., Richmond, VA, USA.

<sup>3</sup> Organic soybean meal, Professional Proteins Ltd., Washington, IA, USA.

<sup>4</sup> Clarkson Grain Co., Inc., Cerro Gordo, IL, USA.

<sup>5</sup> MP Biochemicals Inc., Solon, OH, USA.

<sup>6</sup> 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; and cellulose, 679.64.

<sup>7</sup> 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<sub>12</sub>, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D<sub>3</sub> (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; and alpha-cellulose, 955.589.

<sup>8</sup> Hoffman-La Roche Vitamins Inc., Parsippany, NJ, USA.

<sup>9</sup> Analyzed values.

formulated using EPSM and was supplemented with 0.05% methionine. Methionine level was equal to 0.58% in all diets or 1.81% of dietary protein except in the diet that did not contain either FM or methionine supplements. In this diet (Diet 2), methionine level was 0.53% of the diet or 1.66% of dietary protein. Tilapia (4.78 ± 0.07 g) were randomly stocked into 12 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 Corp., Yellow Springs, OH, 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, OH, USA). TAN 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 0800–0900 and 1600–1700 h. They were weighed weekly to adjust rations. Six weeks after the start of the experiment all fish were harvested, counted, and group weighed.

### *Experiment 2*

Because 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 meat and bone meal (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

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

Ingredient (g/100 g)	Diet			
	1 (MBM)	2 (MBM + Met)	3 (No MBM)	4 (No MBM + Met)
CSM <sup>1</sup>	15.00	15.00	15.00	15.00
DSESM <sup>2</sup>	27.00	27.00	37.00	37.00
MBM <sup>3</sup>	10.00	10.00	0.00	0.00
Menhaden fish oil <sup>4</sup>	3.25	3.25	4.20	4.20
Wheat starch <sup>5</sup>	33.15	33.09	30.40	30.34
Whole wheat <sup>5</sup>	9.00	9.00	9.00	9.00
Trace mineral premix <sup>6</sup>	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free) <sup>7</sup>	1.00	1.00	1.00	1.00
Stay C (25% vitamin C activity) <sup>8</sup>	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic <sup>5</sup>	1.00	1.00	2.80	2.80
L-methionine <sup>5</sup>	0.00	0.06	0.00	0.06
Methionine (% of the diet) <sup>9</sup>	0.46	0.52	0.49	0.55
Cystine (% of the diet) <sup>9</sup>	0.49	0.49	0.51	0.51

CSM = cottonseed meal; DSESM = dehulled solvent-extracted soybean meal; EPSM = expeller pressed soybean meal; MBM = meat and bone meal.

<sup>1</sup> Faithway Feed Co. Inc., Guntersville, AL, USA.

<sup>2</sup> Southern Sates Cooperative Inc., Richmond, VA, USA.

<sup>3</sup> Griffin Industries, Inc., Cold Spring, KY, USA.

<sup>4</sup> Omega Protein Inc., Reedville, VA, USA.

<sup>5</sup> MP Biochemicals Inc., Solon, OH, USA.

<sup>6</sup> 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; and cellulose, 679.64.

<sup>7</sup> 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<sub>12</sub>, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D<sub>3</sub> (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; and alpha-cellulose, 955.589.

<sup>8</sup> Hoffman-La Roche Vitamins Inc., Parsippany, NJ, USA.

<sup>9</sup> Analyzed values.

second diet was the same as the first diet but was supplemented with 0.06% methionine. The last two diets contained 15% CSM, 37% DSESM, and without and with 0.06% methionine supplementation. Eighteen tilapia ( $3.90 \pm 0.05$  g) were randomly stocked into each of the twelve 60-L aquaria of an indoor recirculation system for the first 5 wk. In this system, water temperature was maintained at approximately 28 C using a submerged 3600-W heater (Aquatic Eco-Systems Inc., Apopka, FL, 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 0800–0900 and 1600–1700 h. 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 by the same procedures as described in the first experiment. At the end of the 10th week, fish were harvested, counted, and group weighed.

#### Statistical Analysis

Statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC, USA). Data from both experiments were analyzed using one-way ANOVA to determine if there were significant differences ( $P \leq 0.05$ ) in growth and survival. Student–Newman–Keuls multiple

comparison test (Steel and Torrie 1980) was used to determine differences among treatment means.

## Results

### Experiment 1

Proximate analysis of all test diets in the first and second experiments confirmed that protein and lipid contents have matched the calculated values. The mean ( $\pm$ SD) of water quality variables of the first experiment was as follows: dissolved oxygen,  $6.74 \pm 0.43$  mg/L; water temperature,  $27.0 \pm 0.9$  C; TAN,  $0.157 \pm 0.074$  mg/L; nitrite-N,  $0.033 \pm 0.013$  mg/L; and pH,  $7.9 \pm 0.1$ . These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal 1988; Wangead et al. 1988; Watanabe et al. 1993; El-Shafai et al. 2004). At the end of the first experiment, final mean weights of tilapia varied from 23.89 to 26.59 g. High survival rates were obtained in all treatments, from 96.7 to 100.0%. Feed conversion ratios (FCRs) were from 1.22 to 1.33. 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 because of 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). This experiment was terminated after 6 wk because there were no notable

trends or statistically significant differences ( $P > 0.05$ ) in final mean weight, survival rate, and FCR among the treatments.

### Experiment 2

The mean ( $\pm$ SD) of water quality variables over the first 5 wk of the second experiment was as follows: dissolved oxygen,  $6.83 \pm 0.47$  mg/L; water temperature,  $28.5 \pm 1.2$  C; TAN,  $0.125 \pm 0.050$  mg/L; nitrite-N,  $0.094 \pm 0.049$  mg/L; and pH,  $8.2 \pm 0.2$ . During the last 5 wk, these values were dissolved oxygen,  $6.65 \pm 0.50$  mg/L; water temperature,  $27.2 \pm 1.1$  C; TAN,  $0.256 \pm 0.176$  mg/L; nitrite,  $0.042 \pm 0.043$  mg/L; and pH,  $7.8 \pm 0.2$ . These values were within optimum ranges for normal growth and health of juvenile tilapia (El Gamal 1988; Wangead et al. 1988; Watanabe et al. 1993; El-Shafai et al. 2004).

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%. FCRs 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

SBM is the best plant protein source in terms of protein content and EAA profile. However, it

TABLE 3. Initial weight, final weight, survival, and FCR of tilapia fed four different practical diets in the first experiment.<sup>1</sup>

Diet	Initial weight (g)	Final weight (g)	Survival (%)	FCR <sup>2</sup>
1 (DSESM + Met)	4.77 $\pm$ 0.10	25.64 $\pm$ 8.43a	100.0 $\pm$ 0.0a	1.23 $\pm$ 0.03a
2 (DSESM)	4.83 $\pm$ 0.06	23.89 $\pm$ 0.36a	100.0 $\pm$ 0.0a	1.33 $\pm$ 0.01a
3 (EPSM + Met)	4.76 $\pm$ 0.04	25.75 $\pm$ 2.78a	100.0 $\pm$ 0.0a	1.22 $\pm$ 0.11a
4 (FM)	4.77 $\pm$ 0.03	26.59 $\pm$ 2.29a	96.7 $\pm$ 4.7a	1.28 $\pm$ 0.16a
PSE		1.30	1.67	0.07
P		0.54	0.44	0.65

FM = fish meal; FCR = feed conversion ratio; DSESM = dehulled solvent-extracted soybean meal; EPSM = expeller pressed soybean meal; MBM = meat and bone meal; PSE = pooled standard error.

<sup>1</sup> Values reported are mean  $\pm$  SD of three replicates. Means in the same column with the same superscript letters are not significantly different ( $P > 0.05$ ).

<sup>2</sup> FCR = dry weight of feed offered/wet weight gain.

TABLE 4. Initial weight, final weight, survival, and FCR of tilapia fed four different practical diets in the second experiment.<sup>1</sup>

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

FCR = feed conversion ratio; MBM = meat and bone meal; PSE = pooled standard error.

<sup>1</sup> Values reported are mean ± SD of three replicates. Means in the same column with the same superscript letters are not significantly different ( $P > 0.05$ ).

<sup>2</sup> FCR = dry weight of feed offered/wet weight gain.

is potentially limiting in sulfur-containing amino acids (methionine and cystine) and contains some antinutrient substances such as trypsin inhibitor, hemagglutinin, and antivitamin (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% protein reduced growth of blue tilapia, while SBM could totally replace FM if protein level was 36% because the EAA 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* × *Oreochromis aureus*) because of 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* × *O. aureus*) offered diets devoid of FM was significantly lower ( $P < 0.05$ ) than those fed diets containing FM because of 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 because of 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 defatting 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 because of TSAA levels of non-FM diets already met or exceeded the requirement of juvenile tilapia. TSAA and methionine levels of a non-FM diet without methionine supplementation (Diet 2) were 1.01 and 0.53% of the diet and 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, *Oreochromis 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 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). Because 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 the 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 use 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 or requiring methionine supplementation. To date, the demand for organic products from aquaculture has been increasing because of 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 nonorganic ones and tilapia is not an exception. If organic protein sources, such as EPSM, have reasonable prices compared with DSESM, it may be more profitable to produce organic tilapia. Because 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. CSM is an important protein source for terrestrial animals. However, its utilization in fish feeds is limited, mainly because of 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* × *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 because of 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.

MBM, 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. MBM 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 using CSM, MBM, and DSESM as primary protein sources. Tilapia diets could also be formulated without an 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. Because 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.

### Acknowledgments

The authors would like to thank Ronald Phelps, graduate students: Luke Roy, Herbert Fonsecao, Jesus Venera, and staffs of E.W. Shell Fisheries Center, Department of Fisheries and Allied Aquacultures, Auburn University, for their support during this study. This study was partially funded by project number 322, Ministry of Education and Training, Viet Nam government.

### Literature Cited

- Abdelghany, A. E.** 2003. Partial and complete replacement of fish meal with gambusia meal in diets for red tilapia (*Oreochromis niloticus* x *O. mossambicus*). *Aquaculture Nutrition* 9:145–154.
- Borgeson, T. L., V. J. Racz, D. C. Wilkie, L. J. White, and M. D. Drew.** 2006. Effect of replacement fish meal and oil with simple or complex mixtures of vegetable ingredients in diets fed to Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* 12: 141–149.
- Davis, A. T. and R. R. Stickney.** 1978. Growth responses of *Tilapia aurea* to dietary protein quality and quantity. *Transactions of the American Fisheries Society* 107: 479–483.
- El Gamal, A.-R.** 1988. Reproductive performance, sex ratios, gonadal development, cold tolerance, viability and growth of red and normally pigmented hybrids of *Tilapia aurea* and *T. nilotica*. PhD Dissertation. Auburn University, Auburn, Alabama, USA.
- El-Saidy, D. M. S. D. and M. M. A. Gaber.** 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquaculture Research* 34: 1119–1127.
- El-Saidy, D. M. S. D. and M. M. A. Gaber.** 2004. Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish meal in Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquaculture Research* 35:859–865.
- El-Sayed, A.-F. M.** 1998. Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.), feeds. *Aquaculture Research* 29:275–280.
- El-Sayed, A.-F. M.** 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture* 179:149–168.
- El-Sayed, A.-F. M.** 2006. Tilapia culture. CAB International, Wallingford, UK.
- El-Shafai, S. A., F. A. El-Gohary, F. A. Nasr, N. P. van der Steen, and H. J. Gijzen.** 2004. Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*). *Aquaculture* 232:117–127.
- Fasakin, E. A., A. M. Balogun, and B. E. Fasuru.** 1999. Use of duckweed, *Spirodela polyrrhiza* L. Schleiden, as a protein feedstuff in practical diets for tilapia, *Oreochromis niloticus* L. *Aquaculture Research* 30:313–318.
- Fasakin, E. A., R. D. Serwata, and S. J. Davies.** 2005. Comparative utilization of rendered animal derived products with or without composite mixture of soybean meal in hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*) diets. *Aquaculture* 249:329–338.
- Gaber, M. M.** 2006. The effects of plant-protein-based diets supplemented with yucca on growth, digestibility, and chemical composition of Nile tilapia (*Oreochromis niloticus*, L) fingerlings. *Journal of the World Aquaculture Society* 37:74–81.
- Jauncey, K. and Ross, B.** 1982. A Guide to Tilapia Feeds and Feeding. Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK. 111 pp.
- Kasper, C. S., M. R. White, and P. B. Brown.** 2000. Choline is required by tilapia when methionine is not in excess. *Journal of Nutrition* 238:238–242.
- Li, M. H. and E. H. Robinson.** 2006. Use of cottonseed meal in aquatic animal diets: a review. *North American Journal of Aquaculture* 68:14–22.
- Lim, C. E. and C. D. Webster.** 2006. Nutrient requirements. Pages 469–501 in C. E. Lim and C. D. Webster, editors. *Tilapia: Biology, culture and nutrition*. Food Products Press, New York, USA.
- Lim, C., M. Yildirim, and P. H. Klesius.** 2002. Effect of substitution of cottonseed meal on soybean meal on growth, hematology and immune response of tilapia (*Oreochromis niloticus*). *Global Aquaculture Advocate* 5:28–32.
- Novoa, M. A. O., F. P. Pacheco, L. O. Castillo, V. P. Flores, L. Navarro, and J. C. Samano.** 1997. Cowpea (*Vigna unguiculata*) protein concentrate as replacement for fish meal in diets for tilapia (*Oreochromis niloticus*) fry. *Aquaculture* 158:107–116.
- Parsons, T. R., Y. Maita, and C. M. Lalli.** 1985. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, USA.
- Richter, N., P. Siddhuraju, and K. Becker.** 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture* 217:599–611.

- Santiago, C. B. and R. T. Lovell.** 1988. Amino acid requirements for growth of Nile tilapia. *Journal of Nutrition* 118:1540–1546.
- Shiau, S.-Y., C. C. Kwok, J. Y. Huang, C. M. Chen, and S. L. Lee.** 1989. Replacement of fish meal with soybean meal in male tilapia (*Oreochromis niloticus* x *O. aureus*) fingerling diets at a suboptimal protein level. *Journal of the World Aquaculture Society* 20:230–235.
- Solorzano, L.** 1969. Determination of ammonia in natural waters by the Phenolhypochlorite method. *Limnology and Oceanography* 14:799–801.
- Steel, R. G. D. and J. H. Torrie.** 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, USA.
- Tacon, A. G. J.** 1993. Feed ingredients for warm water fish: Fish meal and other processed feedstuffs. FAO Fisheries Circular No. 856. FAO, Rome, Italy.
- Tacon, A. G. J. and D. J. Brister.** 2002. Organic aquaculture – current status and future prospects. Pages 163–176 in N. E.-H. Scialabba and C. Hattam, editors. Organic agriculture, environment and food security. Environment and Natural Resources Series No.4. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Tacon, A. G. J. and A. J. Jackson.** 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. Pages 119–145 in C. B. Cowey, A. M. Mackie, and J. G. Bell, editors. Nutrition and feeding in fish. Academic Press, London, UK.
- Tacon, A. G. J., K. Jauncey, A. Falaye, M. Pentah, I. MacGowen, and E. Stafford.** 1983. The use of meat and bone meal and hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. Pages 356–365 in J. Fishelton and Z. Yaron, editors. Proceedings of the 1st International Symposium on Tilapia in aquaculture. Tel Aviv University, Tel Aviv, Israel.
- Viola, S. and G. Zohar.** 1984. Nutritional study with market size tilapia hybrid *Oreochromis* in intensive culture. Protein levels and sources. *Israeli Journal of Aquaculture* 36:3–15.
- Viola, S., Y. Arieli, and G. Zohar.** 1988. Animal-protein-free feeds for hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) in intensive culture. *Aquaculture* 75:115–125.
- Wangead, C., A. Greater, and R. Tansakul.** 1988. Effects of acid water on survival and growth rate of Nile tilapia (*Oreochromis niloticus*). Pages 433–438 in R. S. V. Pullin, T. Bhukaswan, K. Tonguthai and J. L. Maclean, editors. Proceedings of the Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings No. 15, Department of Fisheries, Bangkok, Thailand. ICLARM, Manila, Philippines.
- Wassef, E. A., G. Plummer, and M. Poxton.** 1988. Protease digestion of the meals of ungerminated and germinated soybeans. *Journal of the Science of Food and Agriculture* 44:201–214.
- Watanabe, W. O., D. H. Ernst, M. P. Chasar, R. I. Wicklund, and B. L. Olla.** 1993. The effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. *Aquaculture* 112:309–320.
- Wu, Y. V., K. Y. Tudor, P. Brown, and R. R. Rosati.** 1999. Substitution of plant protein and meat and bone meal for fish meal in diets for Nile tilapia. *North American Journal of Aquaculture* 61:58–63.
- Wu, G. S., Y. M. Chung, W. Y. Lin, S. Y. Chen, and C. H. Huang.** 2004. Effect of substituting de-hulled or fermented soybean meal for fish meal in diets on growth of hybrid tilapia, (*Oreochromis niloticus* x *O. aureus*). *Journal of the Fisheries Society of Taiwan* 30(4):291–297.