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Full Length Research Paper

The effects of replacement of soybean meal by shrimp shell meal on the growth of hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*) reared under brackish water

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To evaluate the effects of complete replacement of soybean meal (SBM) protein by shrimp shell meal (SSM) on the growth performance of hybrid tilapia (*O. niloticus x O. aureus*), five approximately isoenergetic and isonitrogenous (30% crude protein) diets containing 0, 33, 50, 67 and 100% SSM were fed to triplicate groups of ten fry with an initial mean weight 1.54 ± 0.03 g/fish under brackish water (15 ppt) for sixty (60) days. Fish fed diet containing 100% SSM exhibited the lowest specific growth rate (SGR). There was no significant difference of SGR in the fish fed 0, 33, 50 and 67% SSM. The fish fed 0, 33 and 50% SSM had better feed conversion rate (FCR) value than those fish fed with 67 and 100% SSM supplement. The survival of juveniles ranged from 97 to 100%, being 97% for 0, 50, 67% SSM and 100% for 33 and 100% SSM. The weight gain was highest in fish fed diet without SSM, intermediate in fish fed diets with 33, 50 and 67% SSM, and lowest in fish fed diet with 100% SSM. The highest body protein content was observed in fish fed diets with 50 and 67% SSM compared to the initial fish. The lowest body protein content was obtained with the fish fed diet with 100% SSM. The tilapia fed diet containing 33 and 67% SSM had the highest body lipid content compared to the initial fish. There were no significant differences in body lipid content among the fish fed diet with 0, 50 and 100% SSM. No difference in body ash content was observed among treatments.

Key words: Hybrid tilapia, *Oreochromis niloticus x Oreochromis aureus*, soybean meal, shrimp shell meal, brackishwater.

INTRODUCTION

Tilapia is the common name for a diverse group of Cichlids, which are well adapted to enclosed water. They are widely cultured in tropical and subtropical regions of the world such as Egypt, Taiwan, Senegal and formed an important human protein source. The production of tilapia had been over 2500 thousand tons per year in the world (FAO, 2006). The tilapia is an omnivorous species that uses a wide spectrum of foods (Sklan et al., 2004a), efficiently uses dietary carbohydrates and has a great ability to digest plant protein (Olvera-Novoa et al., 2002; Shelton and Popma, 2006; Gatlin et al., 2007).

In most parts of the world, soybean meal (SBM) is the main vegetable source of protein ingredients of commercial aquaculture feeds. Different soybean products, such as soy protein concentrate, extracted and defatted soybean meal, full-fat SBM, or low oligo-saccharide SBM (Refstie et al., 1998) produced contrasting growth

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Abbreviations: SBM, Soybean meal; **SSM,** shrimp shell meal; **SGR,** specific growth rate; **FCR,** feed conversion rate; **CFM,** Cassia fistula meal; **PKM,** palm kernel meal; **SWM,** shrimp wastemeal; **NTOU,** National Taiwan Ocean University; **TFRC,** Taiwan Fisheries Research Center; **SAS,** statistic system; **ANOVA,** analysis of variance; **CWP,** crustacean waste products; **FSHM,** fermented shrimp head meal; **SPH,** shrimp protein hydrolysate.

because of the varied quantities of anti-nutrient compounds (trypsin, lectin, phytic acid and protease inhibitors) and/or lowly digestible carbohydrates present in these products. Different authors have also found problems of palatability or the existence of different components affecting SBM utilization in animals. Besides these variable results, SBM is not a readily available feedstuff worldwide. Therefore, there is a need to investigate the possibility of using other animal or vegetable protein sources with low market prices and nutritive value comparable to that of SBM. Less attention has been paid to the partial substitution of soybean in diets for tilapia. Adebayo et al. (2004) reported that *Cassia fistula* meal (CFM) could be a substitute for SBM up to (170 g /kg) in practical diets without compromising growth of *O.niloticus* fingerlings. Lim et al. (2001) concluded that palm kernel meal (PKM) could substitute up to 50% SBM in practical diet for *O. mossambicus* without much adverse effect to fish growth. Chien and Chin (2003) found that partial replacement of SBM protein with lupin seed protein in juvenile tilapia diets resulted in better growth and feed performance. Chareontesprasit and Jiwyam (2001) reported that, the replacement of soybean meal by wolffia meal could be possible only up to 15% in the ration and an increase beyond 15% would decrease total fish growth, survival rate, feed intake, and feed conversion ratio of Nile tilapia. However, there is no available information on the use of shrimp shell meal (SSM) or soybean and shrimp shell protein ratio as feed for tilapia.

Shrimp waste is basically the dried waste of shrimp industry, consisting of the heads, appendages and exoskeleton and is particularly rich in lysine and chitin (Famino et al., 1996). Stimulated by increasing shrimp production from catches and farming, shrimp waste meal (SWM) has been identified as an animal protein source of considerable potential (Fanimo et al., 2000). However, the use of SWM may be restricted due to its high fiber, chitin and ash contents (Cavalheiro et al., 2007), which have been found to reduce crustacean meal digestibility in tilapia (Koprucu and Ozdemir, 2005), decrease lipid absorption and increase water content in the feces of the Atlantic salmon, *Salmo salar* (Olsen et al., 2006). Products obtained from shrimp processing wastes may serve as a useful source of protein and flavoring in food formulations mainly due to the level of free amino acids (Heu et al., 2003; Ruttanaporn et al., 2005).

In Senegal, shrimp waste which represents about 33% of the shrimp weight is almost completely discarded. The underutilization of the shrimp waste posing a serious disposal problem, contributes to the overall cost of the production. In the year 2003, shrimp waste constituted for about 2,089.48 tons of a total Senegal shrimp production of 6,331.75 tons (Senegal Fisheries year book, 2003). In Senegal, the shrimp waste is collected by Senegal Nitrogen Factory for nitrogen production (fertilizer) or released into the environment where it becomes source of pollution. One of the possible solutions of this problem is to transform processing waste into either silage or flour and use this material in the formulation of animal or fish feeds.

A possibility is the use of shrimp head waste meal, which contains high levels of protein with excellent amino acid profile comparable to that of fish meal (Meyers, 1986). Furthermore, the need for improvement in the quality of shrimp head waste protein has attracted the application of different processing methods (Fox et al., 1994). Cooking requires excessive use of firewood or other scarce fuels and degrades the lipids, vitamins and pigments content of the meal (Fox et al., 1994) while sun-drying is frequently carried out under unhygienic conditions leading to meals with high microbial loading (Wood, 1982). Although formic acid isolation can improve the nutritional quality and feedstuffs value of shrimp heads, the cost of organic acids is high and mineral acid silage needs to be fresh shrimp waste (Raa and Gilberg, 1982). However, prompt preservation through lactic acid bacterial fermentation which has been used successfully in fish insolation (Hall and De Silva, 1994) could be desirable as alternative to cooking, sun-drying and acid isolation continued production of the shrimp head waste without corresponding development of technology utilizing the wastes has resulted in waste collection, disposal and pollution problems (Nwanna et al., 2003). Harnessing of these wastes into fish feed production apart from minimizing the costs of fish production would serve as an excellent means of sanitizing the environment.

The purposes of this present study were to evaluate the nutritional quality of SSM by assessing the growth and protein utilization of hybrid tilapia (O.*niloticus x O.aureus*) reared under brackish water and to investigate the utilization and the possible substitution of SSM for SBM in tilapia diets.

MATERIALS AND METHODS

Animals

The growth trial was conducted in an indoor system at National Taiwan Ocean University (NTOU). The genetically improved farmed hybrid tilapia strain of *O. niloticus x O. aureus* was developed by Taiwan Fisheries Research Center (TFRC) from the selective breeding of stocks. Fry hybrid tilapia (*O. niloticus x O. aureus)* were obtained from a fish farm in Tainan, Taiwan; and acclimatized in the laboratory conditions for one week in brackish water (15 ppt). At the beginning of the experiment 15 glass tanks 60 x 30 x 38 cm containers (36 L) were stocked with 10 fishes each with an average of 1.54 ± 0.03 g. Each aquarium was put in a closed recirculating system maintained at 30 ± 1°C. An air stone continuously aerated each aquarium. All aquaria were cleaned daily in the morning and the afternoon by siphoning off accumulated waste materials. Approximately half of water in each tank was replaced with aerated brackish water twice a day. The fish were fed with 6% of body weight per day and gradually decreased to 4% daily. Each diet was fed twice daily at 08:00 h and 17:00 h for 60 days to triplicate group

Table 1. Composition of experimental diets for tilapia *(Oreochromisniloticus x O. aureus)*.

Composition	Treatment				
	100% SSM	67% SSM	50% SSM	33% SSM	0% SSM
Fish meal	6.6	6.6	6.6	6.6	6.6
Shrimp shell meal	50	37.5	25	12.5	-
Soybean meal	-	11.1	22.2	33.3	44.4
Vit. mix [a]	2	2	2	2	2
Min. mix [b]	4	4	4	4	4
Dextrin	4.5	4.5	4.5	4.5	4.5
Wheat flour	11.3	12.7	14.1	15.5	16.9
α-Starch	5	5	5	5	5
Oil [c]	5	5	5	5	5
Cellulose	11.6	11.6	11.6	11.6	11.6
Energy (Kcal/100g)	354.6	354.6	354.6	354.6	354.6

[a]Calcium carbonate 2.1%, calcium phosphate dibasic 73.5%, citric acid 0.227%, cupric citrate 0.046%, ferric citrate (16-17% Fe)0.558%, magnesium oxide 2.5%, magnesium citrate 0.835%, potassium sulfate 6.8%, sodium chloride 3.06%,sodium phosphate 2.14%, zinc citrate 0.133, potassium iodine 0.001%, potassium phosphate dibasic 8.1%.
[b] Thiamin HCl 0.5%, riboflavin 0.8%, niacinamide 2.6%, D-biotin 0.1%, Ca-pantothenate 1.5%, pyridoxine HCl 0.3%, folic acid 0.5%, inositol 18.1%,ascorbic acid 12.1%, para-aminobenzoic acid 3%, cyanobalmin 0.1%, BHT0.1%, α-cellulose 60.3%.
[c] Cod liver oil / corn oil = 2:1

of fish. Each group of fish was weighed at the beginning and every two weeks and the amount of diet fed was adjusted accordingly. A photoperiod of 12 h light, 12 h dark (08:00-20:00h) was used. Fluorescent ceiling lights supplied the illumination.

Diet preparation

To investigate the utilization and possible substitution of SSM (crude protein 39.40%) for SBM (crude protein 44.05%) in tilapia (*O. niloticus x O.aureus*) diets, five diets were formulated to contain approximately equal amounts of digestible protein (30%) and digestible energy. The replacement levels of SBM by SSM were 100, 67, 50, 33, and 0%. Of the total dietary protein, 10% was from fish meal (FM, crude protein 68.71%) and 90% came from different ratios of SBM and SSM (Table 1).

The process for the preparation of the SSM (of *Penaeus monodon*) was based on the method described by Cavalheiro et al. (2007). The main protein sources (SBM and SSM) already grounded into mill was passed as particles through a no. 40 (425µm) mesh sieve. Mineral mix and vitamin mix were prepared in the laboratory according to Sheen and Wu (1999). After all the ingredients were mixed thoroughly, adequate quantity of water (30% for 100 g of mixed ingredients) and oil (cod liver oil and corn oil in the ratio 2:1) were added. The dough was passed through an extruder to make spaghetti, and dried at 35°C for 8h. The dried diet was packaged into plastic bag and stored frozen at -20°C until use.

Proximate analysis of the diet and the dorsal muscle

The experimental diets and samples of the dorsal muscle were analyzed for proximate composition based on AOAC (1984) methods. Crude protein was determined with a Kjeltec system 1002 (Tecator). Crude lipid was determined by chloroform-methanol (2:1, v/v) extraction method (Folchet al.1957). Crude fiber was determined by the Fibertec system M 1020 hot extractor (FOSS Tecator). Gross energy was obtained by IKA calorimeter system C 2000 basic. Ash and moisture were determined by conventional methods using muffle furnace at 505°C and an oven at 105°C.

Growth parameters

Growth response parameters were calculated as follows: WG (weight gain, %) = 100 (Final mean body weight - Initial mean body weight)/initial mean body weight; specific growth rate (SGR, % day^{-1}) = ((In Wt- In Wi) /T) x 100, where Wt is the weight of fish at time t, Wi is the weight of fish at time 0 and T is the rearing period in days; feed conversion rate (FCR) = total dry feed fed g fish^{-1} / total wet weight gain g fish^{-1}. Survival rate (%) = 100 (number of fish which survived/initial number of fish).

Water quality measurement

Water temperature and dissolved oxygen were measured every other day using a YSI Model 58 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA). pH was monitored twice weekly using an electronic pH-meter (pH pen; Fisher Scientific, Cincinnati, OH, USA).

Statistical analysis

The data were analyzed using the statistic system (SAS-PC) (Joyner, 1985) by one-way analysis of variance (ANOVA). The treatment effects were considered significant at $P<0.05$; Duncan's New-multiple range test was used to compare significant difference among the treatments.

RESULTS

During 60 days feeding trial, the water-quality parameters averaged (±SD) water. Temperature, 30±0.8ºC; dissolved oxygen, 5.0 ± 0.7 mgL^{-1} and pH, 8.2 ± 0.3. The proximate composition of the experimental feeds is shown in Table 2. The results show statistically similar composition (P>0.05) of the experimental feeds with respect to moisture, protein, lipid and fiber. However, the

Table 2. Proximate analysis of experimental diets fed tilapia (*Oreochromis.niloticusxO.aureus*).

Composition	Treatment				
	100% SSM	67% SSM	50% SSM	33% SSM	0% SSM
Moisture	13.01	11.01	10.13	10.06	10.08
Crude protein*	30.08	29.32	30.02	30.01	30.02
Crude lipid*	6.49	6.40	6.43	6.38	6.75
Crude fibre*	10.37	10.27	10.01	10.11	9.27
Ash*	28.16	22.67	17.50	12.75	8.42
Gross energy (Kcal/100g)	369.1	367.1	367.3	368.8	366.8
Calculated energy (Kcal/100g)	354.6	354.6	354.6	354.6	354.6

* Presented as percentage of dry weight.

Table 3. Initial, final weight, weight gain (mean±SE), FCR and survival of Tilapia (*Oreochromisniloticus x O. aureus*).

Treatment	Initial weight	Final weight	Weight gain	SGR	FCR	Survival (%)
100% SSM	1.55±0.02	4.66±0.25	201.21±19.02 [d]	1.83±0.14 [b]	2.42	100
67% SSM	1.54±0.04	5.29±0.29	242.97±16.19 [c]	2.06±0.17 [a]	2.32	97
50% SSM	1.54±0.05	6.31±0.59	308.60±35.57 [b]	2.35±0.32 [a]	1.94	97
33% SSM	1.54±0.02	5.86±0.23	280.02±17.87 [bc]	2.23±0.13 [a]	1.86	100
0% SSM	1.55±0.03	7.01±0.20	353.00±8.22 [a]	2.52±0.12 [a]	1.73	97

[abcd] Means in the same column with the different letter are significantly different.
(P＜0.05). SSM: shrimp shell meal.

ash increases when increasing the SSM diet content. The mean weight gain, FCR, SGR and survival, of the hybrid tilapia fed treatments diets are shown in Table 3. The highest growth performance (P<0.05) was obtained with the control (diet without SSM), followed by 67, 50 and 33% SSM. Lowest responses were obtained with 100% of SSM replacement. There was no significant difference (P>0.05) in weight gain among the fish fed diets with 67, 50 and 33% SSM. Fish fed diet containing 100% SSM showed the lowest SGR. There was no significant difference (P>0.05) of SGR in the fish fed 0, 67, 50 and 33% SSM. The FCR of the fish fed the tests diets followed the same general pattern as the weight gain. The fish fed 50, 33 and 0% SSM had better FCR value than those fish fed with 100 and 67% SSM supplement. During the 60-day feeding trial, mortality was observed with each type of feed, including the control feed. The survival of juveniles ranged from 97 to100%, being 97% for 0, 50, 67% SSM and 100% for 33 and 100% SSM (Table 3).

Table 4 displays the initial and final body compositions of whole fish. Statistically (P>0.05), the moisture and ash content of tilapia fed the different feeds did not show any appreciable variation. The inclusion of SSM in hybrid tilapia diets significantly (P<0.05) affected final fish body composition. The body protein content was significantly high in fish fed diet containing 67 and 50% compared to the body protein content of the initial fish. The lowest body protein was obtained in the fish fed diet containing 100% SSM. There were no significant differences (P>0.05) in body protein content among the fish fed diet with 33 and 0% SSM and the initial fish. No difference (P>0.05) was observed in the body moisture content of the fish fed the tests diets and the initial fish. The tilapia fed diet containing 33 and 67% SSM had the highest body lipid content (P<0.05) compared to the body lipid content of the initial fish. There were no significant differences (P>0.05) in body lipid content among the fish fed diet with 100, 50 and 0% SSM. There was no difference (P>0.05) in body ash content among treatments.

DISCUSSION

Under the experimental conditions, the results of the study indicated that tilapia growth increases when increasing the soybean diet content. In other words, it decreases when increasing the SSM diet content. In fact, there is a polysaccharide in SSM called chitin, which is not readily digestible. The chitin can reduce tilapia growth regardless of the supplementation level (Shiau and Yu, 1999). The SSM in tilapia diets was provided up to 60% of the total protein (substituting SBM) without compromising growth in nutrient utilization. In this study the partial replacement of SBM with SSM is also recommended. The better growth rate noted in terms of weight gain, SGR and FCR may be due to the supplementation

Table 4. Proximate analysis of dorsal muscle of tilapia (*Oreochromisniloticus x O. aureus)*.

Composition	Treatment					
	Initial	**100% SSM**	**67% SSM**	**50% SSM**	**33% SSM**	**0% SSM**
Moisture	77.46	76.27	77.12	77.36	76.21	77.06
Crude protein*	74.89	62.01	80.23	80.09	76.16	77.16
Crude lipid*	10.50	11.18	13.84	12.61	14.24	12.63
Ash*	4.39	5.88	4.92	5.23	4.20	5.20

* Presented as percentage of dry weight.

of amino acids by FM in the formulated diet without SSM.

In our first growth trial, tilapia fed soybean meal as main protein source (24% crude protein) had lower weight gain and high mortality when reared in brackish water (20%) (Fall et al., 2011). This was attributed to decreasing level of some amino acid balance in soybean protein (Shiau et al., 1989). This is in agreement with the findings of Furuya et al. (2004). Furthermore, it is true that soybean meal could be replaced by shrimp meal in diets for fish without depressing performance according to Cruz-Suarez (1993).

Chemical analysis (moisture, protein, lipid and ash content) at the end of a feeding trial is frequently used to determine the influence of feed on fish composition. According to Hepher (1990), endogenous factors (size, sex, and stage of life cycle) and exogenous factors (diet composition, feeding frequency, and temperature) affect the body composition of fish. It should be noted that within exogenous factors, the composition of the feed is the only factor, which could have influenced the difference chemical composition of the fish, as other endogenous factors were maintained uniform during the study.

Although SSM inclusion resulted in carcass protein and fat deposition (Table 4), these results are similar to those reported by Plascencia-Jatomea et al. (2002), who found body crude lipid content ranging from 53.6 to 67.2 in Nile tilapia fed diets containing shrimp head hydrolysate by fermentative silage. Whole body composition reflected diet composition only with regard to ash content. Grenat (2001) showed that properly processed shrimp meal can be used in relatively high levels in place of SBM in layer diets without negatively affecting bird performance. Ozogul (2000) indicated that crustacean waste products (CWP) may be recommended for use as an alternative to FM sparing in rainbow trout diets, but that partial replacement requires amino acid supplementation. The use of shrimp by-product meal in the diet supported good growth and survival of rainbow trout. Feed consumption increased slightly with diet supplemented with CWP, although no significant differences were detected between control diet and supplemented diet with CWP. Meyers (1987) stated that crustaceans and mollusks have attractants that contain nitrogenous compounds, for instance, amino acid, peptides, nucleotides and chitin, which promote consumption and thus increase growth. In the present study, the FCR was improved with diet supplemented with 67 and 100% SSM. This may be attributed to the direct effect of increased palatability. The mean FCR of 2.02 reported from the study is in line with the observations of Nwanna (2003) which stated that the mean FCR was 2.21. Further, the mean SGR of 2.20 obtained in the present study similar to the SGR of 2.28 reported by Fagbenro and Jauncey (1995) from *Clarias gariepinus* juveniles fed fish silage blended with hydrolyzed feather meal. Unremarkable changes in the carcass ash, moisture obtained from the present study validates the findings of Fox et al. (1994) and Fagbenro and Jauncey (1995).

Nwanna (2003) indicated that the best profit margin would be realized by replacing fish meal with 30% fermented shrimp head meal (FSHM) in the diet of *C. gariepinus*. In addition, Plascencia-Jatomea et al. (2002) concluded that shrimp protein silage could be included in tilapia diets at concentrations as high as 15%, improving fish growth rate. They demonstrated that 6% of shrimp protein hydrolysate (SPH) can be included in diets for Nile tilapia without reducing growth performance. In the present study total replacement of SBM by SSM depressed growth performance of the tilapia. This discrepancy may be attributable to the method for processing the shrimp waste meal other than nutritional. Moreover, these results may be attributed to the high ash content of the diet, the high levels of chitin found in SSM. The exoskeleton of the shrimp is composed mainly of chitin, an N-acetylated glucosamine polysaccharide that forms part of the protein complex, and is considered to have low digestibility when fed to animals (Austin et al., 1981). Due to this low digestibility, chitin physically blocks the access of digestive enzymes to lipids and proteins, thus affecting the utilization of these nutrients (Castro et al., 1989; Karasov, 1990).

From the results of this study we concluded that SSM is a promising alternative protein source to SBM in hybrid tilapia fry. Based on the estimated economic benefits and nutrient utilization indices, biologically SSM can effectively replace SBM up to 60% in the diet of hybrid tilapia fry.

The present study showed that the survival of juvenile hybrid tilapia was 97% for 0, 50 and 67% SSM. Similarly, the results of feeding trials and digestibility studies conducted to assess the effect of replacing fish meal with 0, 5, 10, 20, 30 and 40% FSHM in practical diets for African catfish revealed that *C. gariepinus* fingerlings fed the various diets had similar survival of 95% (Nwanna, 2003).

Conclusion

The use of SSM as a replacement for fishmeal at 0–100% levels showed that the proximate composition of the feeds did not differ statistically, except for ash content. Nutritional evaluation of these feeds did show significant statistical difference among themselves, in terms of weight gain. The mortality rate during the feeding trial did not present any conclusive trend. Chemical analysis of tilapia fed on the diets for a period of sixty days also differs statistically in terms of protein and lipid content. The results of this study show that SSM could be used as a substitute for SBM up to 60% in a practical diet for tilapia fry. Further research is required to evaluate the influence of higher SSM inclusion on the digestive enzyme profile and evaluate the influence on the digestive enzyme profile and the effects of these diets on the innate immune system of hybrid tilapia.

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