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Full Length Research Paper

The effects of different protein sources on the growth of hybrid tilapia (*Oreochromis niloticus × O. aureus)* reared under fresh water and brackish water

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To evaluate the effects of different protein sources on the growth of hybrid tilapia (*Oreochromis niloticus × O. aureus*), three growth trials were conducted. In the present study, four approximately isonitrogenous (24% crude protein) and isoenergetic diets were fed to triplicate groups of 9 fish with a mean initial body weight of 1.85 ± 0.02 g/fish and 2.41 ± 0.06 g/fish in the brackish water and fresh water, respectively for 6 weeks. In fresh water, the weight gain was higher in the fish fed the control diet containing (FM) and lower in the fish fed diet with squid meal (SQM). There were no significant difference in weight gain for fish fed diet with shrimp meal (SM) and soybean meal (SBM). The fish fed diet containing SM and FM had higher body protein content but lower lipid than the fish fed diet SBM and SQM. In the brackish water, the growth performance of the fish fed the test diets FM and SM was significantly ($P < 0.05$) higher than that of the fish fed diets containing SBM and SQM. Fish fed SBM showed a significantly ($P < 0.05$) lower growth and higher mortality than those fed the other treatments. The fish fed diet SM had higher body protein content than those fed the other treatments. There were no differences in body lipid content among treatments. The blood osmolarity of the brackish water fish (340 mOsm/L) was higher than that of the fresh water fish (300 mOsm/L).

Key words: Hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, nutrition, fresh water, brackish water.

INTRODUCTION

Fishmeal (FM) still constitutes a substantial part of the feed formula for tilapia and many other species cultivated at commercial levels. The price of fishmeal raised up to 1600 US$ per ton in 2006 and it was forecasted to be higher in the future due to higher freight costs and due to government decision to reduce the fish catch in order to replenish fish supplies (Goettl, 2003). The rising cost and uncertain availability of FM reduced its use as a protein source for fish diets. This reduction has forced nutritionists and feed manufacturers to use less expensive, readily available plant protein to substitute fish meal (Lim and Dominy, 1989). Therefore, replacement of fishmeal with alternative proteins with sustainable supplier or less expensive protein sources would be beneficial in reducing feed costs.

Soybean is widely available, economical protein source with relatively high digestible protein and energy contents (Hertrampf and Piedad-Pascual, 2000). In most part of the world, soybean meal (SBM) is the main vegetable source of protein ingredients of commercial aquaculture feeds. However, different authors have reported problems of palatability or the existence of different components affecting SBM utilization in animals.

A few studies have been conducted to use shrimp meal as partial or total protein source for tilapia. El-Sayed (1998) reported that shrimp meal (SM) could totally replace fishmeal in test diets for Nile tilapia. Plascencia-Jatomea et al. (2002) demonstrated that shrimp head hydrolysate is a promising alternative protein source for tilapia feeding, improving growth ratio at dietary inclusion levels as high as 15%.

Asgard (1987) revealed that squid meal has the advantage of contributing to the palatability of the diet and improving the growth rate of trout and salmon when squid replaced fish meal in the diet. Kolkovski and

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Table 1. Composition of experimental diets for tilapia (*O. niloticus* × *O. aureus*) of the experiment.

Ingredients	Treatments			
	Fish meal	**Soybean meal**	**Shrimp meal**	**Squid meal**
Fish meal	31.5	-	-	-
Soybean meal	-	45.2	-	-
Shrimp meal	-	-	37.8	-
Squid meal	-	-	-	46.2
Vit. mix[a]	1.5	1.5	1.5	1.5
Min. mix[b]	4	4	4	4
Dextrin	4.5	4.5	4.5	4.5
Wheat flour	20	20	20	20
Corn starch	18	5.76	13.38	15.45
Oil[c]	6.5	7.85	7.75	3.1
Cellulose	14	11.19	11.07	5.25
Energy (Kcal/100 g)	354.5	354.5	354.5	354.5

[a]Calcium carbonate 2.1%, calcium phosphate dibasic 73.5%, citric acid 0.227%, cupric citrate 0.046%, ferric citrate (16 to17% 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 0.1335%, potassium iodine 0.001% and 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%, BHT 0.1% and α-cellulose 60.3%.[c]Cod liver oil / corn oil = 2:1.

Tandler (2000) reported that the use of squid protein hydrolysate (crude protein more than 50%) as a protein source in diets for sea bream larvae is not recommended.

Because of the deficiency of fresh water in the world, managing to conserve scarce fresh water resources for human consumption is necessary. The possibility of raising fast-growing tilapia in brackish water looks promising. Therefore, to obtain reference values, relationships between salinity and blood parameters values (osmolarity); in addition the effect of diet quality as reflected by protein level on those relationships should be taken into investigation (Verdegem et al, 1997).

The objectives of this present study were to evaluate the effects of plant and animal protein sources on the growth of hybrid tilapia (O*reochromis niloticus* × *O. aureus*) reared under fresh water and brackish water.

MATERIALS AND METHODS

Diets preparation

To evaluate the effects of total replacement of fish meal (crude protein 66%, crude fat 6.7%) by plant soybean meal (crude protein 48%**,** crude fat 7%) and animal shrimp meal (crude protein 58%, crude fat 18%) and squid meal (Crude protein 70%, Crude fat 15%) protein sources on the growth of hybrid tilapia (*O. niloticus x O. aureus*) reared under fresh water and brackish water, four isonitrogenous (24% crude protein) and isoenergetic diets (FM, SBM, SM, and SQM) were formulated (Table 1). The main protein sources (fish meal, soybean meal, shrimp meal and squid meal) already ground into meal were passed as particles through an n°40 (425 μm) mesh sieve. Mineral mix and vitamin mix were prepared in the laboratory (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. Then, the dough was passed through an extruder to make spaghetti, and dried at 35° for 8 h. The dried diet was packaged into plastic bag and stored frozen at -20°C until use. The proximate composition of the experimental diets is given in Table 2

Culture conditions

Fry hybrid tilapia (*O. niloticus* × *O. aureus)* were obtained from the Fresh Water Aquaculture Research Center (FARC) Taiwan and conditioned in our laboratory for three weeks in two fiber glass tanks (2000 L), one fresh water and the other containing brackish water. During this period fish were fed with a commercial diet obtained from Tairoun feed company in Tainan, Taiwan. At the beginning of the experiment, 24 glass tanks containing 68, 4 L of water (60 × 30 × 38 cm) were stocked with 9 fish each. The average fish weight was 1.85 ± 0.02 g in 12 glass tanks containing fresh water and 2.41 ± 0.06 g other 12 glass tanks containing in brackish water (20%). Each aquarium was part of a closed recirculating system maintained at 28 ± 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 1/2 of water in each tank was replaced with aerated fresh water or brackish water twice a day. The fish were fed 6% of body weight per day. Each diet was fed twice daily at 08:00 h and 17:00 h for 6 weeks to triplicate group of fish. Each group of fish was weighed at the beginning and every two weeks and the amount of diet fed was adjusted accordingly.

Laboratory analyses

Osmolarity analytical method

At the end of the first experiment I, blood was collected from the caudal vessels of all fish by using heparinized syringe and the samples kept in a refrigerator at 4°C for 24 h. After centrifugation at

Table 2. Proximate analysis of experimental diets fed tilapia (*O. niloticus* × *O. aureus)* of the first experiment.

Composition	Treatments			
	Fish meal	Soybean meal	Shrimp meal	Squid meal
Moisture	4.33	4.50	4.85	4.49
Crude protein*	24.28	24.02	25.53	25.68
Crude lipid*	7.99	6.67	10.47	8.56
Crude fiber*	10.62	10.80	9.30	7.23
Ash*	8.95	6.82	6.76	7.56
Gross Energy (Kcal/100 g)	441.0	423.7	446.5	454.6
Calculated Energy (Kcal/100 g**)**	354.5	354.5	354.5	354.5

*Presented as percentage of dry weight.

6000 rpm for 10 min, 150 µl of the supernatant was aspirated in a kit. Osmolality was determined by automatic osmometer system (KNAUER) calibrated at 400 mOsm/Kg using NaCl 12.687 g/kg distilled water (calibration solution). Following the blood sample the fish of each treatment were sacrified, a dorsal muscle sample was taken and frozen at -20℃ before analysis.

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 (Folch et 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℃ and a 105℃ oven.

Statistical analysis

The data were analyzed using the Statistical Analysis System (SAS-PC) (Joyner, 1985) by Two-way ANOVA (Analysis of Variance). 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

Mean weight gain, food conversion ratio (FCR), and survival for each treatment is shown in Table 3. The fresh water fish fed diet containing squid meal (SQM) gained weight significantly ($P < 0.05$) lower than those fed diet containing fishmeal (FM). Fish fed diet containing shrimp meal (SM) or soybean meal (SBM) gained weight slightly less than those fed diet containing fishmeal. There were no significant differences in weight gain for fish fed shrimp meal (SM) and soybean meal (SBM). There is no significant difference in the survival between fish raised in the fresh water. The FCR of the fish generally followed the same pattern as the weight gain did. The body composition of the tilapia fed with tests diets are shown in Table 4. The fish fed diet containing SM and FM had higher body protein content but lower lipid content than those fed diet with SBM and SQM. There was no difference in body ash content among treatments.

The brackish water fish fed diet with soybean showed higher mortality than the fish fed diet with fishmeal, shrimp meal and squid meal, where no mortality was observed during the experimental period. The fish fed diet containing fishmeal and shrimp meal gained weight significantly higher ($P < 0.05$) than fish fed diet with soybean meal and squid meal. Fish fed diet with SBM had the lowest weight gain. The fish fed diet containing SM had higher body protein content. The lowest body protein content was observed in fish fed diet with SBM (Table 4). There were no significant differences in body lipid and ash content among treatments.

Dietary protein sources have effects on the weight gain of tilapia (*O. niloticus* × *O. aureus*). The fish fed with FM had significantly higher weight gain ($P < 0.05$). The weight gain of Fish fed SM and SQM were not significantly different ($P > 0.05$). The lowest weight gain was observed in the fish fed with SBM. There were no different effects of salinities on the weight gain of tilapia (*O. niloticus* × *O. aureus*) (Table 5).

The brackish water fish showed high blood osmolality (340 mOsm/kg) than the fresh water fish (300 mOsm/kg).

DISCUSSION

The results of the present study showed that shrimp meal could totally replace FM in practical diets (24% crude protein and 5% crude lipid) for hybrid tilapia reared under fresh water and brackish water. In agreement with this finding, El-Sayed (1998) reported that shrimp meal (SM) could totally replace fishmeal in test diets for Nile tilapia. Shrimp head silage fed to *O. niloticus* is reported to be a promising alternate protein source in aqua-feeds at inclusion levels of 10 to 15% to improve fish growth significantly (Plascencia-Jatonea et al., 2002).

Recent efforts to incorporate plant ingredients into tilapia diets have concentrated on replacing fishmeal with a single ingredient. Goda et al. (2007) found that growth performance of Nile tilapia were reduced when replaced fishmeal by soybean meal (SBM), full-fat soybean (FFSB)

Table 3. Initial, final weight, weight gain (mean ± SE), FCR and survival of tilapia (*O. niloticus* × *O. aureus)* fed the experimental diets.

Treatments	Fresh water					Brackish water				
	Initial weight	Final weight	Weight gain	FCR	Survival (%)	Initial weight	Final weight	Weight gain	FCR	Survival (%)
Fish meal	2.42±0.10	9.86±0.44	308.73±34.01[a]	1.43	100	1.85±0.03	7.21±1.42	288.48±71.12[a]	1.08	100
Soybean meal	2.40±0.06	8.91±0.05	270.73±10.75[ab]	1.63	100	1.85±0.01	5.02±0.43	170.90±24.01[b]	1.82	33
Shrimp meal	2.41±0.05	9.34±0.77	287.95±25.27[ab]	1.53	100	1.85±0.04	7.30±0.65	294.52±42.76[a]	1.05	100
Squid meal	2.41±0.05	8.45±0.73	250.46±28.33[b]	1.76	100	1.85±0.02	6.37±0.55	243.58±25.34[ab]	1.27	100

[ab]Means in the same column with the different letter are significantly different ($P < 0.05$).

Table 4. Proximate analysis of dorsal muscle of tilapia (*O. niloticus* × *O. aureus)* fed the experimental diets.

Composition	Fresh water				Brackish water			
	Fish meal	Soybean meal	Shrimp meal	Squid meal	Fish meal	Soybean meal	Shrimp meal	Squid meal
Crude protein*	85.59	84.46	85.28	83.55	87.31	86.72	89.01	88.29
Crude lipid*	3.69	6.20	3.82	5.27	4.07	4.64	5.11	4.52
Ash*	6.90	6.84	6.84	6.46	5.77	5.65	5.49	5.46

* Presented as percentage of dry weight.

Table 5. Effects of dietary protein sources and salinities on the weight gain of tilapia *(O. niloticus* × *O. aureus).*

Variation	FM	SBM	SM	SQM	
Freshwater	308.73	270.73	291.19	250.46	280.28 [a]
Brackish water	288.48	170.90	294.52	243.58	299.37 [a]
	298.60 [a]	220.81 [c]	292.86 [ab]	247.02 [bc]	

abc: Mean in the same column with different letters are significantly different ($P<0.05$).

or corn gluten meal (CGM) under ideal protein profile. This is in agreement with the results of the present study which demonstrated that soybean meal as a sole protein source could not totally replace FM.

This study revealed that squid meal could not totally replace FM in the test diet containing 24% crude protein and 5% crude lipid. These results are in contradiction with those of Rodriguez-Serna et al. (1996) and Ajani et al. (2004) who found that animal by-product can replace up to 100% of fishmeal in the diets of tilapia. There is no available reference on the use of squid meal as main protein source for hybrid tilapia. However, Kolkovski and Tandler (2000) reported that squid protein hydrolysate included in micro diet sat 50% or more cannot serve as an efficient dietary component in seabream larvae. Asgead (1987) concluded that squid meal is considered to be a valuable feedstuff in diets for rainbow trout and salmon based on the chemical composition of squid meal and its organoleptic properties.

The present study showed that there were non-significant effects of salinities on the weight gain of tilapia (*O. niloticus* × *O. aureus*). Similarly, Larumbe-Moran et al. (2010) reported a non-significant tendency to increased weight gain was observed as water salinity increased, suggesting that the salinity of the culture environment does not influence dietary protein requirements in Nile tilapia *O. niloticus* fry.

The growth data obtained in the present study were generally higher than that obtained by some previous studies (Shiau et al., 1987; Shiau and Huang, 1989; Shiau and Huang, 1990). For instance, hybrid tilapia *O. niloticus* × *O. aureus* at initial weight of 1.24 g had a weight gain of 143.84% in 8 weeks when the fish were fed 24% protein diets (Shiau et al., 1987). Shiau and Huang (1989) reported that tilapia at an initial weight of 2.88 g fed 24% protein diet had reached a weight gain of 188.82% in 8 weeks. In another trial, a weight gain of 141.06% was observed within 8 weeks with an initial weight of 1.61 g fed two dietary protein levels, 24 and 21% (Shiau and Huang, 1990). In the present study, tilapia with an initial weight of 1.85 ± 0.02 g in brackish water and 2.41 ± 0.06 g in fresh water fed diets with 24% protein diet had weight gains of 238.12 and 356.66%, respectively in 6 weeks.

In this experiment, we found that osmolality of the brackish water (20 $^{0}/_{00}$) fish (340 mOsm/kg) was higher than that of the fresh water fish (300 mOsm/kg). Verdegem et al. (1997) reported plasma osmolality 300 mOsm/kg for fresh water tilapia, which is similar to our results and 305 mOsm/kg for fish cultured in brackish water (19 $^{0}/_{00}$). Magdeldin et al. (2007) reported that plasma osmolality showed averages of 315 mOsm/kg for fish acclimated to fresh water (FW), which is a bit higher than our results, and 325 mOsm/kg for the fish cultured in 100% SW. The brackish water treatment groups in the present study showed higher osmolalities in the proportion of the higher water salinity. According to Al-Amoudi (1987), the higher blood osmolality in the brackish water fish (340 mOsm/kg) is the overall result of dehydratation, ions influx and intestinal uptake of monovalent ions. It implies that the fish cultured in saline environment need more energy for maintenance. Because of the higher maintenance requirements, it is expected that fast growing tilapia cultured in brackish water will also exhibit a higher maintenance metabolism (Verdegem et al., 1997). Moreover, it was reported that euryhaline tilapia reacted to increase salinity by increasing total plasma protein. Plasma osmolality is considered an excellent indicator of the adaptability of fish to changes in environmental salinity (Verdegem et al., 1997).

The results of this study indicate that replacement of fishmeal with a single component of processed plant ingredients results in lower growth performance of tilapia cultured in the FW and SW. The formulation of complex diets for tilapia will provide new opportunities for the value-added processing of regionally available crops, reduce the requirement of the aquaculture feed industry for fishmeal and enhance the sustainability of the industry.

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