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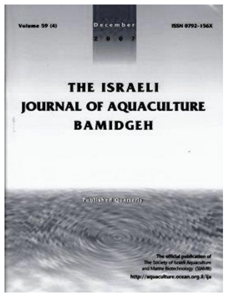

Use of Oyster Processing Byproduct to Replace Fish Meal and Minerals in the Diet of Nile Tilapia *Oreochromis niloticus* Fry

Ernestina M. Peralta[1], Barry Leonard M. Tumbokon[2], Augusto E. Serrano, Jr.[1,2]

[1]Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines

[2]National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao, Iloilo, Philippines

[3]Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines

Keywords: oyster processing residue; broken line model; fish meal substitute, mineral replacement; Nile tilapia fry

Abstract

A byproduct from processing oysters (*Crassostrea iridalei*) for human consumption was evaluated as a possible protein source to replace fishmeal in the Nile tilapia diet. In an 8 week-feeding trial, fish were fed 7 experimental diets containing various inclusion levels of oyster byproduct (OBP) which replaced fish meal. Diet 1 contained 0% OBP, while Diets 2-6 contained 5%, 10%, 15%, 20%, 25% OBP respectively; Diet 7 was a fishmeal-based diet with OBP as the only source of dietary minerals. The optimum amount of OBP inclusion levels producing maximal responses i.e. final average body weight (FABW), weight gain (WG), specific growth rate (SGR), food conversion rate (FCR) and protein efficiency rate (PER) was estimated using the Broken Line Model of analysis. Average optimum level was 17.0% of dietary OBP (fishmeal replacement of 63.8%). Diet 4 (15% OBP= 56.4% fishmeal replacement) resulted in statistically similar growth and feed efficiency parameters to Diets 1-3 and were closest to the estimated optimum inclusion level. Total mineral replacement by OBP (Diet 7) resulted in differing results; SGR values were statistically similar to those of the other dietary treatments; however FABW was inferior, and values for WG, FCR and PER were intermediate. In conclusion, OBP could replace as much as 63.8% by weight of dietary fishmeal and probably a large proportion of the mineral mix in the diet of Nile tilapia fry.

* Augusto E. Serrano, Jr., e-mail: aeserrano@up.edu.ph

Introduction

Increasing demand for fishmeal in animal industries including aquaculture has contributed to its present scarcity and high price. This is exacerbated by the fact that capture fisheries are nearing or exceeding maximum sustainable yields and yields are diminishing. Aquaculture is increasingly filling the shortfall of fish production for human consumption however it is also suffering from the conception that it is a protein-consuming industry rather than a protein-producing one. In addition many countries, including the Philippines, import fishmeal. Aquaculture is heavily dependent on imported fishmeal especially from the USA, Peru, and Chile (Sumagaysay-Chavoso, 2007). There is a need to reduce the use of fishmeal in aquafeeds and replace it with non-conventional protein sources and if possible, with resources which have reduced demand. Oysters are the most common bivalves farmed in the Philippines where they are traditionally cultured in natural beds in rivers and coasts. Compared to other aquaculture farming (e.g milkfish and shrimp culture), oyster culture is economically viable in coastal areas due to low input and capitalization. Oyster production in the Philippines in 2011 was 21.5 MT (BAS 2011). Despite their low cost and high nutritional content, oysters are not popular due to the inherent characteristic flavor. It has been a challenge to fish processing scientists to develop new attractive oyster-based products for human consumption with higher market value. Oyster powder which is available year round in the Philippines, is currently being incorporated in food. A protein rich byproduct of oyster powder production appears to be a good partial replacement product for fishmeal in aquafeeds. Since this byproduct is an animal protein source the EAA levels are superior to those of plant sources. The present study aims to evaluate the oyster processing byproduct as a cost effective, fishmeal replacement in the diet of Nile tilapia fry.

Materials and Methods

Sample collection and treatment. Live oysters (*Crassostrea iredalei*) were harvested from commercial farms in Brgy Basiao Ivisan, Capiz Philippines and immediately transported to the laboratory. Oysters were scrubbed with clean water to remove mud and debris from their shells. They were then dipped for 15 min in hot water (60^{o}C) to facilitate shell opening and shucking; oyster meat (8.9 $\pm$ 1.02 g) was removed from the shell and the intravalvular fluid was drained prior to extraction.

Approximately 5 kg drained meat was mixed with water at a ratio of 1:0.5 (meat: water) and boiled for 5 min. The sample was allowed to cool prior to homogenization in a household blender, and then filtered using a fine mesh separating the residue (insoluble) and extract (soluble). The residue was then finely chopped, dried in a cabinet drier at 60^{o}C for 8 h, ground into fine powder using a mechanical grinder, packed in polyethylene bags, and stored at room temperature until feed preparation for the Nile tilapia fry.

Preparation of experimental feeds. Seven practical diets were formulated to contain between 33-36% crude protein which fulfill juvenile tilapia requirements. The diets were designed to provide 5%-7% crude fat containing varying amounts of the oyster byproduct (OBP). Diets containing graded inclusion levels of OBP replacing fish meal were prepared as follows: Diet 1 (control diet, 0% OBP) while Diets 2-6 contained 5%, 10%, 15%, 20%, 25% OBP; Diet 7 was a fishmeal-based diet similar to the control with OBP as the only source of dietary minerals. The diets were formulated based on a basal diet described by Santiago et al. (1982)(Table 1). The ingredients were pulverized and passed through a sieve (150 μm) prior to mixing. All dry ingredients were thoroughly mixed and then liquid ingredients were added. Gelatinized cornstarch was added as a final step before pelletizing. The moistened mixture was pelleted in a meat grinder and oven-dried at 60^{o}C for 16–18 h to about 10% moisture. Diets were then crumbled into appropriate sizes, sealed in plastic bags, and stored at -20^{o}C until use.

Table 1. Composition and proximate analysis of practical diets fed to *O. niloticus* fry for 8 weeks.

Diet	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Ingredient (g)	*0*	*50*	*100*	*150*	*200*	*250*	*0-MIN*
Danish fish meal	265.8	215.8	165.8	115.8	65.8	15.8	252.8
Squid meal	81.0	81.0	81.0	81.0	81.0	81.0	81.0
Soybean meal	260.0	260.0	260.0	260.0	260.0	260.0	260.0
Oyster BP	0.0	50.0	100.0	150.0	200.0	250.0	13.0
Copra meal	152.5	152.5	152.5	152.5	152.5	152.5	152.5
Corn starch	187.5	187.5	187.5	187.5	187.5	187.5	217.5
Cod liver oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mix	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Mineral mix	30.0	30.0	30.0	30.0	30.0	30.0	0.0
Antioxidant	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
	Proximate composition (%, dry weight basis)						
Moisture	6.30	6.39	6.77	7.29	7.51	6.88	8.45
Crude protein	40.51	40.62	41.36	40.80	41.19	42.20	40.77
Crude Fat	4.12	4.77	4.70	4.70	4.01	4.84	3.48
Crude Fiber	3.36	3.42	3.48	3.56	3.62	3.60	2.56
Ash	9.45	8.84	8.46	8.02	7.51	7.09	7.81

Experimental tilapia and set up. Nile tilapia fry (21 days old) were purchased from SEAFDEC-AQD, Tigbauan, Iloilo, acclimatized to the experimental conditions and the basal diet for 10 days. The fish were randomly stocked in twenty 60 L tanks (25 fry/tank). Experimental diets were fed to 3 replicate groups of fish twice daily. At the start and every 15 days, fish were bulk-weighed, and feeding rate was adjusted for the next 15-day feeding period until termination of the experiment after 8 weeks. The feeding trial was conducted in a closed recirculating system. 70% of the water was replaced and physical filters cleaned every other day. Uneaten feed and feces were siphoned-off each morning before feeding. Replacement water was chlorinated tap water (100 ppm NaClO) left to aerate for 3 days for dechlorination. Water quality indices were monitored by measuring temperature and pH twice a day, dissolved oxygen (D.O.) twice a week, and nitrite and total ammonia weekly, using commercial kits.

Growth performance parameters. Growth performance and feed utilization were estimated using the following formulae:

Weight gain, WG (g) **=** FABW – IABW

Specific Growth Rate (SGR, %/day) = 100*(ln FABW−ln IABW)/(T_2– T_1)]

FCR = FI(g) /WG (g)

PER = WG (g) / FI*dietary CP in decimal

Survival (%) = 100*(Survived fish / Initial fish stocked)

Where: FABW = final average body weight (g) and
IABW = initial average body weight (g)
T_2 = Final time (in days)
T_1 = Initial time (in days)
FI= total individual feed intake (g)

Proximate analysis. Oyster powder, experimental feeds, and carcass were subjected to proximate analysis. To obtain final carcass, fish from each replicate tank were sacrificed and pooled. Samples were oven-dried at 60°C and pulverized before proximate analysis. Moisture was measured using a thermo-balance (Mettler Toledo HB43 halogen moisture analyzer). Ash content was determined after incineration in a muffle furnace at 550°C for 12 h (AOAC, 1990). Crude protein was measured after block digestion and steam distillation using Foss Tecator™ digestion system and Foss Kjeltec™ 8200 auto-distillation unit. Crude fat was extracted using Foss Soxtec™ 2050 automatic system and fiber was determined using Foss Fibertec™ 2010 system.

Statistical analysis. Data were presented as mean ± standard error of the mean (SEM) and were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Data that passed the tests were subjected to one way Analysis of Variance (ANOVA) while those that did not were subjected to transformation before performing one-way ANOVA tests. Growth indices (FABW, WG, SGR), feed utilization parameters (FCR, protein, lipid and energy retention rates), body composition and survival rates were subjected to ANOVA using α=0.05. Tukey test was performed to rank the mean values.

Results

Effects of increasing OBP inclusion. Survival of experimental fish was 100% for all dietary treatments. The effects of increasing levels of OBP inclusion could be observed from the results of fish fed diets 1-6. FABW and WG results in fish fed diets 1-4 were similar, while fish fed diet 6 had lower values but not significantly different from those fed diet 5 (Table 2). SGR was similar in fish fed Diets 1-5, but was inferior in Diet 6 group. FCR of fish fed Diets 1-5 was not statistically different; FCR in fish fed Diet 6 was significantly different (FCR was worst). Fish fed Diets 1-5 exhibited similar PER values while lowest value was seen in fish fed Diet 6 but was not statistically different to those fed Diet 5. The inclusion level of OBP that resulted in the maximum values of performance indicators (i.e. FABW, WG, SGR, FCR and PER) were determined by the Broken Line analysis (Table 3) and the average value was 16.9% ± 0.3% of OBP.

Increasing OBP inclusion did not significantly affect whole body moisture and protein composition. In contrast, both whole body fat and ash were affected by increasing dietary OBP. Diets containing 15%-25% (Diets 4 to 6) resulted in significantly higher whole body fat than the other diets. Whole body ash was lower in fish fed diets containing OBP from 5%-25% than in those fed Diets 1 and 7 which contained no or minimal OBP.

Table 2. Performance of young Nile tilapia fed with diets containing various inclusion levels of oyster byproducts (OBP) replacing fish meal.

Diet	*IABW*	*FABW*	*WG*	*SGR*	*FCR*	*PER*	*Survival (%)*
1 (0%)	0.23 ± 0.00	12.89 ± 0.53^{a}	12.66 ± 0.54^{a}	4.98 ± 0.06^{a}	0.96 ± 0.01^{c}	2.58 ± 0.03^{a}	100.0
2 (5%)	0.23 ± 0.00	11.43 ± 0.23^{ab}	11.20 ± 0.23^{ab}	4.89 ± 0.03^{a}	1.0 ± 0.01^{bc}	2.46 ± 0.03^{ab}	100.0
3 (10%)	0.23 ± 0.01	10.93 ± 0.42^{bc}	10.71 ± 0.42^{bc}	4.90 ± 0.05^{a}	1.01 ± 0.03^{bc}	2.41 ± 0.07^{ab}	100.0
4 (15%)	0.23 ± 0.01	11.29 ± 0.41^{ab}	11.06 ± 0.41^{ab}	4.87 ± 0.11^{a}	1.01 ± 0.04^{abc}	2.43 ± 0.09^{ab}	100.0
5 (20%)	0.23 ± 0.01	9.87 ± 0.45^{bcd}	9.64 ± 0.45^{bcd}	4.79 ± 0.02^{ab}	1.06 ± 0.02^{abc}	2.29 ± 0.04^{bc}	100.0
6 (25%)	0.23 ± 0.01	8.52 ± 0.22^{d}	8.29 ± 0.21^{d}	4.61 ± 0.02^{b}	1.11 ± 0.03^{a}	2.13 ± 0.06^{c}	100.0
7 (-min)	0.22 ± 0.01	9.44 ± 0.39^{cd}	9.22 ± 0.38^{cd}	4.76 ± 0.04^{ab}	1.07 ± 0.01^{ab}	2.31 ± 0.03^{bc}	100.0

Table 3. Whole body composition of *O. niloticus* fry fed the various experimental diets for 60 days (g/kg)

Diet	Protein	Lipids	Ash	Moisture
1 (0%)	147 ± 7	32.9 ± 1.0^{cd}	35.0 ± 2.5^{a}	774 ± 8
2 (5%)	139 ± 4	30.7 ± 0.3^{d}	32.4 ± 0.2^{ab}	786 ± 6
3 (10%)	139 ± 4	33.8 ± 2.4^{cd}	32.4 ± 1.0^{ab}	779 ± 8
4 (15%)	143 ± 2	38.7 ± 0.3^{bc}	29.9 ± 0.2^{ab}	776 ± 1
5 (20%)	152 ± 1	44.0 ± 0.4^{ab}	27.9 ± 0.4^{b}	767 ± 3
6 (25%)	142 ± 2	46.2 ± 2.3^{a}	27.0 ± 1.7^{b}	770 ± 3
7 (-min)	147 ± 7	25.9 ± 0.9^{d}	34.8 ± 2.2^{a}	781 ± 10

Table 4. Estimated I_{max} values (% inclusion) of oyster byproduct and equivalent fish meal replacement calculated using the broken line model.

Indicator	I_{max} *(%)*	*Equivalent fishmeal replacement (%)*
FABW	17.6	66.2
WG	17.5	65.8
SGR	16.3	61.3
FCR	16.3	61.3
PER	16.9	63.6
Average	16.9	63.6
SEM	± 0.3	± 1.1

Effect of complete replacement of minerals by OBP. The effect of mineral replacement by OBP could be observed by comparing results of Diet 1 and Diet 7 (Table 2). Diet 1 contained the maximum level of fishmeal and the required vitamin mix but no OBP while Diet 7 was composed of the same maximum level of fishmeal but the mineral mix was wholly replaced by OBP. Almost all performance indicator values were superior in fish fed Diet 1 to those fed Diet 7; the exception was SGR where values were similar.

Discussion

Results indicated that 17% inclusion rate (equivalent to 64% FM replacement) oyster byproduct constituted a suitable replacement level for FM (Table 4). Poultry byproducts replaced 67% FM protein in Florida pompano (Riche 2015) and gibel carp *Carassius auratus gibelio* (Yang et al 2006). 67% FM protein replacement was also found suitable for Nile tilapia (Fasakin et al 2005) and red drum *Sciaenops ocellatus* when substituted in an FM/soybean meal diet (Kureshy et al 2000). In these studies 67% replacement was the highest rate of substitution and a reduction from their estimated values was not observed. The present study included dietary treatments containing the ingredient of interest at a higher inclusion level than the estimated optimum level similar to a study on Florida pompano (diet formulation of Riche (2015). Performance parameter values decreased in Nile tilapia fed either Diet 4 or Diet 5 and were strongly reduced in Diet 6 (equivalent to 56%, 75% and 94% FM substitution) in the present study. Broken line analysis was used to estimate the I_{max} for the OBP.

Vegetal protein and rendered animal protein combined, replaced total fish meal protein in catfish (Havasi et al., 2015), while more than 40% substitution of poultry byproduct (PBM) for FM resulted in reduced weight gain and protein retention in the African catfish *Clarias gariepinus*. This was probably due to decreasing dietary Lysine and Methionine with increasing substitution rates (Abdel-Warith et al 2001). This could also be a possible cause of decreased performance in tilapia when FM substitution was between 56%-94%. Digestibility of the substituted ingredient, which is affected by the quality of protein, is also critical. For poultry byproduct, the rendering process can result in loss of nutritional value due to reduction in AA availability (Riche 2015); Lysine level is particularly sensitive. In the present study, spray drying may have contributed to major loss of nutritional value. The same observation was made in Florida pompano *Trachino tuscarolinus* L. (Rossi and Davis 2002) and rainbow trout (Steffens 1994) where decreased performance was observed with increasing PBM; the reduced performance was ameliorated by the addition of supplemental Lysine and Methionine.

Whole body ash content decreased as inclusion level of OBP increased; this may be the result of decreased contribution of ash by the fish meal. One of the problems in fish meal-based diets is that the bones and scales go into the process of producing the meal. This was one of the advantages of incorporating OBP in the tilapia diet. The reason for increased whole body fat of fish fed diets containing 15% to 25% OBP (Diets 4, 5, and 6) is not very clear since the lipid content of OBP was moderate (8.65% ± 0.02, unpubl. data) and could be similar to that of fish meal (8-11%). It could be that the OBP contained certain minerals or vitamins that play as cofactor of enzymes involved in lipid synthesis; this remains to be studied in detail.

In general, OBP could not completely replace the mineral requirements of Nile tilapia. However, although the values of growth parameters significantly different, the actual values were similar as were the SGR results, it could be deduced that a large portion of the dietary minerals could be replaced by OBP. Future experiments in which replacement levels are varied are needed to determine this level.

In conclusion, the estimated optimum amount of OBP inclusion levels giving maximal response was 17.0% of dietary OBP, equivalent to fishmeal replacement of 63.8%. Increasing dietary OBP resulted in decreased whole body ash and increased whole body fat. OBP could partially but not totally replace the dietary mineral requirement in the diet of the Nile tilapia.

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