

Use of Biochemically Improved Shrimp Industry Waste in Fry Tilapia (*Oreochromis Niloticus*, Linnaeus 1758) Diets: Effects on Growth Performance and Carcass Composition

Dior DIOP

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop

UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR

Tel: 221-33-864-5981

Jean FALL (Corresponding author)

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop

UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR

Tel: 221-77-956-4339 E-mail: kagoshima77@yahoo.com

Mariama SAGNE

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop,

UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR,

Tel: 221-33-864-5981

Abdoulaye LOUM

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop,

UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR,

Tel: 221-33-864-5981

Diégane NDONG

Agence Nationale de l'Aquaculture Cité Comico 3 VDN Liberté 6 Extension LOT N1

Tel: 221-338-698-450

Malick DIOUF

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop,
UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR,

Tel: 221-33-864-5981

Alassane SARR

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh
Anta Diop, UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR

Tel: 221-33-864-5981

Nicolas AYEISSOU

Laboratoire d'Analyses et d'Essais/ Ecole Supérieure Polytechnique

Université Cheikh Anta Diop BP 5085 Dakar Fann

Tel: +221-825-0894

Omar Thiom THIAW

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop,
UCAD II bâtiment pédagogique/Rez de chaussée BP 5005 DAKAR,

Tel: 221-33-864-5981

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Abstract

To evaluate the nutritional quality of the shrimp by-products four approximately isonitrogenous ($32 \pm 2\%$ crude protein) diets containing different types of shrimp waste meal were fed to duplicate groups of ten fry with an initial mean weight $1.43 \pm 0.22\text{g}$ /fish for forty two (42) days. Fishes fed with diet D had the highest mean weight gain (MWG) and specific growth rate (SGR) and those fed with the control diet A had the lowest MWG and SGR.

There were no noticeable change in food conversion ratio (FCR) of the fishes fed with diets B, C and D which are significantly different from the FCR of those that fed with control diet A. The survival of fry tilapia ranged from 70 to 100%, being 70% for D, 80% for B, 90% for C and 100% for A. There were no significant differences in body protein content among the fish fed with diets B, C, D and the initial fish. The lowest body protein was obtained in the fish fed with control diet. The tilapia fed with control diet had the highest body lipid content as compared to the initial fish. However, there were no significant differences in body lipid content among the fish fed test diets B, C and D as compared to the initial fish. The variations in body ash content of initial fish and those fed with test diets were significantly different. The initial fish had the highest body ash content.

Keywords: Tilapia, *Oreochromis niloticus*, Shrimp waste Meal, Recirculating system

1. Introduction

Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) is one of the most cultured fish in tropical and subtropical regions in the world. Tilapia production (except Africa) was 2.4 million metric tons in 2008 representing 8% of the total freshwater and brackish water fish production (FAO, 2010). Tilapia production in Senegal was only 200 metric tons in 2010 (NAA, 2011).

Shrimp world production was 6 million tons in 2006 (FAO, 2009). In Senegal, shrimp production was estimated to be 4657 metric tons (DPM, 2009). The shellfish-processing industry in Senegal generates about 2794.2 metric tons of waste per year. Shrimp waste considered as an important animal protein source (Fanimio *et al.* 2000) is essentially composed of heads, shells, appendages and tails, which are potential feedstuff due its minerals, pigments, lipid, and flavors contents. Prawn shell waste protein is rich in essential amino acids (Forster 1975; Penafiora 1989) and the oil extracted from shrimp head contains polyunsaturated fatty acids (PUFA) essential for fish and shellfish (Joseph & Meyers 1975; Joseph & Williams 1975). The most important waste material in shrimp processing industries is head waste, which represents approximately 35–45% of the whole shrimp weight (Meyers, 1986). Enhanced nutritional value and digestibility of fermented shrimp head waste by fish was reported by Plascencia-Jatomea *et al.* (2002) and Nwanna (2003). Synowiecki & Al-Khateeb (2000) revealed that shrimp head waste contents high chitin (11%, dry weight basis).

This abundant waste may pose a disposal problem due to the ease of spoilage and contributes to the overall cost of the production. To solve this problem it is important to transform waste into either silage or mill and use this material in the formulation of fish feeds. However, the use of shrimp head meal in the formulation of fish feed is not recommended due to its high fiber and ash contents, which results in the formation of weak pellets (Meyers, 1986) with poor stability in water. According to Fagbenro & Bello-Olusoji (1997), fiber and ash content could be reduced by transformation of shrimp head waste into silage by the process of fermentation. A similar observation on the reduction of ash and fiber in silage was also made by Fox (1994) using formic acid. Consequently, the pellets made of shrimp head silage possessed better structural stability in water.

Shrimp waste is an excellent source of protein (50–65%, dry weight basis), nutritive

components and enzymes (Fanimó *et al.* 2000 and Heu *et al.* 2003). Therefore, using of such shrimp wastes has drawn much interest from researchers in recent years. Enzymatic hydrolysis method (Cano-Lopez *et al.* 1987), trypsin (Synowiecki & Al-Khateeb 2000) and certain proteolytic enzymes such as alcalase (Mizani *et al.* 2005 and Guerard *et al.* 2007) have been used to extract the proteins from shrimp waste. These methods are very expensive in commercial enzymes. There is now a need to develop an efficient and economical method for extracting proteins from shrimp waste. Little is known on the protein quality of this important feedstuff.

The biochemical improvement of shrimp waste in the feed processing plant for fish is of economic and environmental benefits. This provides peptides that are more digestible than proteins (Heu *et al.* 2003). The generation of peptides anti-oxidative activity leads to improved food preservation by inhibiting the oxidation of nutrients, especially lipids and fatty acids (Zo Randriamahatody, 2011). The small-scale production can be achieved through the establishment of a small unit of less expensive feed manufacture on the farm.

The aim of the present study is to evaluate the nutritional value of the diets with respect to weight gain, survival and carcass composition in a feeding trial with Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758).

2. Material and Methods

2.1 Culture Conditions

Male sex-reversed tilapia fry (*Oreochromis niloticus*) of initial weight 1.43 ± 0.22 g were collected from the tilapia hatchery in Richard Toll, Saint Louis, Senegal. Fish were acclimated to the experimental conditions for a period of two weeks. During this period, they were fed with a commercial diet as previously occurred at the above mentioned hatchery. To determine the initial body composition, 20 selected fish on a random basis were collected, filleted and stored at $-18\text{ }^{\circ}\text{C}$ for proximate analysis according to the AOAC (1984). At the beginning of the experiment, 100 tilapia fry were randomly divided into five different groups with two replicates containing 10 fish/each. Fish were kept in 10 glass tank (50 x 40 x 30 cm) containers (50 L). Each aquarium was put in a re-circulating system maintained at $30 \pm 1\text{ }^{\circ}\text{C}$. An air stone continuously aerated each of both aquariums. All aquaria were cleaned up every day in the morning and the afternoon by siphoning off accumulated waste materials. Fish were then fed with 10% of body weight per day and gradually decreased to 4% per day. Each diet was fed twice a day at 08:00 (a.m.) and 17:00 (p.m.) for 42 days to duplicate groups of fish. On the other hand, 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, while fluorescent ceiling lights supplied the illumination. After 6 weeks of feeding, fish were taken out from each treatment; the dorsal muscle tissue of each was dissected and used for carcass composition analysis purposes.

2.2 Raw Materials

Shrimp (*Farfantepenaeus notialis*) were caught in the southern Casamance in Senegal and brought to the processing company of fishery products IKAGEL located at Mballing in the

department of Mbour, Senegal. Once landed, shrimp were checked to confirm their quality level. After processing, heads, shells, tails and appendages are generated. These wastes were kindly donated for our study. The wastes were frozen and transported to the IUPA (Institut Universitaire de Pêche et d'Aquaculture) laboratory.

2.3 Preparation of shrimp meal

At the laboratory, the shrimp by-products underwent different treatments namely cooking, steam and silage. Shrimp by-products were divided into 3 batches of 5 kg each after thawing.

In the first batch, shrimp byproducts were cooked in water at 100 °C for 20 minutes. After centrifugation, the supernatant was drained and the solid part was dried under the sunlight for 3 days before grinding and sieving.

The second batch was treated according to Cavalheiro *et al.* 2007. Briefly, shrimp by-product were mixed with 17% acetic acid and stirred at regular intervals of 2 h for 24 h at room temperature (30 ± 1 °C) to accelerate the liquefaction process by the enzymes naturally present. After this period, the silage thus obtained was dried with forced air circulation for 3 days and then ground before being sieved to obtain flour.

The third batch was treated according to Mirzah, 1997 with some modifications. In fact, shrimp byproducts were steamed using a steamer for 30 minutes. After this operation, the steamed shrimp product was dried under sunlight for 2 days and then ground and sieved.

The different flours obtained were analyzed biochemically according to the standard method AOAC (1984) to know their protein levels.

2.4 Diet Preparation

Four diets were formulated to contain 32 ± 2% digestible proteins. Main protein sources were fishmeal: 57.7 % crude protein, cooked shrimp byproduct: 48.12 % crude protein, Shrimp byproduct silage: 45.5 % crude protein, steamed shrimp byproduct: 39.38 % crude protein and maize meal: 10.8 % crude protein. There were grounded into mill and passed as particles through n°. 40 (425 µm) mesh sieve. Mineral and vitamin premix were purchased from Aquavet feed Company, Thiès, Senegal. After all, ingredients were thoroughly mixed, and appropriate quantity of water provided (30% for 100 g of mixed ingredients). Diets were supplemented with 6% of mixture of fish oil (FO) and vegetable oil (VO) (Table 1).

Table 1. Composition of experimental diets for tilapia (*Oreochromis niloticus*)

Ingredients	A	B	C	D
Fishmeal	340	170	170	170
Maize meal	480	300	280	200
Shrimp waste meal	0	350	370	450
Cellulose	100	100	100	100
Fish oil	20	20	20	20
Vegetable oil (PO +SO)	40	40	40	40
Vit mix ^a	10	10	10	10
Min mix ^b	10	10	10	10

PO= Peanut Oil

SO= Soybean Oil

^a= vit A 250000 UI; vit D3 250000UI; vit E 5000mg ; vit B1 100mg ; vit B2 400mg ; vit B3(pp) 1000mg ; vit B5 pantode Ca2000mg ; vit B6 300mg ; vit K3 1000g ; vit C 5000mg ; H biotin 15mg ; choline 100g ; anti-oxydant (BHT), crushed and calcined attapulгите qs 1000mg;;

^b= phosphorus 7% ; calcium 17% ; sodium 1,5% ; potassium 4,6% ; magnesium 7,5% ; manganese 738mg ; zinc 3000mg ; iron 4000mg ; copper 750mg ; iodine 5mg ; cobalt 208mg ; calcined and ground attapulгите qs 1000g; fluorine 1.5% (approximately).

Dough was passed through an extruder to produce spaghetti and dried at 37 °C for two days. So, the concerned dried diet was packaged into plastic bag and stored frozen until its usage. 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 Kjeltex 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). Ash and moisture were determined by conventional methods using muffle furnace at 505 °C and an oven at 105 °C.

2.5 Growth Parameters

Growth response parameters were calculated as follows: Weight gain (%) = 100* ((final mean body weight - initial mean body weight)/ initial mean body weight); Specific Growth Rate (SGR, % /day) = 100* ((ln Wt- ln Wi) /T), 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 / total wet weight gain g/ fish. Survival rate (%) = 100* (number of fish which survived/initial number of fish).

2.6 Water Quality Measurement

Water temperature and dissolved oxygen were measured each following day using YSI Model 58 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA).

2.7 Statistical Analysis

Data were analyzed using the following statistic system (SAS-PC) (Joyner, 1985) and subjected to one-way analysis of variance (ANOVA). Treatment effects were considered significant at P<0.05; Duncan's test was used to compare significant difference among treatments.

3. Results

3.1 Water Quality Control

Water quality parameters monitored were similar in all treatments and the values (Temperature 30 ± 1 °C) and dissolved oxygen (6 ± 1 mg/L).

3.2 Comparison of the Diet Composition

The proximate composition of the diets are marginally ($P > 0.05$) different. Crude protein levels of the diets varied from 30.00 to 34.46 and the lipid content from 7.66 to 11.23, while crude fiber ranged between 2.54 and 8.99 and. Ash of the diets varied from 15.28 to 22.72. There was no significant difference ($P > 0.05$) in dry matter of the various diets (Table 2).

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

Treatments				
Composition	A	B	C	D
Dry matter*	96	95	94	95
Crude fiber*	3	6	7	9
Crude lipid*	9	10	11	8
Crude protein*	30	32	33	34
Ash*	15	21	18	23
Calculated Energy (cal/kg)	213	243	262	243

* Presented in percentage of dry weight.

3.3 Comparison of the Growth Parameters

Fishes fed with diet D had the highest mean weight gain (MWG) and specific growth rate (SGR) which did not vary significantly ($P > 0.05$) from the MWG and SGR of fishes fed with diets B and C but differed remarkably ($P < 0.05$) from the MWG and SGR of fishes fed with control diet A. However, there were no noticeable change ($P > 0.05$) in food conversion ratio (FCR) of the fishes fed with diets B, C and D which are significantly different from the FCR of those that fed with control diet A. The survival of fry tilapia ranged from 70 to 100%, being 70% for D, 80% for B, 90% for C and 100% for A (Table 3).

Table 3. Initial and final mean weight, mean weight gain, SGR, FCR and survival of Tilapia (*Oreochromis niloticus*)

Treatments				
	A	B	C	D
Initial mean weight g/ fish	1.26 ± 0.00	1.23 ± 0.01	1.26 ± 0.03	1.26 ± 0.01
Final mean weight g/fish	3.31 ± 0.51	4.95 ± 0.63	5.44 ± 0.44	6.22 ± 0.45
Mean weight Gain g/fish	2.05 ± 0.52	3.72 ± 0.63	4.18 ± 0.47	4.96 ± 0.47
Mean weight Gain (%)	163.47 ± 41.95 ^b	303.94 ± 48.40 ^{ab}	331.85 ± 44.34 ^{ab}	393.88 ± 41.35 ^a
SGR	2.29 ± 0.38 ^b	3.31 ± 0.29 ^{ab}	3.48 ± 0.24 ^a	3.79 ± 0.20 ^a
FCR	3.35 ^b	2.17 ^a	2.18 ^a	1.69 ^a
Survival	100	80	90	70

^{ab} Means in the same column with the different letter are significantly different ($P < 0.05$).

3.4 Comparison of the Carcass Composition

Table 4 displays the initial and final body compositions of whole fish. There were no significant differences ($P>0.05$) in body protein content among the fish fed with diets B, C, D and the initial fish. The lowest body protein was obtained in the fish fed with control diet without shrimp waste meal. The tilapia fed with control diet 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 B, C and D compared to the body lipid content of the initial fish. The variations in body ash content of initial fish and those fed with test diets were significantly different. The initial fish had the highest body ash content. However there were no differences ($P>0.05$) in body ash content among treatments.

Table 4. Proximate analysis of dorsal muscle of tilapia (*Oreochromis niloticus*)

Treatments					
Composition	Initial Fish	A	B	C	D
Protein (%)	83.77	77.63	86.43	87.00	85.93
Lipid (%)	8.82	10.98	9.16	9.07	9.06
Ash (%)	10.10	8.27	8.02	7.91	8.52

4. Discussion

Water quality parameters monitored were similar in all treatments and the values (Temperature 30 ± 1 °C) and dissolved oxygen (6 ± 1 mg/L), conform to the recommendation of Boyd (1982) for warm water fish culture.

The uses of animal protein sources from fishery by-catch and byproducts for aquaculture have been widely studied by many researchers (Goddard *et al.* 2003, Li *et al.* 2004, Goddard & Perret 2005; Whiteman & Gatlin III 2005).

The present study showed that the incorporation of shrimp waste product in tilapia diets gave better growth performance compared to the control diet. SGR (3.79) obtained in fish fed on diet D which contains 45% of shrimp waste product are higher than 2.28 and 1.81 SGR reported respectively by Fagbenro & Jauncey (1995) and Nwanna 2003. However, the results obtained in this trial are in line with those of Özogul Yesim (1999) who found that the incorporation of proteins of shrimp waste gave the best SGR in the rainbow trout.

Better Leal *et al.* (2009) demonstrated that the incorporation of protein hydrolysate of shrimp at 60% did not affect the growth performance of tilapia (*O. niloticus*). Furthermore, Tibbets *et al.* (2011) found that the best performance was obtained with the 100% of krill meal in cod as in the halibut Atlantic. In contrast, Fall *et al.* (2012) found that the incorporation of shrimp waste meal to replace soybean meal at a concentration greater than 60% decreased growth performance of hybrid tilapia. Chitmanat *et al.* (2008) showed that the inclusion of fishery by-product meal more than 25% was detrimental to growth performance of tilapia. The results of this experiment showed that the steamed shrimp waste product could be included up to 45% in the diet of tilapia. Cavalheiro *et al.* (2007) showed that shrimp head silage could substitute

for fish meal in tilapia fish feed without affecting the nutritional quality of the feed and, at the same time, being economical. The inclusion rate of the present study is higher than that of Mirzah (1997) who included steamed shrimp product in the diet of chickens only up to 18%. However it should be aware that the difference in incorporation rate also depends on the species to be cultured.

In this experiment, the diet D which gave the best growth performance contains the highest fiber (8.99%). These fibers are mainly constituted of chitin (30% to 40% of the shrimp shell) and chitosan. These results are in contradiction with the observations of Shiau & Yu (1999), Fall *et al.* (2012) and Leal *et al.* (2009). According to these authors, the decrease in growth performance observed from high inclusion level of shrimp waste meal could be explained by poor digestion of chitin. In fact, chitin is known to absorb fat and bile in the gastrointestinal tract, thereby reducing the digestion and absorption of lipids (Tharanathan & Kittur 2003). It seems that cichlids in general do not have enzymes called chitinases able to digest chitin (Fines & Holt 2010). In this study, the growth performance recorded may be related to different treatments performed on shrimp waste meal but also the rate of incorporation into diets. In fact, it is known that cooking either steam or boiled transforms the fibers making them more soluble and digestible. Better fiber and ash of a food can be reduced by the process of fermentation (silage) as demonstrated by Fagbenro & Bello-Olusoji (1997).

The present trial revealed that FCR obtained from fish fed with diets B, C and D did not show any significant differences. The best FCR (1.69) obtained from fish fed with diet D is slightly similar to those obtained by Tibbets *et al.* 2011. These authors found that the FCR of cod and halibut juveniles fed on diet containing krill meal was around 1.1. In the present experiment, the FCR of fish fed on the different tests diets showed significant variation as compared to that of tilapia fed on the control diet. Contrary, Nwanna (2003) reported that there was no significant difference between FCR of fish fed on the control diet containing no shrimp head silage and other diets containing different inclusion rate of shrimp head hydrolysate in Nile tilapia.

It is known that the survival and growth of fish are closely related to the quality of the feed distributed and, in particular, the protein source (Cruz-Suárez *et al.* 1992; Cahu & Zambonino-Infante, 2001). In our study, regardless of the feed distributed, survival rate is relatively high and ranged from 70 to 100 %. Similarly, Cavalheiro *et al.* 2007 revealed that the survival of tilapia varied from 73.0% to 100%. Moreover, Nwanna, (2003) found survival rates of 95% during the feeding trials and digestibility studies conducted to evaluate the effect of replacing fish meal by shrimp head meal silage at different levels of 0, 5, 10, 20, 30 and 40% in the diet of African catfish. According Sumi *et al.* (2011), a survival rate above 80% is excellent in nursery.

Biochemical analysis (protein, fat and ash) of the carcass composition of tilapia at the beginning and the end of the experiment are used to determine the influence of diet on the composition of the fish. In the present study, there were no significant differences in body protein content among the fish fed diet with diets B, C, D and the initial fish. The lowest body protein was obtained in the fish fed with control diet without shrimp waste

meal. The tilapia fed with control diet had the highest body lipid content compared to the body lipid content of the initial fish. There were no significant differences in body lipid content among the fish fed with diets B, C and D compared to the body lipid content of the initial fish. The variations in body ash content of initial fish and fish fed with test diets were significantly different. The initial fish had the highest body ash content. However there were no differences in body ash content among treatments. Nwana (2003) made similar observation in catfish fed shrimp head silage meal. Contrary to our results, Leal *et al.* (2009) reported that the carcass protein, ash and fat content of initial fish decreased compared to the carcass composition of fish fed with different diets containing 0, 5, 10 and 20% of shrimp protein hydrolysate in the Nile tilapia.

5. Conclusion

Water quality parameters monitored were similar in all treatments and the values (Temperature 30 ± 1 °C) and dissolved oxygen (6 ± 1 mg/L). The proximate composition of the diets is marginally different. The evaluation of nutritional quality of the different shrimp waste meal showed significant difference among treatments in terms of MWG, SGR and survival. The FCR did not demonstrate noticeable change in fishes fed test diets. Chemical analysis of the carcass of the fry tilapia fed the diets containing diverse shrimp waste meal for 42 days differs statistically in terms of protein and lipid content. The results of this study show that shrimp waste meal are important feedstuff for tilapia and steamed shrimp waste meal can be included in tilapia diets up to 45%.

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