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Effects of Partial Substitution of Fishmeal by Crustacean (*Callianassa*) Meal on the Growth Performance, Feed Efficiency and Survival Rate of Nile Tilapia (*Oreochromis niloticus*)

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Abstract

The effect of substituting fishmeal with crustacean (Callianassa) meal in the diets of mixed sex Nile tilapia (*Oreochromis niloticus*) fry (0.2 g) was evaluated. Three isonitrogenous and isolipidic diets were formulated to contain 30% CP and 15% CL where fishmeal was substituted with *Callianassa* meal at 0, 25 and 50% in diets R1, R2 and R3 respectively. The experiment lasted for 6 weeks. The stocking density was 10 fish per tank in an isolated system and the capacity of each tank was 50L. Each treatment was administered in duplicates and fish were fed three times per day (09h, 13h, and 17h). The daily ration corresponds to 10% of the live weight of the fish during the first 2 weeks and was reduced to 8% and then 6% for the second and last two weeks of the trial period. At the end of the experiment, fish fed on R1 (control) diet and R2 diet (25% fishmeal replacement) had similar final weight (0.62 g), absolute mean weight gain (0.42 g), relative mean weight gain (210 %) and specific growth rate (2.50 %) and were significantly different from the values obtained from those fed on R3 diet (50% fishmeal replacement). R3 diet had the best FCR (1.42) and was significantly different from the values obtained from R1 and R2 diets but the values of the diets were not significantly different. The dry matter content of the initial fish (96.33%) and those of the R1 (94.12%) and R2 (95.71%) diets did not differ significantly but were slightly higher than that of the value obtained from fish fed on R3 diet (91.15). The protein content of the initial fish (49.63%) was significantly lower than the values obtained from fish fed on R3 diet (61.95%), R1 diet (54.74%) and R2 diet (52.59%). The fat content of the initial fish (41.51%) was higher than the values obtained when fish were fed on the experimental diets. Among the tested diets, the higher the crustacean meal in the diet, the lower the fat content of the fish muscle. The fat content of R1 (control) diet (29.56%) was slightly higher than the value of R3 diet (24.44%) and slightly lower than R2 diet (32.56%). It is concluded that crustacean meal can replace 25% of fishmeal in the diet of Nile tilapia (*Oreochromis niloticus*) fry at 0.2 g without any effect on the growth performances and feed efficiency.

Keywords: fishmeal, substitution, *Oreochromis niloticus*, crustacean meal, growth and muscle composition

1. Introduction

The strong demographic growth is increasing the demand for fishery products, a large part of which is provided by capture fisheries. At the moment, the productivity of this sector is declining because most of the fish stocks are either over-exploited or fully exploited and only a few stocks are under-exploited. Declining catches is characterizing the current situation of scarcity of fish in the world.

Therefore, aquaculture is considered to be the sector capable of increasing fish production and animal protein intake for the ever-growing population. The world production of farmed fish for human consumption has been remarkably increasing and stood at 73.8 million tons in 2014 while in 1960, it was just 1.6 million tons (FAO, 2016). The pivotal role aquaculture is playing in supplying fish to the market during these decades has made it to be considered as one of the most highly productive animal producing activities. The 73.8 million tons produced by aquaculture in 2014 was estimated at US $ 160.2 billion at first sale which included 49.8 million tons of fish (US$ 99.2 billion), 16.1 million tons of shellfish (US $ 19 billion), 6.9 million tons of crustaceans (US $ 36.2 billion), and 7.3 million tons of other aquatic animals (3.7 billion US $) (FAO, 2016). Per capita fish consumption has increased from 10 kg in the 1960s to 19.7 kg in 2013 (FAO, 2016). The sustainable development of aquaculture requires easy access to quality and efficient feed. The growth of aquaculture worldwide is attributed to the increasing use of compound feeds whose protein component is provided mainly by fishmeal. Considering the development of aquaculture and the foreseeable stagnation of pelagic fishmeal production, it is necessary to reduce the proportion of fishmeal in aqua-feeds. Thus, alternatives to fishmeal such as crustacean meal among others are being studied.

The crustacean used in the formulation of the tested feeds has not been identified. Nevertheless, it belongs to the infra-order of Thalassinidea, in the group of decapod crustaceans (Acad, 1973). Some 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%. Fall *et al*. (2012) reported that fish fed diet containing shrimp meal (SM) gained weight slightly less than those fed diet containing fishmeal. Diop *et al.* (2013) revealed that the incorporation of shrimp by-products in the diets of tilapia at 45% gave the best weight gain (4.96 g) compared to the control diet. Several authors (Jung *et al.*, 2006; Wenhong *et al.*, 2008; Uno *et al.*, 2010; Laila *et al.*, 2010) have highlighted that there exist three methods of waste utilization from aquaculture or wild stocks, which are fishmeal, silage and organic fertilizer productions. The crustacean waste (shrimp, Antarctic Krill, crab and lobster processing) is the most important chitin source. It is present in amounts varying from trace quantities up to about 40% of the body weight of the organism. Chitin present in the crustacean waste is associated with proteins, minerals (mainly calcium carbonate) and lipids including pigments.

The objective of this study was to evaluate the effects of the partial substitution of fishmeal by crustacean meal on the growth performance of tilapia (*Oreochromis niloticus*).

2. Material and Methods

2.1 Feed Preparation

Three isonitrogenous (30% protein) and isolipidic (15% lipid) practical diets were formulated in which fishmeal component of the diet was replaced by crustacean meal at 0% (R1), 25% (R2) and 50% (R3). Other ingredients were dried mango peel meal, peanut cake meal, dried mango kernel meal, minerals, and vitamins. *Corchorus tridens* was used as a natural binder. The composition of the experimental diets is shown in Table 1. For the production of each diet, the raw materials were ground, weighed and mixed with the vitamins, minerals then fish oil and water at 30% of the diet weight were added to obtain a homogeneous mixture. The paste was passed through an electric chopper machine (Moulinex) with appropriate die to produce spaghetti like filaments of feed. The feeds were then dried under the sun, crushed, and sieved before being put into bags and stored until use.

Table 1. Formulation of the experimental diets

Ingredients (g/kg)	R1 (0%)	R2 (25%)	R3 (50%)
Mango kernel meal (g)	200	200	200
Mango peel meal (g)	150	140	140
***Corchorus tridens* (g)**	20	20	20
Crustacean meal (g)	0	42.5	85
Fishmeal (g)	170	127.5	85
Fish oil (g)	50	50	50
Peanut cake meal (g)	390	400	400
Vitamines [a] (g)	10	10	10
Minerals [b] (g)	10	10	10
Total	1000	1000	1000

[a] Vit A 250000 UI; Vit D_3 250000 UI; Vit E 5000 mg ;Vit B_1 100 mg; Vit B_2 400 mg; Niacine 1000 mg; Pantothenate Ca 2000 mg; Vit B_6 300 mg; Vit K_3 1000 g ; Vit C 5000 mg; Biotine 15 mg; Choline 100 g; BHT 1000 mg;

[b]Phosphorus 7%; Calcium 17%; Sodium 1.5%; Potassium 4.6%; Magnesium 7.5%; Manganese 738 mg; Zinc 3000 mg; Iron 4000 mg; Copper 750 mg; Iodine 5 mg; Cobalt 208 mg; Calcium and grounded attapulgite qs 1000 g; Fluoride 1.5%.

2.2 Experimental Fish and System

Mixed sex tilapia fry obtained by artificial propagation from Ouakam aquaculture station (west coast of Dakar, Senegal) were used. The fry were fed with commercial feed imported from Ghana (Raanan Fish feed company) up to three weeks old. Then the fish were acclimatized for two weeks given the same imported feed three times per day (9h, 13h, and 17h). The experiment lasted for 6 weeks. The fry of 0.2 g average size were randomly distributed in 6 tanks with a stocking density of 10 fish per tank. Each diet was tested in duplicate. The daily ration corresponds to 10% of the live weight of the fish during the first 2 weeks. For the rest of the experiment, the ration was reduced to 8% and then to 6% according to the live weight of the fish after weighing. Fish were hand fed 3 times per day (09h, 13h, and 17h). The tanks were cleaned twice a day by siphoning suspended particles and those deposited at the bottom. The fish were subjected to a photoperiod of 12 hours of light and 12 hours of darkness. Physicochemical parameters (temperature, pH) were measured daily using an YSI (Yellow Springs Instruments, Yellow Springs, OH, USA), Model 58 meter. Every two weeks, measures of growth parameters (WG, SGR), feed efficiency (FCR) and survival rate (SR) were taken to assess the nutritional quality of diets and the amount of feed given was readjusted. At the end of the experiment, 5 fish are taken at random in each tank and subjected to bromatological carcass analysis.

2.3 Proximate Analysis of Fish Flesh

Bromatological analysis of the fish (before and after the experiment) was carried out according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1984). The samples were analyzed at ENSA laboratory in Thiès region, Senegal for determination of proximate composition of fish (crude protein, crude fat and dry matter). Fish samples were dried to constant weight at 105 °C for 24h to determine moisture. Crude protein (total Nitrogen x 6.25) was determined by using micro-Kjeldahl method (Kjeltec System 1002 Distilling Unit, Tecator, Hoeganaes, Sweden). Crude fat was extracted by Soxhlet method.

2.4 Data Calculation and Statistical Analysis

To estimate growth of fish and characterize the efficiency of the use of experimental diets, different growth parameters were calculated. The growth and feed utilization performance parameters measured were: final mean weight (FMW), absolute mean weight gain (AMWG), relative mean weight gain (RMWG), specific growth rate (SGR) and feed conversion ratio (FCR).

The formulae used in calculations were:

Absolute mean weight gain (AMWG (g)) = final mean weight – initial mean weight

Relative mean weight gain (RMWG (%)) = 100 × (final mean weight – initial mean weight) / initial mean weight.

Specific growth rate (SGR (%/d)) =100 (ln (final mean weight) – ln (initial mean weight)) / number of days.

Feed conversion ratio (FCR) = total dry feed fed (g) / total wet weight gain (g).

The data were analyzed using SAS software program for windows (V.9 SAS Institute, Cary, North Carolina, USA) and the results were presented as means of the two replicates after subjecting them to one-way analysis of variance (ANOVA) using GLM procedure. In case of significant differences, Duncan’s multiple-range test was conducted and the difference was considered significant at *P*-values < 0.05.

3. Results and Discussion

3.1 Results

During the experiment, there were no pathological signs or symptoms. The fish accepted the diets. The survival of the fish fed on experimental diets ranged from 50 to 90%. The average temperature ranged between 27.5 and 27.8 °C whereas pH values were between 8.74 and 8.75, both were within the tolerant limits of Nile tilapia (*Oreochromis niloticus*).

Table 2. Growth and feed efficiency parameters.

Parameters	**Diets**		
	R1 (0%)	**R2 (25%)**	**R3 (50%)**
IMW (g)	0.2	0.2	0.2
FMW (g)	0.62	0.62	0.56
AMWG (g)	0.42ᵃ	0.42ᵃ	0.36ᵇ
RMWG (%)	210ᵃ	210ᵃ	180ᵇ
SGR (%/d)	2.50ᵃ	2.50ᵃ	2.29ᵇ
FCR	2.84ᵇ	2.93ᵇ	1.42ᵃ
SR (%)	90	85	50

Values are means of the duplicates; values within the same row without a common superscript are significantly different (p <0.05).

IMW *= initial mean weight;* ***FMW*** *= final mean weight;* ***AMWG*** *= absolute mean weight gain;* ***RMWG (%)*** *= relative mean weight gain;* ***SGR (%/d)*** *= specific growth rate; FCR = feed conversion ratio;* ***SR*** *= survival rate.*

Table 2 shows the parameters of growth (absolute mean weight gain, relative mean weight gain, specific growth rate), feed efficiency performance (feed conversion ratio), and the survival of tilapia.

The fish fed with R1 diet (control) and R2 diet (25% crustacean meal replacement for fishmeal) had similar and better final weight (0.62 g), absolute mean weight gain (0.42 g), relative mean weight gain (210 %), specific growth rate (2.50 %) compared to those fed with R3 diet (50% crustacean meal replacement for fishmeal) which are 0.56 g, 0.36 g, 180 %, 2.29% respectively.

For the feed conversion ratio (FCR), the lowest value (1.42) was obtained from fish fed on R3 diet and it was significantly different from the values obtained with R1 and R2 diets. The fish fed on R1 and R2 diets had higher FCR of 2.84 and 2.93 respectively but there was no significant difference between the two.

The bromatological composition of the flesh of fish

Table 3. Results of the biochemical analysis of the flesh of *O. niloticus*.

	Dry Matter (%)	**Protein (%)**	**Lipid (%)**
Initial fish	96.33	49.63	41.51
R1 (0%)	94.12	54.74	29.56
R2 (25%)	95.71	52.59	32.56
R3 (50%)	91.15	61.95	24.44

The table above shows the composition of the flesh of the Nile tilapia (dry matter, fat, and crude protein) at the beginning and at the end of the experiment.

Value-wise, differences were observed in the body composition of the fish fed on the different diets. The dry matter content of the initial fish (96.33%) and those of the R1 (94.12%) and R2 (95.71%) diets did not differ significantly. On the other hand, these values are higher than that of the value obtained from fish fed on R3 (91.15) regime. The protein content of the flesh was significantly higher in fish fed on R3 diet (61.95%), followed by those fed on R1 diet (54.74%) and then R2 diet (52.59%) compared to that of the initial fish (49, 63%).

For the fat content, the lowest value was observed from the fish fed with the R3 diet (24.44%), followed by R1 diet (29.56%) and then R2 diet (32.56%) compared to the initial fish (41.51%).

3.2 Discussion

During the trial, the mean temperature ranged from 27.50 to 27.80 °C and it was within the range for best growth performances (24 - 28 °C). The pH values recorded (8.74 to 8.75) were also within the range in which Tilapia (*O. niloticus)* can survive (8 to 11) as reported by Balarin and Hatton, 1979.

The fish that received R1 diet containing 0% crustacean meal and R2 diet containing 25% crustacean meal had similar weight gain (0.42 g) but different from that obtained with the R3 diet containing 50% crustacean meal (0.36 g). This shows that fishmeal could be partially replaced with crustacean meal (in this case, 25%) in Nile tilapia feed without any negative effect on the growth. These results are contrary to those of Diop *et al.* (2013) who reported that the incorporation of shrimp by-products in the diets of tilapia at 45% gave the best weight gain (4.96 g) compared to the control diet. It is also inconsistent with the results of Fall *et al.* (2012) who found significant difference between the control and test diets when soybean meal was replaced with shrimp shell meal in the diet of hybrid tilapia. For other fish species, the present result is not in line with the result of Raja Nandini *et al.* (2014) who achieved 50% replacement of fishmeal with shrimp waste meal in the diet of Koi carp *(Cyprinus carpio haematopterus)*.

The best SGR (2.50% / day) in this study obtained from R1 and the R2 diets is significantly different from the one obtained from R3 diet (2.29% / day). This is different from the result of Diop *et al.* (2013) who had the best SGR (3.79% / day) from tilapia fed with a diet containing 45% shrimp by-products. It is also inconsistent with the result of Fall *et al.* (2012) who did not find any significant difference between the control diet and 67% substitution of shrimp shell meal with soybean meal in the diet of hybrid tilapia. For other fish species, the present result differed from the result of Raja Nandini *et al.* (2014) who reported that the replacement of fishmeal with shrimp waste meal up to 50% was significantly better than the control fishmeal based diet for Koi carp *(Cyprinus carpio haematopterus)*. Also, Tibbets *et al.* (2011) found that the best performance was obtained with 100% krill meal in both cod and Atlantic halibut.

In this study, the best feed conversion ratio (1.42) was obtained with R3 diet containing 50% crustacean meal and 50% fishmeal while R2 diet containing 25% crustacean meal and 75% fishmeal produced the worst FCR (2.93). A similar thing was obtained by Diop *et al.* (2013) who reported the best FCR (1.69) from tilapia fed on diet containing 45% shrimp by-products compared to that of the control (3.35). Compared to the results obtained from other fish species, the result of the present study is in agreement with that obtained by Raja Nandini *et al.* (2014) who observed the best FCR (1.30) in Koi carp fed on diet containing 50% shrimp waste meal. It is also consistent with that of Keremah (2013) who showed that African giant catfish fed on diet containing 40% of crab meal had the best feed conversion ratio (1.12) compared to the control diet containing only fishmeal. Toppe *et al.* (2005) also indicated that cod fed with the highest rate of crab meal had the best FCR compared to other groups of fish. The FCR result of this study is not in line with the result of Fall *et al.* (2012) who found the best FCR (1.73) from fish fed on the control diet followed by fish fed with the diet containing 25% shrimp by-products.

The survival percentage obtained in this study varies between 50 to 90%. The R1 diet had the highest survival (90%) followed by R2 diet (85%) containing 25% crustacean meal and then R3 diet (50%) containing 50% crustacean meal. The 85 and 90% survival obtained from fish fed on R2 and R1 diets are considered excellent based on the observations of Sumi *et al.* (2011) who stated that survival greater than 80% is excellent in the nursery. The two values are almost in the same range with the one obtained (95%) by Nwanna (2003) during feeding trials and digestibility studies of evaluating the effect of replacement of fishmeal by shrimp silage meal at different levels: 0, 5, 10, 20, 30, and 40%. The lowest survival 50% obtained in the fish fed diet R3 containing 50% of crustacean meal was probably due to high level of chitin, which is not readily digestible.

The protein and lipid contents of the flesh at the beginning and the end of the experiment were used to determine the influence of the diets on the muscle composition of the fish. Studies have shown that exogenous factors (food composition, temperature, oxygen) and endogenous factors (size, sex, stage of sexual maturity) are likely to influence the body composition of the cultured fish species (Hepher, 1990).

The lipid content of the initial fish (41.51%) decreased after subjecting them to the experimental diets [R1 (29.56%), R2 (32.56%) and R3 (24.44%)]. This could mean that fish subjected to the experimental diets spent much energy during the trial period to cope with the environment conditions of the cultured medium resulting to the reduction of the body lipid. Moreover, the low lipid content of the experimental fish can be caused by poor digestion of the dietary fat in the feeds. Indeed, well digested dietary lipids generally lead to lipid deposition in the muscle. This plays a significant role in the supply of energy from digestion of complex carbohydrates, a role that is more important for fish (Guillaume *et al.*, 1999). The result of this study is contrary to that of Fall *et al.* (2012) who observed reduction of fat in the flesh of the experimental fish compared to the initial fish. It is also not in line with the result of Diop *et al.* (2013) who reported that the lipid content of the initial fish (83.73%) was almost the same as those found in the experimental fish (85.93%, 86.43%, and 87%).

Fish muscle protein level increased after trial from initial (49.63%) to 54.74%, 52.59% and 61.95% for R1, R2 and R3 respectively. This indicates that the fish were able to convert the dietary protein of the experimental feeds into body protein. This result agrees with that of Fall *et al.* (2012) who observed an increase in the protein content of the dorsal muscle of the fish when fed on the tested diets compared to the initial fish. Compare to other fish species, the result of the present study is concurrent with that of Nwanna (2003) who also made similar observation when *Clarias* were fed on the shrimp ensiled head meal.

4. Conclusion and Perspectives

Global aquaculture continues to grow and intensify. Reducing the use of fishmeal, the primary source of protein in the diet of farmed fish through substitutions for meeting sustainable development is one of the significant challenges for future aquaculture development worldwide. The present study reveals the importance of crustacean meal as an essential ingredient in tilapia feed.

The results obtained showed the importance of the partial substitution of fishmeal by crustacean meal in the diet of tilapia (*Oreochromis niloticus*). The fish fed on diet containing 25% crustacean meal had the same growth performance as those fed on diet containing fishmeal. This shows that crustacean meal can partially replace fishmeal in the tilapia (*O. niloticus*) feed. However, it seems that the higher the incorporation rate of crustacean meal, the lower the growth performance. The results of this experiment showed that crustacean meal could replace 25% of fishmeal in the diet of tilapia without affecting the nutritional quality of the feed.

The laboratory study should be repeated in ponds where live feeds contribute to the supply of nutrients. Studies focusing on the digestibility of diet containing crustacean meal may complement the results.

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