

**EFFECT OF REPLACEMENT DIETARY FISH MEAL
BY RICE PROTEIN CONCENTRATE ON
PERFORMANCE, BODY COMPOSITION AND
INTESTINAL HISTOLOGY IN NILE TILAPIA
(*OREOCHROMIS NILOTICUS*)**

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ABSTRACT

The present study was designed to study the effect of the partial or complete substitution of fish meal by rice protein concentrate (RPC) in the diets of Nile tilapia (*O. niloticus*) and their effect on the growth performance parameters, body composition and intestinal histology. A total of 320 *O. niloticus* with an average body weight of 40.50 ± 0.250 g were used. The fish were randomly divided into four equal replicate groups (each replicate contains 20 fish). A basal control diet was formulated with zero replacement of fish meal (G1) and the other experimental groups (G2, G3 and G4) received a basal diet contained fish meal substituted with 25%, 50%, and 100% of RPC, respectively. The fish were fed isonitrogenous and isocaloric diets; three times daily at a rate of 3% of the body weight for 90 days. Body weight was not affected by substituting fish meal by 100% of RPC compared to control, while at this level of replacement, feed intakes, and feed conversion ratios were higher than in the control group. Protein efficiency ratio and the protein retention efficiency were reduced by replacing fish meal with RPC at the level of 25%, 50%, and 100% than control group. The results showed that substitution of 50% and 100% of fish meal with RPC had recorded significant lower protein, calcium and phosphorus and higher fat content than control group. All experimental groups fed RPC -based diet had a significant lowered cysteine, methionine, lysine, threonine and serine than the control group. On the other hand, the experimental groups fed RPC -based diet showed a significant higher intestinal villus height, crypt depth and muscle thickness.

INTRODUCTION

In Egypt, there is an attention for the aquaculture industry to find high nutritional and more economical protein sources to replace the traditional protein ingredients such fish meal (FM). (Katya et al., 2014).

However, the very nice nutritive value of FM, its price was very expensive as well as the unstable production and little availability in the market. Consequently, to allow the sustainability of the aquaculture industry, it is of great significance to explore other protein alternatives to fish meal in the aqua diets. In this approach, vegetable protein meals are very good candidates due to their rich abundance and relatively low cost (Olsen and Hasan, 2012).

Rice protein concentrate (RPC) has been reported to be an alternative protein raw materials of fish meal due to its high content of protein and lipid profile (Cai et al., 2018). Unfortunately, lysine was the first limiting indispensable amino acid in RPC, as limits its utilization in the aqua diets (Gatlin et al., 2007). Accordingly, synthetic lysine could be supplemented for balancing the amino acid profile of the aqua diet to enhance the usage of RPC. Fish meals could be substituted by rice protein concentrate tell 50% without deteriorative effect on the growth performance parameters, hematological parameters, and the nutrient digestibility up to 20% in the rainbow trout (Palmegiano et al., 2006). Also RPC could replace fish meal tell 64% in the blackspot seabream (Dapra et al., 2009) and up to 90% in the gilthead seabream (Sanchez-Lozano et al., 2009). In shrimp RPC reached up to 50% fish meal replacement without adverse effect on performance (Oujifard et al., 2012), up to 25% in the European sea bass juveniles (Guroy et al., 2013), up to 18% with microcapsule lysine addition in the Chinese soft-shelled turtle (Sun et al., 2018) and up to 100% with lysine addition (Cai et al., 2018).

The aim of this study was to evaluate the effect of the partial or total replacement dietary fish meal protein with rice protein concentrate on *O. niloticus*'s growth performance parameters, body and amino acids composition and intestinal histology.

MATERIALS AND METHODS

Ethical Statement

The ethics of the experimental protocol were performed following the recommendations of the local experimental fish care committee of the Faculty of Veterinary Medicine, Zagazig University (ZU-IACUC) with Approval No. ZU-IACUC/2/F/116/2020)

Experimental Fish

A total number of 320 healthy live *O. niloticus* with an average body weight of 40.50 ± 0.250 g were obtained from a private fish farm, Abbassa village, Abu-Hammad district, Sharkia Governorate, Egypt. Fish

were stocked in a clean concrete ponds (3 x 1 x 1 meter) and filled with de-chlorinated freshwater and aerator. The water temperature ($^{\circ}\text{C}$), the dissolved oxygen (DO, mg/L), pH, ammonium (NH_4 , mg/l), and nitrite (NO_2 , mg/L) were measured by using bag of water aquarium analysis and found to be $27 \pm 2^{\circ}\text{C}$, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02 mg/l, respectively. Fish were divided into four equal replicate groups (each replicate contain 20 fish). The fish were adapted to the experimental conditions for two weeks before starting of the experiment.

Fish diets and feeding

The control group received a basal diet without rice protein concentrate (G1) and other experimental groups (G2-4) received a basal diet contained fish meal substituted with 25%, 50%, and 100% RPC, respectively. All fish were fed their respective diet at a level of 3% of the body weight three times daily for 90 days. Feedstuffs used in diets formulation were analyzed for moisture, dry matter (DM), crude protein (CP), Ether extract (EE), and crude fiber (CF) according to the standard procedures of the International guidelines (**Feldsine et al., 2002**). The results of the chemical analysis, as well as, the nutritive value in the diets were listed in Table 1. Isocaloric and isonitrogenous diets were prepared at Fish Research Center, Faculty of Veterinary medicine, Zagazig University, Egypt. It contained (3007 kcal/kg metabolizable energy and 32 % crude protein) in the form of dry pellets and were formulated to match the nutrient requirements of *O. niloticus* set by **National Research Council (1993)** as shown in Table 1.

Growth performance traits

The all-experimental fish (80) of each group were weighed at the start and the end of the experiment. The average body weight was calculated by dividing the total weight of fish by the number of the fish in each group. Body weight gains, feed conversion ratios (**Siddiqui et al., 1988**), body gains percent (**Jauncay and Ross, 1982**) and specific growth rates % (**Nathanailides et al., 2019**) were detected. The following growth performance parameters indicators were calculated as a protein efficiency ratio (Stuart and Hung, 1989), and Protein retention efficiencies (**Castell and Tiews, 1980**). The mortalities were recorded and the relative survival percentage calculated by subtraction of the mortality from the total number of fish.

Chemical, amino acids analysis of whole-body composition: -

a- Chemical analysis of fish body

For calculation of the whole-body composition, 5 fish from each treatment group at the end of the experiment were minced then dried at 70°C for 72 hours in hot air oven and used for determination of moisture, crude protein, ether extract, and ash according to (**AOAC, 2012**).

Table 1. Chemical composition of the experimental diets.

Ingredients	Experimental diets			
	Control	Rice protein concentrate		
		25%	50%	100%
Fish meal, 66%	20	15	10	0
Rice protein concentrate	0	5	10	20
Soybean meal, 44%	20	20	20	20
DDGS, 28%	10	10	10	10
Yellow corn	15	15	15	15
Corn gluten, 62%	4.55	5.20	6.25	8
Rice bran	26.45	25.80	25.30	24.15
Vegetable oil	3.50	3.50	2.85	2.20
L-Lysine HCL, 78 %	-	-	-	-
DL-Methionine, 98 %	-	-	0.10	0.15
Calcium carbonate	-	-	-	-
Vitamin mineral premix*	0.50	0.50	0.50	0.50
Total, %	100	100	100	100
Calculated composition				
DM, %	86.47	86.48	86.20	85.79
CP, %	32.01	32.04	32.01	32.05
EE, %	11.47	11.11	10.93	10.51
CF, %	4.27	4.30	4.24	4.18
Ash, %	7.60	6.76	5.82	4.01
NFE, %	34.06	35.21	35.79	37.26
Ca, %	0.89	0.71	0.79	0.77
P, %	1.19	1.07	0.94	0.68
Lysine, %	1.85	1.69	1.63	1.60
Methionine, %	0.71	0.76	0.81	0.90
DE, Kcal/ kg**	3007.46	3008.87	3006.77	3008.69

* Vitamin and mineral mixture sourced from Trouw nutrition, Netherlands (5kg/diet) {Vit. A 6000 I.U, D3 2.000 I.U, E 300 mg, k3 7.5 mg, C 500 mg. B₁ 12 mg, B² 24 mg, B₆ 24 mg, B₁₂ 0.036 mg, Biotin 0.21 mg, Folic acid 4.5 mg, choline 1500 mg inositol, 150 mg, pantothenic acid 72 mg, Nicotinic acid 90 mg, iron 3mg, copper 5 mg, zinc 150 mg, Sodium selenite 0.2 mg and, potassium iodide 2 mg}.

** Digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm according to (Santiago *et al.*, 1982).

b- Amino acids analysis of fish body by HPLC amino acids analyzer

Analysis of amino acids by HPLC amino acids analyzer

Before HPLC analysis of amino acids, the fish samples were subjected to a process of sample preparation. Samples of fish body were

homogenized using an Ultra Turrax grind mixer (IKA, Staufen, Germany) at high speed (11.93 m/s for 1 min). The amino acids analysis was done by the method described by (Garde-Cerdán et al. 2014). Free amino acids were analyzed by HPLC (Agilent, Palo Alto, USA). Each sample (5 ml of supernatant) was mixed with 100 µl of norvaline and 100 µl of sarcosine (internal standards). The mixture was submitted to an automatic precolumn derivatisation with o-phthaldialdehyde (OPA Reagent, Agilent) and with 9-fluorenylmethylchloroformate (FMOCl Reagent, Agilent). The injected amount from the derived sample was 10 µl, and a constant temperature of 40 °C was maintained. All separations were performed on a Hypersil ODS (250 × 4.0 mm, I.D. 5 µm) column (Agilent). Two eluents were used as mobile phases: eluent A: 75 mM sodium acetate + 0.018% triethylamine (pH 6.9) + 0.3% tetrahydrofuran; eluent B: water, methanol, and acetonitrile (10:45:45, v/v/v). Detection was performed by fluorescence detector FLD, and DAD detector. Identification of compounds was performed by comparison of their retention times with those of pure reference standards. The pure reference compounds and internal standards were from Sigma-Aldrich (Madrid, Spain).

c- Calcium and phosphorus analysis

Atomic absorption measurements were made with a Perkin-Elmer 4000 Atomic Absorption Spectrophotometer. Flame photometry measurements were made with an Instrumentation Laboratory (IL) Model 343 Digital Flame Photometer. The method described by (Dipietro, et al., 1988). 2 ml of hydrochloric and 3ml of nitric acids were used for each sample. Atomic absorption standard solutions (1000 mg) were certified accurate within 1% by the manufacturer. The samples homogenate were mixed with 2ml hydrochloric and 3ml nitric acids incubated for 24hr then filtrated, the filtrate used for minerals measurement using the flame atomic absorption spectroscopy according to (Bayse, et al., 1981).

Intestinal histological examination

The intestine (from the middle part) was collected for histometric evaluation. The samples were immediately fixed in a paraformaldehyde solution, followed by slide processing that included washing, dehydration using different grades of alcohol, clearing by xylene, and embedding in paraffin wax. The wax blocks were sectioned into five-micron thick sections; the sections were stained with haematoxylin and eosin (H&E)

to prepare histological slides according to the method by (Roberts 2001). The villi length, crypt depth and muscle thickness were measured.

RESULT AND DISCUSSION

Growth performance

Effect of the replacement fish meal with RPC in *O. niloticus* diets on overall growth performance was shown in (Table 2). The final body weight of the control group had slight non-significant increase with the other three groups of RPC levels 25%, 50%, and 100%. In addition, final body weight gain and gain percentage of the control group significantly increased ($P \leq 0.05$) in group fed RPC 50% but no significant differences were observed among the control group and the other groups (25% and 100%). Regarding to the total feed intake, the control group had marked decrease in total feed intake ($P \leq 0.05$) when compared with the group fed 100% of RPC but there were no significant differences with the other experimental groups. The feed conversion ratio of the control group was significantly decreased ($P \leq 0.05$) when compared with both 50% and 100% RPC fed groups, respectively, but no significant differences with the group of RPC 25% was detected. Regarding to the specific growth rates, the control group had marked increase ($P \leq 0.05$) specific growth rate than the both of the experimental groups of 25%, and 50% RPC but no significant changes were marked with the group of RPC 100%. On the other hand, protein efficiency ratios and protein efficiencies were decreased with substitution of the fish meal by RPC 25%, 50%, and 100% than control group. The relative survival percentage was not significant between all experimental groups. The survival rate was 96.67% in G1, 95% in G2, 93.33% in G3 while 93.33% in G4. In the same line, RPC could be used without adverse effect on the growth performance reductions, within the inclusion rate of up to 20% in the rainbow trout diets (Palmegiano et al., 2006). At the same time, these results were agreed with the finding of Sanchez-Lozano et al. (2009) who discovered that there were no differences in the growth performance parameters of growing the gilthead seabream when fish meal substituted with RPC. Our results were confirmed by published data which concluded that up to 50% FM could be replaced RPC as an alternative protein raw material in the commercial shrimp diets without any effect on the growth (Oujifard et al., 2012). More interestingly, the RPC had potentials as sustainable feed raw materials for uses in the European sea bass juvenile's diets. Dietary substitution at levels up to 25% replacement of fish meal (14%) could be used without effects on the growth traits (Guroy et al., 2013). In addition, it had been concluded that RPC with microcapsule lysine supplementation could be replaced by a dietary 18% FM without growth changes in the Chinese soft-shelled turtle (Sun et al., 2018).

Table 2. Effect of the replacement fish meal with rice protein concentrates in the diet of Nile tilapia (*O. niloticus*) on overall performance during 0- 12 week (means \pm SE).

Parameters	Experimental groups			
	Control	Rice protein concentrates (RPC)		
		25%	50%	100%
Initial body weight, g	40.33 \pm 0.22	40.97 \pm 0.26	41.05 \pm 0.15	40.53 \pm 0.27
Final body weight, g	74.69 \pm 0.16	74.17 \pm 0.09	74.17 \pm 0.18	74.02 \pm 0.08
Total gain, g	34.35 \pm 0.36 ^a	33.20 \pm 0.32 ^{ab}	33.12 \pm 0.05 ^b	33.49 \pm 0.35 ^a
Gain, %	85.19 \pm 1.35 ^a	81.06 \pm 1.28 ^{ab}	80.68 \pm 0.28 ^b	82.64 \pm 1.44 ^a
Total feed intake, g	69.78 \pm 0.84 ^b	71.51 \pm 1.28 ^b	73.37 \pm 0.32 ^b	74.12 \pm 1.02 ^a
Feed conversion ratio	2.03 \pm 0.03 ^b	2.15 \pm 0.02 ^{ab}	2.22 \pm 0.01 ^a	2.15 \pm 0.01 ^a
Specific growth rate	0.68 \pm 0.01 ^a	0.66 \pm 0.01 ^b	0.66 \pm 0.002 ^b	0.67 \pm 0.01 ^{ab}
Protein efficiency ratio	3.81 \pm 0.06 ^a	3.54 \pm 0.01 ^b	3.44 \pm 0.02 ^b	3.48 \pm 0.02 ^b
Protein retention efficiency	27.30 \pm 0.33 ^a	25.72 \pm 0.30 ^b	25.24 \pm 0.07 ^b	24.70 \pm 0.19 ^b
Relative survival, %	96.67 \pm 1.67 ^a	95.00 \pm 2.89 ^b	93.33 \pm 1.67 ^c	93.33 \pm 1.67 ^c

^{a, b} Means with different superscripts in the same column are significantly different ($p < .05$).

Chemical, amino acids analysis of whole-body composition: -

Effect of the replacement fish meal with RPC on Nile tilapia (*O. niloticus*) body composition at the end of experiment is shown in Table (3). The results showed that replacement of the 50% and 100% of fish meal with RPC recorded significant ($P \leq 0.05$) lower protein content than the control group, while group fed 25% RPC as replacement for fish meal not significantly differ than control group. On the other hand, fish meal replacement with 50% and 100% RPC recorded a significant ($P \leq 0.05$) higher fat content than control group while the group with 25% fish meal replacement with RPC not significant differ than the control group. While the all-experimental group which fed RPC-based diet recorded a significant ($P \leq 0.05$) lower Ash, calcium, and phosphorous contents than the control group. Meanwhile, the results showed that there was no significant ($P \leq 0.05$) difference in some of the amino acids like arginine, histidine, isoleucine, and valine among all experimental groups and the control group. While the all-experimental group which fed RPC-based diet showed a significant ($P \leq 0.05$) lower cysteine, lysine, methionine, serine, and threonine than the control group. In convenience with our results (Oujifard et al., 2012) reported that replacement fish meal by RPC by 25, 50, 75 and 100% not affect shrimp tail-muscle chemical composition (moisture, protein, lipid, and ash), while the dispensable and indispensable amino acids of the tail muscle of shrimp fed with 25, 50, and 75% RPC were significantly higher than the FM (0%) and 100% RPC diets. Moreover (Cai et al., 2018) reported that RPC supplemented with micro-lysine or crystalline lysine could replace fish meal with higher muscle fiber frequency in the 20 to 50 μ m class but lower 50 μ m class and higher cooking loss than that of the other groups.

Furthermore, no significant difference was found in whole-body proximate compositions of blunt snout bream.

Table 3. Effect of the replacement fish meal with rice protein concentrates in the diet of Nile tilapia (*O. niloticus*) on body composition at the end of experiment (means \pm SE).

Parameters	Experimental groups			
	Control	Rice protein concentrates (RPC)		
		25%	50%	100%
DM, %	23.12 \pm 0.07	23.00 \pm 0.14	23.21 \pm 0.11	23.18 \pm 0.05
CP, %	61.68 \pm 0.17 ^a	60.99 \pm 0.14 ^{ab}	61.37 \pm 0.21 ^a	60.85 \pm 0.23 ^b
EE, %	6.17 \pm 0.04 ^b	6.20 \pm 0.05 ^b	6.28 \pm 0.06 ^{ab}	6.49 \pm 0.12 ^a
Ash, %	28.40 \pm 0.22 ^a	26.90 \pm 0.21 ^b	26.55 \pm 0.09 ^b	24.46 \pm 0.17 ^c
Calcium, %	7.12 \pm 0.17 ^a	6.16 \pm 0.04 ^b	6.14 \pm 0.02 ^b	5.83 \pm 0.02 ^c
Phosphorus, %	4.07 \pm 0.02 ^a	3.99 \pm 0.06 ^a	3.40 \pm 0.13 ^b	3.11 \pm 0.04 ^c
Amino acids percent of crude protein				
Arginine, %	3.46 \pm 0.12	3.41 \pm 0.03	3.45 \pm 0.10	3.43 \pm 0.07
Cystine, %	0.48 \pm 0.01 ^a	0.44 \pm 0.02 ^{ab}	0.45 \pm 0.02 ^{ab}	0.42 \pm 0.01 ^b
Histidine, %	0.98 \pm 0.01	0.98 \pm 0.01	0.98 \pm 0.02	0.95 \pm 0.01
Isoleucine, %	2.07 \pm 0.05	2.11 \pm 0.09	2.02 \pm 0.01	2.02 \pm 0.04
Lysine, %	3.87 \pm 0.03 ^a	3.45 \pm 0.03 ^b	3.44 \pm 0.02 ^b	3.42 \pm 0.01 ^b
Methionine, %	1.69 \pm 0.02 ^a	1.59 \pm 0.03 ^b	1.56 \pm 0.02 ^b	1.53 \pm 0.05 ^b
Serine, %	2.36 \pm 0.01 ^a	2.28 \pm 0.02 ^b	2.25 \pm 0.003 ^b	2.25 \pm 0.01 ^b
Threonine, %	2.51 \pm 0.01 ^a	2.44 \pm 0.01 ^{ab}	2.42 \pm 0.01 ^b	2.38 \pm 0.04 ^b
Valine, %	2.46 \pm 0.02	2.68 \pm 0.32	2.68 \pm 0.34	2.33 \pm 0.01

a, b, c Means with different superscripts in the same column are significantly different ($p < .05$).

Intestinal histology:

Effect of the replacement fish meal with RPC on Nile tilapia (*O. niloticus*) intestinal histology at the end of experiment is shown in Table (4) and figure (1). The intestinal histology revealed that villus height, crypt depth and muscle layer thickness were increased respectively with increasing the percent of RPC in the rations. This result was in agreement with finding of (Shi et al., 2019) as reported that replacing fish meal with plant protein source had improved intestinal histology of rice field eel.

Table 4. Effect of the replacement fish meal with rice protein concentrates on tilapia nilotica intestinal histomorphology (means \pm SE).

Parameters	Experimental groups			
	Control	Rice protein concentrate	Rice protein concentrate	Rice protein concentrate
		25%	50%	100%
Villi height (μ m)	222.35 \pm 0.86 ^d	273.50 \pm 1.02 ^c	335.75 \pm 1.45 ^b	452.60 \pm 1.29 ^a
Crypt depth (μ m)	88.35 \pm 1.02 ^d	98.10 \pm 0.98 ^c	101.66 \pm 1.02 ^b	131.70 \pm 1.04 ^a
Muscle thickness (μ m)	36.94 \pm 0.42 ^b	44.60 \pm 0.68 ^a	44.60 \pm 0.81 ^a	48.46 \pm 0.81 ^a

a, b, c, d Means with different superscripts in the same column are significantly different ($p < .05$).

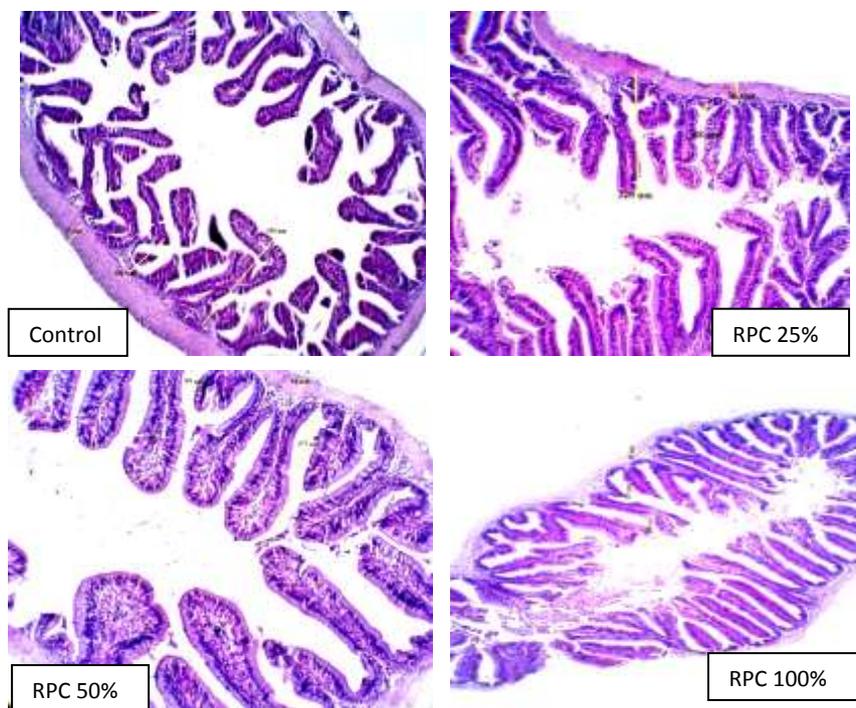


Figure 1: Effect of the replacement fish meal with rice protein concentrates on tilapia nilotica intestinal histomorphology

CONCLUSION

RPC could replace fish meal in Nile tilapia feed till 100% without adverse effect on fish performance while lowered cp, calcium, phosphorus and higher fat content of muscles as well as improved intestinal histomorphology.

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CONFLICT OF INTEREST

The authors declared that there are no conflicts of interest

AUTHORS CONTRIBUTION

All authors contributed equally to this work

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تأثير استبدال مسحوق السمك بمركز بروتين الارز علي الاداء و مكونات الجسم و الاحماض الامينية و الصفات الهستولوجية للأمعاء في اسماك البلطي النيلي

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هدفت الدراسة الحالية لدراسة تأثير الاستبدال الجزئي أو الكلي لمسحوق السمك بمركبات بروتين الأرز (RPC) في علائق البلطي النيلي (*O. niloticus*) وتأثيرها على معدلات النمو ومكونات الجسم ودراسة الصفات الهستولوجية للأمعاء. تم استخدام 320 سمكة من اسماك البلطي النيلي بمتوسط وزن جسم 40.50 ± 0.250 جم. قسمت الأسماك عشوائياً إلى 4 مجموعات متساوية وكل مجموعة تحتوي علي 4 مكررات (كل مكرر يحتوي علي 20 سمكة). تم تكوين عليقة ضابطة احتوت علي مسحوق السمك (G1) اما المجموعات التجريبية الأخرى (G2-4) تم استبدال مسحوق السمك بـ 25% و 50% و 100% مركبات بروتين الأرز علي التوالي. غذيت اسماك البلطي النيلي مرتين يوميا بمعدل 3% من وزن الجسم لمدة 90 يوما.

لم يتأثر وزن الجسم باستبدال مسحوق السمك بمركبات بروتين الأرز بنسبة 100% مقارنة بالمجموعة الضابطة، بينما في هذا المستوى من الاستبدال، كانت استهلاك ومعدلات تحويل العلف أعلى مما كانت عليه في المجموعة الضابطة. انخفضت نسب كفاءة البروتين وكفاءة الاحتفاظ بالبروتين عن طريق استبدال مسحوق السمك بمركبات بروتين الأرز بنسبة 25% و 50% و 100% مقارنة بالمجموعة الضابطة

أظهرت النتائج أن استبدال 50% و 100% من مسحوق السمك بمركبات بروتين الارز سجل انخفاضاً معنوياً في البروتين والكالسيوم والفوسفور ومحتوى الدهون أعلى من المجموعة الضابطة. جميع المجموعات التجريبية التي تم تغذيتها على النظام الغذائي المعتمد على مركبات بروتين الارز كان لها انخفاض معنوي في السيستين ، الميثيونين ، اللايسين ، الثريونين والسيرين من المجموعة الضابطة. من ناحية أخرى ، أظهرت المجموعات التجريبية التي تغذت على النظام الغذائي المعتمد على مركبات بروتين الارز ارتفاعاً ملحوظاً في طول وعمق خملات الامعاء و سماكة العضلات.