Research Article



Performance, and Economic Efficiency of Replacing Fish Meal with Rice Protein Concentrate in *Oreochromis niloticus* Diets

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Abstract The present work was designed to study the effect of partial or complete replacement of fish meal by rice protein concentrate (RPC) in the diets of Oreochromis niloticus (O. niloticus) and its effect on growth performance traits, some blood biochemical parameters, nutrients digestibility, and economic efficiency. A total of 320 O. niloticus with an average body weight of 40.50±0.270 gm were used. The fish were randomly divided into 4 equal replicate groups (Each replicate contained 20 fish). A basal control diet was formulated with zero replacement of fish meal (G1) and other experimental groups (G2-4) received a basal diet containing fishmeal replaced with 25%, 50%, and 100% RPC, respectively. The fish were fed isonitrogenous, isocaloric diets 4 times daily at a rate of 5% of body weight for 12 weeks. Bodyweight gains were not affected by replacing fish meal by RPC 100% compared to control, while at this level of replacement, feed intakes, and feed conversion ratios were higher than in the control group. Protein efficiency ratios and protein retention efficiencies were decreased by replacement fish meal with RPC at 25%, 50%, and 100% than the control group. Protein and lipid profile parameters in fish serum were not significantly different between all experimental groups except triacylglycerol and very-low-density lipoproteins were lowered in RPC 100% than the other groups. Dry matter digestibility wasn't different between experimental groups except in RPC 100% it was decreased, while crude protein digestibility was lowered with all percent of fish meal replacement with RPC and EE digestibility was lowered in 50 and 100% RPC groups compared to control. It could be concluded that RPC could replace up to 100% of FM in O. niloticus diets without any adverse effects on growth parameters as well as high economic efficiencies and high net return.

Keywords | O. niloticus, Rice protein concentrate, Growth, Digestibility, Economics.

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INTRODUCTION

In Egypt, there is a a strong attention within the aquaculture industry to utilize high nutritional and more economical protein sources to replace the traditional marine protein ingredients such as fish meal (FM). (Suarez et al., 2013; Katya et al., 2014). However the very good nutritive value of FM, its price are quite high as well as the inconstant production and uncertain availability in the aftertime. Accordingly, to allow the sustainable expansion of the aquaculture industry, it is of great significance to explore other protein alternatives to FM in aqua diets. In this approach, plant protein meals are perfect candidates

due to their rich abundance and relatively low cost (Tacon and Metian, 2008; Olsen and Hasan, 2012).

Rice protein concentrate (RPC) have been reported to be an alternative protein ingredient of FM because of its high contents of protein and lipid profile (Palmegiano et al., 2006, 2007; Cai et al., 2018). Unfortunately, lysine is the first limiting indispensable amino acid in RPC, as limits its utilization in aqua diets (Gatlin et al., 2007). Consequently, synthetic lysine should be supplemented to balance the amino acid profile of aquadiets to boost the utilization of RPC.



Fish meals can be replaced by RPC without any marked difference on the growth performance traits, hematological parameters, and nutrient digestibility by up to 20% in rainbow trout (Palmegiano et al., 2006), 64% in blackspot seabream (Daprà et al., 2009), up to 90% in gilthead seabream (Sánchez-Lozano et al., 2009), up to 50% in shrimp (Oujifard et al., 2012), up to 25% in European sea bass juveniles (Güroy et al., 2013), up to 18% with microcapsule lysine addition in Chinese soft-shelled turtle (Sun et al., 2018), up to 100% with lysine addition (Cai et al., 2018) or 1.5% xylooligosaccharide addition in blunt snout bream (Abasubong et al., 2019).

The objective of this study was therfore to evaluate the effect of partial or total replacement of the *O. niloticus* dietary FM protein with RPC on the growth performance traits, some blood biochemical parameters, nutrient digestibility, and economic efficiency.

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 ± 0.270 g were procured from a private fish farm, Abbassa village, Abu-Hammad district, Sharkia Governorate, Egypt. Fish were stocked in clean concrete ponds (3 x 1 x 1 meter) filled with de-chlorinated freshwater and aerator. The water temperature (°C), dissolved oxygen (DO, mg/L), pH, ammonium (NH₄, ppm), and nitrite (NO₂, mg/L) were measured by using bag of water aquarium analysis and found to be 27 \pm 2 °C, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02 mg/l, respectively.

Fish were divided into 4 equal replicate groups (Each replicate contained 20 fish). The fish were adapted to the experimental conditions for two weeks before the start of the experiment.

FISH DIETS AND FEEDING

The control group received a basal diet without RPC (G1) and other experimental groups (G2-4) received a basal diet containing fishmeal replaced with 25%, 50%, and 100% RPC, respectively. All fish were fed their respective diet at a level of 5% of body weight three times daily for 12 weeks. 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 was formulated to meet the nutrient requirements of *O. nilo*ticus 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 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 determined. The following growth performance traits indicators were determined as a protein efficiency ratios (Stuart and Hung, 1989), and Protein retention efficiencies (Castell and Tiews, 1980). Mortalities were recorded as it occurred to calculate the relative survival % by subscribtion of mortality from total number of fish.

BIOCHEMICAL EVALUATION IN EXPERIMENTALLY USED FISHES

Blood was obtained from caudal blood vessels into plastic Eppendorf tubes (0.5 mL; capacity) for sera preparation. Blood was collected into Eppendorf tubes without anticoagulant in the syringe then centrifuged (Heavy Duty Doctor Model; model name: DM3000BCMWSR; 3,000 R.P.M. for 15 min). Sera were collected and stored immediately in a deep freezer (-20°C) until use. Sera were examined to determine some biochemical metabolites using diagnostic kits (Roch Diagnostics, GmbH, USA) such as total protein and albumin (Burtis et al., 2006), while globulin was calculated by the difference between total protein and albumin. Total cholesterol (Pisani et al., 1995); triglyceride (Stein and Myers, 1995); HDL-cholesterol (Nitschke and Tall, 2005); LDL-cholesterol and VLDL-cholesterol (Sonntag and Scholer, 2001). Serum AST; aspartate aminotransferase (Murray, 1984) and ALT; alanine aminotransferase (Young, 2001).

DIGESTIBILITY TRIALS

As 0.50 % chromic oxide was incorporated at the end of the experiment, large individuals of each fish group were reared in glass aquaria (30 x 40 x 150 cm) and given diets containing chromic oxide two times daily for one week as adaptation period, then for two weeks as a collection period for determination of nutrients digestibility. The feces



Table 1: Composition of 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-Lysin HCL, 98%	-	-	-	-
D L- Methionine	-	-	0.10	0.15
Calcium carbonate	-	-	-	-
Vitamin. mineral premix*	0.50	0.50	0.50	0.50
Total, %	100	100	100	100
Calculated compostion				
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
Lysin, %	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 (kg/ diet) {Vit .A 6000 I.U, D3 2.000 I.U, E 500 mg, k3 12.0 mg, C. 1.000 mg. B_1 10 mg, B_2 15.0 mg, B_3 7.5 mg, B_{12} 0.1 mg, Biotin 0.2 mg, Folic acid 0.4 mg, cholin Hcl 1.0 g inosit. 3000.0 mg, pantothemic acid 50.0 mg, Nicotinic acid 100 mg, P-Aminobenzonic acid 50 mg, iron 80 mg, copper 5 g, zinc 40 g, Sodium selenite 100 mg and, potassium iodide 300 mg, and cobalt sulphate 100 mg}.

Table 2: Effect of the replacement fishmeal with RPC on *O. niloticus* overall performance during 0- 12 weeks (means ±SE).

Parameters	Experimental groups				
	Control	Rice protein concentrates			
		25%	50%	100%	
Initial body weight, g	40.33 ± 0.22	40.97 ± 0.26	41.05 ± 0.15	40.53 ± 0.27	
Final body weight, g	74.69 ± 0.16 a	74.17 ± 0.09 b	74.17 ± 0.18 b	74.02 ± 0.08 b	
Total gain, g	34.35 ± 0.36 a	33.20 ± 0.32 ab	33.12 ± 0.05 b	33.49 ± 0.35 a	
Gain,%	85.19 ±1.35 a	81.06 ± 1.28 ab	80.68 ± 0.28 b	82.64 ± 1.44 a	
Total feed intake, g	69.78 ±0.84 b	71.51 ±1.28 b	73.37 ±0.32 b	74.12 ± 1.02 a	
Feed conversion ratio	2.03 ± 0.03 b	2.15 ± 0.02 ab	2.22 ± 0.01 a	2.15 ± 0.01 a	
Specific growth rate	0.68 ± 0.01 a	0.66 ± 0.01 b	0.66 ± 0.002 b	0.67 ± 0.01 ab	

^{**} digestible energy calculation based on values of protein 3.5 kcal/g, fat 8.1 kcal/ g, NFE 2.5 kcal/g according to (**Santiago** *et al.*, **1982**).

Protein efficiency ratio	3.81 ± 0.06 a	3.54 ± 0.01^{b}	3.44 ± 0.02 b	3.48 ± 0.02 b
Protein retention efficiency	27.30 ± 0.33 a	$25.72 \pm 0.30^{\mathrm{b}}$	25.24 ± 0.07 bc	24.70 ± 0.19 °
Relative survival, %	96.67 ± 1.67	95.00 ± 2.89	93.33 ± 1.67	93.33 ± 1.67

^{abc} Means within the same row carrying different superscripts are significantly different at $(P \le 0.05)$.

were collected after giving diets by about 5-6 hours using a fine net; feces were collected after cleaning the aquaria to ensure no residue of diets were left. The collected feces from each group were dried at 70°C for 72 hours then at 105°C for chromic oxide analysis. Before analysis, the samples were dried in a forced-air oven at 55°C for 72 hours, followed by fine grinding. The excreta samples for each replicate from the 2 weeks were pooled and then frozen for storage. The chromic oxide concentration of the excreta and diet samples were measured according Petry and Rapp (1971). Diets and excreta were analyzed for DM, CP, and EE. The digestibility coefficients for DM, CP, and EE were determined using the equations for the indicator method described by Cho and Singer (1979).

ECONOMIC EFFICIENCY (Y)

It was calculated according to the following formula: Y= (A-B)/B x 100 where A was the selling cost of the obtained gain and B was the feeding cost of this gain. (El-Kerdawy, 1997).

STATISTICAL ANALYSIS

Data were statistically analyzed using one way ANOVA, LSD (Least significant difference) based on Snedecor and Cochran (1982) using model {Y ik = μ + Li + eik} where Y ik = observation, μ = over all means, and Li = effect of groups as G1 (group 1) (j = 1, 2, 3 and 4); in our experiment, 1 = control, 2 = RPC 25%, 3 = RPC 50% and 4 = RPC 100%. eik = random error, after normality was verified using the Kolmogorov–Smirnov test. Comparing differences among different means were detected with Duncan's multiple range tests (Duncan, 1995). Data were shown as mean ± SE and the significance was considered at p < 0.05.

RESULT AND DISCUSSION

Effect of the replacement fishmeal with RPC on *O. niloticus* on overall growth performance during the whole period of the experiment (Table 2). The final body weights of the control group had marked elevates ($P \le 0.05$) with the other three groups of RPC levels 25%, 50%, and 100%. Also, the total body weight gain and the gain percentage of the control group had a marked increases ($P \le 0.05$) with the group of RPC 50% but no significant changes were recorded between the control group and the other groups of the level of 25% and 100%. Regarding to the total feed intake, the control group had marked decreases ($P \le 0.05$) with

the group of RPC 100% but there was no significant differences with the other experimental groups. The feed conversion ratios of the control group had marked decreases (P ≤ 0.05) with both groups of 50%, and 100%, respectively, while no significant changes with the group of RPC 25% was observed. Regarding to the specific growth rates, the control group had marked elevates (P ≤ 0.05) SGR than both of the experimental groups of 25%, and 50% while no significant changes were detected with the group of RPC 100%. On the other hand, protein efficiency ratios and protein effeciencies were lowered with replacement of the fish meal by RPC 25%, 50%, and 100% than control group. The relative survival percentage wasn't 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 line, RPC could be used without any effect on the growth performance reductions, within the inclusion rate of up to 20% in rainbow trout diets (Palmegiano et al., 2006). At the same time, these results were in agreement with the finding of Sánchez-Lozano et al. (2009) who concluded that there were no changes in growth parameters of growing gilthead seabream when FM replaced with RPC. Our results were assured by published data which reported that up to 50% FM can be replaced with RPC as an alternative protein ingredient in the commercial shrimp diets without affecting growth (Oujifard et al., 2012). More interestingly, the RPC has potentials as a sustainable feed ingredients for using in European sea bass juveniles diets. Dietary inclusion at levels up to 25% replacement of FM (14%) could be used without any observed effects on the growth performance (Güroy et al., 2013). Also, it has been reported that RPC with microcapsule lysine supplementation could be replaced by a dietary 18% FM without any growth degradation in Chinese soft-shelled turtle (Sun et al., 2018).

Effect of the replacement fishmeal with RPC on *O. niloticus* serum biochemical tests (Table 3). The results showed that there were no marked ($P \le 0.05$) differences between all experimental groups in the total protein, albumin, globulin, cholesterol, HDL-cholesterol, and LDL-cholesterol. On the other hand, replacement of the 100% of fishmeal with RPC had recorded significant ($P \le 0.05$) lower triacylglycerol and VLDL-cholesterol than other experimental groups. Furthermore, RPC could replace as much as 64% of FM without any detrimental effects on the intestinal morphology and physiology of black spot seabream, *Pagellus bogaraveo*, while dietary RPC supplement caused marked increases in trypsin activity, whereas lipase activity was reduced (Daprà et al., 2009). As obtained by this study,





Table 3: Effect of the replacement fishmeal with RPC on O. niloticus serum biochemical tests (means ±SE).

Parameters	Experimental groups			
	Control	ntrol Rice protein cond		
		25%	50%	100%
Total protein, g/dl	6.89 ± 0.11	6.82 ± 0.08	6.79 ± 0.13	6.79 ± 0.09
Albumin, g/dl	4.32 ± 0.05	4.09 ± 0.06	4.24 ± 0.04	4.16 ± 0.09
Globulin, g/dl	2.57 ± 0.16	2.73 ± 0.10	2.55 ± 0.12	2.63 ± 0.10
Cholesterol, mg/dl	90.17 ± 2.31	88.14 ± 1.83	86.90 ± 0.66	86.62 ± 1.88
Triacylglycerol, mg/dl	92.36 ± 0.52 a	92.48 ± 0.92 a	90.37 ± 0.26 ab	88.62 ± 0.32 b
HDL-Cholesterol, mg/dl	38.52 ± 0.52	35.69 ± 0.73	37.05 ± 1.15	37.23 ± 0.28
LDL-Cholesterol, mg/dl	29.28 ± 0.55	28.65 ± 0.24	30.14 ± 0.35	29.96 ± 0.47
VLDL-Cholesterol, mg/dl	18.47 ± 0.10 a	18.50 ± 0.18 a	18.07 ± 0.05 ab	17.72 ± 0.06^{b}
ALT, U/L	31.77 ± 0.17	31.49 ± 0.27	30.91 ± 0.22	31.48 ± 0.24
AST, U/L	37.24 ±0.10	37.27 ± 0.36	36.68 ±0.18	37.02 ± 0.42
Creatinine, U/L	0.22 ± 0.04	0.22 ± 0.08	0.21 ± 0.07	0.22 ± 0.01
Uric acid, mg/dl	1.98 ± 0.01	2.01 ± 0.04	1.97 ± 0.004	2.03 ± 0.03
Urea, mg/dl	3.51 ± 0.07	3.50 ± 0.03	3.57 ± 0.06	3.55 ± 0.04

^{ab} Means within the same row carrying different superscripts are significantly different at $(P \le 0.05)$.

Table 4: Effect of the replacement fishmeal with RPC on *O. niloticus* nutrient digestibility (means ±SE).

Parameters	Experimental groups				
	Control	Rice protein concentrates			
		25%	50%	100%	
Indicator in feed, %	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	
Indicator in feces, %	2.38 ± 0.018	2.39 ± 0.01	2.37± 0.01	2.38 ± 0.01	
DC of DM	79.02 ± 0.16	79.04 ± 0.05	78.92 ± 0.10	78.99 ± 0.08	
CP in feed, %	32.24 ±0.02	32.23 ± 0.04	32.27 ±0.03	32.25 ± 0.05	
CP in feces, %	16.88 ± 0.54	17.08 ± 0.60	17.70 ± 0.82	18.87 ± 0.18	
DC of CP	89.01 ± 0.40	88.89 ± 0.38	88.43 ± 0.57	87.71 ± 0.13	
EE in feed, %	11.49 ± 0.11^{a}	11.14 ± 0.05^{b}	$10.99 \pm 0.05^{\rm b}$	$10.53 \pm 0.05^{\circ}$	
EE in feces, %	5.18 ± 0.22	5.12 ± 0.23	5.37 ± 0.20	5.46 ± 0.26	
DC of EE	90.54 ± 0.41	90.36 ± 0.44	89.70 ± 0.43	89.10 ± 0.59	

^{abc} Means within the same row carrying different superscripts are significantly different at $(P \le 0.05)$.

Table 5: Effect of the replacement fishmeal with RPC on *O. niloticus* economic efficiency (means ±SE).

Parameters	Experimental groups			
	Control	Rice protein concentrates		
		25%	50%	100%
Feed price/kg, LE	9.56 ± 0.00	8.86 ± 0.00	8.18 ± 0.00	6.78 ± 0.00
Total feed cost/fish, LE	0.67 ± 0.01^{a}	0.63 ± 0.01^{b}	$0.60 \pm 0.003^{\circ}$	$0.50 \pm 0.01^{\rm d}$
Feed cost / kg gains, LE	19.42 ±0.26 ^a	19.08 ± 0.16^{a}	18.12 ± 0.05^{b}	15.01 ± 0.08^{c}
Profit/ kg gains, LE	$2.58 \pm 0.26^{\circ}$	$2.92 \pm 0.16^{\circ}$	3.88 ± 0.05^{b}	6.99 ± 0.08^{a}
Benefit-cost ratios	0.27 ± 0.03^{d}	$0.33 \pm 0.02^{\circ}$	$0.47 \pm 0.01^{\rm b}$	1.03 ± 0.01^{a}

^{abc} Means within the same row carrying different superscripts are significantly different at $(P \le 0.05)$.

the RPC dietary inclusion at levels up to 25% replacement of FM could be used without marked effects on the growth performance, nutrient utilization, digestibility or hematological parameters (Güroy et al., 2013).

Effect of the replacement fishmeal with RPC on *O. niloticus* on nutrient digestibility (Table 4). The results showed that there were marked decreases ($P \le 0.05$) in the percentage of the indicators in the feces and the DC of DM



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in the groups fed 100%, RPC comparing to the other experimental groups (Table 4). The crude protein content in the feed showed no significant ($P \le 0.05$) differences between all experimental groups, while there were significant increases ($P \le 0.05$) of the protein content in the feces in the groups fed 100%, 50% and 25% RPC compared to the control group fed fishmeal based diet. Meanwhile, there was marketly ($P \le 0.05$) lower of the DC of CP and EE in feed in the groups fed 100%, 50%, and 25% RPC compared to the control group fed fishmeal based diet. On the other hand, replacement of the 50% of fishmeal with RPC had recorded significant ($P \le 0.05$) higher EE in feces than control diet and 25% RPC replacement but lower than 100% RPC replacement. Meanwhile replacement of the 50% of fishmeal with RPC had recorded marked (P ≤ 0.05) lower DC of EE than control diet and 25% RPC replacement but higher than 100% RPC replacement. Replacement with higher levels of RPC than 20% resulted in a linear reduction in the apparent digestibility coefficients (ADC) of the nutrients and energy and this was mirrored in the growth performance of the fish (Palmegiano et al., 2006). However, the replacement of FM by RPC up to 50% resulted in an almost linear reduction in the ADC of the nutrients (Oujifard et al., 2012). Additionally, the dietary inclusion of RPC at levels up to 25% could be used without negative effects on the growth performance, nutrient utilization, digestibility, or hematological parameters (Güroy et al., 2013).

Effect of the replacement fishmeal with RPC on *O. niloticus* economic efficiency (Table 5). The results showed that the control group fed fishmeal-based diet had recorded marked ($P \le 0.05$) higher total feed cost per fish than the rest of the experimental groups. Meanwhile, the control group and the group of fishmeal replacement with 25% RPC concentrate had recorded marketly ($P \le 0.05$) higher feed cost per kilogram-fish weight gains than the rest of the experimental groups. On the other hand, fishmeal replacement with 100% RPC concentrate had recorded marketly ($P \le 0.05$) higher profit per kilogram-fish weight gain and benefit-cost ratio than the rest of the experimental groups.

CONCLUSION

RPC could replace fish meal in Nile tilapia feed tell 100% without any adverse effect on fish performance, nutrients digestibility, blood chemistry as well as high economic efficiencies and high net return.

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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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