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Research Article
Impact of Partial Replacing of Dietary Fish Meal by Different Protein Sources on the Growth Performance of Nile Tilapia (*Oreochromis niloticus*) and Whole Body Composition

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Abstract
Background and Objective: The present study aimed to assess the appropriate level of replacement of fish meal with alternative protein sources in the feed fed to *Oreochromis niloticus* to evaluate the growth performance. Five diets were formulated. The control diet using fish meal at level 20%, other diets using 10% of fish meal and other 10% using *Chlorella vulgaris* algae, blood meal, hydrolyzed feather meal and rapeseed meal. **Materials and Methods:** One hundred and fifty Nile tilapia (21.8 g) were randomly distributed into five groups of three replicates of 15 fish/aquaria for 8 weeks. Body weight gain, feed conversion, specific growth rate, protein efficiency ratio and chemical analysis of fish body were measured. **Results:** No significant differences in growth performance between experimental groups were observed. There were significant decrease in ash% of whole fish body fed rapeseed meal and hydrolyzed feather meal. However, the ether extract percentage of the whole fish body fed rapeseed meal was higher than other experimental groups. No significant differences in amino acids composition of whole fish body fed the experimental diets. **Conclusion:** It can be concluded that fish meal can be replaced by 50% of its level with other protein sources without any negative effects on growth performance of Nile tilapia.

Key words: Protein sources, diet, growth performance, Nile tilapia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquaculture production is known as a fast growing sector with a high quality source of protein[1]. About 40-60% of the aquaculture intensive system costs account for aqua feed with special focus of dietary protein which represents the major and most expensive component of formulated aqua feeds[2]. Fish meal (FM) is the commonest source, however, it is often rare and expensive[2]. Fish meal is characterized by its high protein content, balanced amino acid profile and high digestibility thus, it is considered the most desirable animal protein ingredient in aqua feeds[3]. There is a need to find alternative for FM for aqua feeds, especially after global FM has been increased more than two fold in recent years[1].

Simple aquatic plants as algae have different sizes ranging from the microscopic (microalgae) to macroscopic algae[4]. Inclusion of algae (2.5-10% of the diet) into fish diets resulted in enhances growth, feed utilization, gut health and carcass quality[4]. Total protein content of algae has a wide variation (8-50% dry weight), besides it has all essential amino acids (EAAs) and n-3 long-chain polyunsaturated fatty acids[5]. It has been suggested that *C. vulgaris* (CVA) contain supreme level of crude protein, carbohydrate, lipid, essential amino acids and minerals[6].

Blood meal (BM) is an animal waste product, produced from animal blood which usually is collected from locally abattoirs. The source of blood comes from various domestic animals such as cattle, pig and chicken[7]. It was reported that BM is considered as an alternative high quality protein source in fish feed formulations[8]. Blood meal contains high levels of protein (80-86%) and may have a favorable essential amino acids profile[2]. Moreover, it was postulated that BM is considered as a rather low cost and easy available product worldwide[8].

It has been reported that hydrolyzed feather meal (HFeM) is a by product from poultry production. The HFeM contains a complex protein (keratin), which can be hydrolysed to improve bioavailability[9]. Feather meal is an economical protein ingredient mostly used in aqua-feeds[10]. Moreover, the required EAAs for tilapia are found HFeM in a proper quantity[11,12].

Rapeseed meal (RM) is made from oilseeds from the genus *Brassica*. It was reported that RM contains around 3.5% residual oil, 35% crude protein, 6% ash and 12% crude fiber, also containing 4% of phytic acid and glucosinolate and other factors considered to have anti-nutritional effects[13].

It has been reported that tilapia fish are the second most important cultured fish in the world after carps and there are not any reports of cultural or religious restrictions on tilapia consumption all over the world[14,15]. Moreover, tilapia is often referred to as "aquatic chicken" due to its rapid growth rates, ease in culture, ability to eat many types of feeds, disease resistance, hardiness and tolerance to a wide range of environmental conditions[15].

Owing to the limited supply and high cost of FM, nutritionists have to find alternative protein sources to be included in fish diet production instead of FM. Numerous studies have investigated the potential use of alternative protein sources instead of FM in fish diets. Subsequently, the objectives of the present work were to evaluate the effects of using alternative dietary source of protein for Nile tilapia fish on performance and while body composition.

MATERIALS AND METHODS

Egyptian regulations of health guide for the care and use of laboratory animals were considered during performing the animal experiments and did not require any notification or approval in accordance with the Animal Protection Act. Interventions before dissection were not carried out.

Diet preparation: Five isonitrogenous (32% CP) diets were formulated to satisfy the nutritional requirements of Nile tilapia (*O. niloticus*)[11]. Nile tilapia fish were fed diets with different sources of protein as FM, CVA (*Chlorella vulgaris*-powder, Roquette Klötze GmbH and Co.KG, Klötze, Germany), BM (Haemoglobin blood of pigs, Acontex GmbH, Germany), HFeM and RM. Control basal diet contains 20% FM, while other experimental groups had partial (50%) replacement of FM with CVA, BM, HFeM and RM (10%).

Experimental design: One hundred and fifty unsexed fish weighing approximately (21.8 g) were received and stocked in 15 glass aquaria (80×35×40 cm), 15 fish in each and reared for 2 months (June-July). The experiment was done in the Nutrition and Nutritional Deficiency Diseases Laboratory, Faculty of Veterinary Medicine, Mansoura University. Each diet was fed to the fish in triplicate aquaria at 3% body weight (BW) twice daily (9.00-10.00 and 13.00-14.00 h) for 60 days. Fish were subjected to a photoperiod regimen of 12-13 h light and 12-11 h dark/day and the temperature during the experimental period was about 25-27°C. Daily cleaning for each aquarium was carried out with partial replacement of water by previously stored (for 48 h) dechlorinated tap water.

Sample collection: At the end of the experimental period (60 days), random fish samples (5 fish/aquarium) from all experiment groups were collected and minced for whole body chemical analysis.

Chemical analysis: The proximate analysis of diets and fish was performed in Animal Nutrition Institute, University of Veterinary Medicine Hannover, Germany according to the standard methods of AOAC[16]. Moisture content was determined by drying in a hot air oven at 105°C until constant weight, crude protein (N×6.25) was determined by Kjeldahl method after acid digestion, ash content was determined by incineration a muffle furnace at 500°C for 18 h. Ether extract was determined by soxhlet apparatus. Ash was determined by combusting dry samples in a muffle furnace at 550°C for 6 h. Calcium and phosphorus in fish whole body and in formulated diets were analyzed using Flame Atomic Absorption Spectrometry (Spectra 55B AAS) after wet ashing and acid digestion. A high performance liquid chromatography (HPLC) reverse phase system was used to separate and quantify amino acids[17].

Growth parameters measurements: The following equations were used to evaluate fish growth performance:

$$\text{Body weight gain (g)} = \text{Mean final weight (g)} - \text{Mean initial weight (g)}$$

$$\text{Feed conversation ration (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Specific growth rate (SGR, \%/day)} = \frac{\text{Ln (mean final body weight)} - \text{Ln (mean initial body weight)}}{\text{Culture period (days)}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain}}{\text{Crude protein fed (g)}}$$

Statistical analysis: The results were subjected to a one-way ANOVA to test the impact of partial replacement of FM by different sources of protein (CVA, BM, HFeM and RM) on fish growth performance and whole body composition. Data were analyzed using statistical SPSS v20 (SPSS Inc., Chicago, IL, USA). Differences between means were compared using Duncan's multiple range test at significance of differences ($p<0.05$) among dietary treatments.

RESULTS

Ingredient and diets composition: The nutrient profiles of the protein sources (FM, CVA, BM, HFeM and RM) are reported in Table 1. The protein content was higher in BM (90%) than in FM (64.5%), CVA (52.7%), RM (32.2%) and FEM (89%). The lipid contents of protein sources are 9.5, 0.10, 9.9, 3.85 and 1.4% for FM, BM, CVA, RM and HFeM, respectively.

Table 2 shows the chemical composition of the experimental diets. The percent of FM included in the control diet was 20% while FM level in experimental diets was decreased to the 10% and other 10% from other alternative protein sources.

Table 1: Percentage of nutrients composition of protein sources used in experimental diets

	Sources of protein				
Parameters (%)	FM	CVA	BM	HFeM	RM
DM	96.0	96.5	95.10	95.0	87.3
Crude protein	64.5	52.7	90.00	89.0	32.2
Ether extract	9.5	9.9	0.10	1.4	3.85
Ash	20.0	6.1	2.14	1.9	7.62
Crude fiber	0.8	0.0	0.84	0.9	8.87

Sources of protein are fish meal (FM), algae meal (CVA), blood meal (BM), hydrolysed feather meal (HFeM) and rapeseed meal (RM)

Table 2: Percentage of ingredients and nutrients composition of experimental diets

	Experimental diets*				
Ingredients (%)	FM	CVA	BM	HFeM	RM
Yellow corn (8.5%)	11.97	15.00	18.00	15.00	18.00
Soybean meal (44%)	20.30	23.66	15.00	16.00	24.70
Fish meal	20.00	10.00	10.00	10.00	10.00
Algae	0.00	10.00	0.00	0.00	0.00
Blood meal	0.00	0.00	10.00	0.00	0.00
Feather meal	0.00	0.00	0.00	10.00	0.00
Rapeseed meal	0.00	0.00	0.00	0.00	10.00
Wheat bran	40.00	30.00	39.66	41.66	20.46
Corn gluten	1.00	3.00	0.00	0.00	8.00
Gelatin	2.00	2.00	2.00	2.00	2.00
Vegetable mixed oil	3.00	4.50	3.50	3.50	5.00
**Minerals and vitamins premix	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin C	0.10	0.10	0.10	0.10	0.10
antioxidant	0.02	0.02	0.02	0.02	0.02
Dicalcium phosphate	0.10	0.10	0.10	1.00	0.10
Methionine	0.21	0.32	0.32	0.32	0.32
Chemical composition (analyzed)					
Crude protein(%)	32.00	32.10	32.10	32.40	32.00
Ash (%)	7.28	6.84	6.52	5.86	6.71
Ether extract (%)	5.83	5.97	6.48	6.45	6.00
Crude fiber (%)	2.62	2.80	2.24	2.89	3.39
Calcium (%)	1.18	1.00	1.04	0.95	0.98
Phosphorus (%)	0.97	1.00	0.93	0.77	0.92

*FM, CVA, BM, HFeM, RM represent diets of Nile containing 100% of FM or diets containing 50% FM and 50% of CVA, BM, HFeM, RM. **Trace minerals and vitamins premixes were prepared[11]. Vitamins premix (IU or mg kg^{-1} diet), Vit. A: 5000, Vit. D3: 1000, Vit. E: 20, Vit. K3: 2, Vit. B1: 2, Vit. B2: 5, Vit. B6: 1.5, Vit. B12: 0.02, Pantothenic acid 10, Folic acid 1, Biotin 0.15, Ni acid 30. Mineral mixture (mg kg^{-1} diet), Fe: 40, Mn: 80, Cu: 4, Zn: 50, I: 0.5, Co: 0.2, Se: 0.2

Table 3: Analyzed amino acids composition of the experimental diets

Amino acids (%)	Experimental diets FM	CVA	BM	HFeM	RM	EAAs req of Nile tilapia[#]
Non-essential amino acids						
Asp	3.23	3.41	4.30	3.14	3.49	-
Ser	1.78	1.79	1.65	2.39	1.95	-
Glu	6.89	6.24	5.48	6.31	7.50	-
Gly	2.16	2.34	2.20	2.70	2.34	-
Ala	1.73	1.96	2.25	1.71	1.97	-
Prol	2.62	2.38	1.97	2.84	2.79	-
Tyr	1.20	1.20	0.98	1.09	1.29	
Essential amino acids						
Val	1.64	1.57	2.17	1.91	1.81	0.78
Met+Cys	1.44	1.24	1.11	1.79	1.34	0.90
Ileu	1.44	1.43	0.98	1.51	1.58	0.87
Leu	2.84	2.97	3.20	2.67	3.39	0.95
Thr	1.31	1.23	1.16	1.42	1.33	1.05
Phe	1.4	1.77	1.87	1.64	1.89	1.05
His	0.80	0.99	1.37	0.67	0.87	0.48
Lysin	2.03	2.20	2.30	1.72	2.04	1.43
Arg	2.29	2.64	2.30	2.43	2.48	1.18
NH_3	0.64	0.64	0.52	0.59	0.64	

[#]Amino acids requirement of Nile tilapia. Source: NRC[11]

Table 4: Influence of partial replacement of FM with different sources of protein on growth performance of Nile tilapia

Parameters	Experimental diets FM	CVA	BM	HFeM	RM
IW (g)/fish	21.92±0.96	22.31±0.83	21.44±0.75	21.22±0.77	21.85±0.81
FW (g)/fish	54.80±1.86	54.27±2.50	49.83±2.30	50.02±2.20	50.65±2.84
BWG (g)/fish	32.70±1.24	31.00±1.62	29.81±1.75	29.55±1.42	29.77±1.64
FCR	1.95±0.04	2.15±0.43	2.09±0.09	2.16±0.27	2.23±0.42
SGR	1.52±0.04[a]	1.33±0.03[b]	1.54±0.06[a]	1.49±0.03[a]	1.33±0.02[b]
PER	1.89±0.07[a]	1.62±0.09[b]	1.75±0.10[ab]	1.68±0.08[ab]	1.59±0.10[b]

Values in the same row with different superscript are significantly different from each other (p<0.05). Mean±SE, IW: Initial weight, FW: Final weight, BWG: Body weight gain

The protein percent for different experimental diets were almost equal (32%). Essential amino acid (EAA) composition of five experimental diets and the EAAs requirement of Nile tilapia were presented in Table 3. The EAAs contents of all the diets were sufficient to satisfy the EAAs requirements for Nile tilapia[11]. The RM diet had the highest levels of isoleucine, leucine and phenylalanine (1.58, 3.39 and 1.89%, respectively) compared to other diets.

Growth performance: The results of growth performance of fingerlings Nile tilapia fed on diets containing 20% FM or diets with partial replacement (10%) of FM with 10% of HFeM, BM, RM and CVA are presented in Table 4. There were no significant differences (p>0.05) in FW, BWG, FCR, SGR and PER between fish fed 100% FM and fish fed 50% replaced of FM by BM. Furthermore, there were no significant (p>0.05) differences in growth performance (FW, BWG, FCR) between Nile tilapia fed 100% FM and fish fed 50% RM in the present work. However, there were significant (p<0.05) decrease in SGR and PER in fish group fed 50% RM. In addition, no significant differences were indicated in growth performance (FW, BWG, FCR, SGR and PER) of Nile tilapia fed diet replaced 50% of FM by HFeM.

Also, results in this study indicated the SGR and PER were decreased significantly (p<0.05) in fish fed 50% CVA as partial substitute of FM in comparison to fish group fed 100% FM.

Body composition: The impact of partial replacement of FM by different sources of protein on the proximate composition of whole body fish is shown in Table 5 and 6. There was a significant increase of EE% of whole fish body fed 50% replacement of FM with other protein sources (CVA, BM, HFeM and RM) compared with whole fish fed 100% FM. Most of the EAA composition of whole fish fed 100% FM were similar to those fish fed different sources of protein (CVA, BM, HFeM and RM). However, the levels of isoleucine and leucine of whole fish body of fish fed 100% FM were numerically higher than those levels in whole fish body in other experimental groups.

Table 5: Impact of partial replacement of FM by different sources of protein on whole body chemical composition of Nile tilapia (on dry matter basis)

	Experimental diets				
Parameters	FM	CVA	BM	HFeM	RM
Moisture (%)	71.70±0.25	71.50±0.20	71.00±0.23	71.60±0.48	70.30±0.14
Ash (%)	19.60±0.12[a]	18.83±0.013[a]	17.88±0.05[ab]	16.39±0.4[b]	15.25±0.06[b]
Crude protein (%)	65.60±1.32[a]	63.70±0.95[ab]	63.90±1.01[a]	62.60±1.22[ab]	61.10±2.12[b]
Ether extract (%)	12.93±0.04[d]	16.23±0.05[c]	16.94±0.09[bc]	18.46±0.06[b]	20.85±0.08[a]
Calcium (%)	5.94±0.43	5.55±0.23	5.36±0.45	5.08±0.30	4.42±0.15
Phosphorus (%)	3.49±0.15	3.39±0.34	3.19±0.23	2.90±0.10	2.55±0.07

Values in the same row with different superscript are significantly different from each other ($p<0.05$). Mean±SE

Table 6: Amino acids profile of whole body of Nile tilapia as affected by partial replacement of FM with different protein sources

	Experimental diets*				
Amino acids (%)	FM	CVA	BM	HFeM	RM
Non-essential amino acids					
Asp	6.21±0.45	6.37±0.34	6.37±0.54	6.22±0.75	5.96±0.65
Ala	4.19±0.23	4.21±0.56	4.26±0.14	4.02±0.14	3.97±0.24
Serine	2.88±0.12	2.67±0.15	2.72±0.24	2.76±0.16	2.49±0.09
Glu	8.98±0.54	8.87±0.65	8.99±0.67	8.88±0.23	8.36±0.54
Gly	5.15±0.23	5.20±0.23	5.08±0.24	4.92±0.17	4.82±0.14
Tyr	2.11±0.09	2.03±0.4	2.13±0.7	2.28±0.10	2.46±0.3
Proline	3.36±0.25	3.12±0.16	3.20±0.23	3.37±0.25	2.94±0.06
Essential amino acids					
Cyst	0.34±0.03	0.37±0.02	0.33±0.04	0.33±0.01	0.35±0.06
Meth	1.17±0.27	1.30±0.12	1.55±0.12	1.42±0.10	1.39±0.15
Ileu	3.55±0.05	2.52±0.27	2.45±0.14	2.43±0.14	2.61±0.23
Leu	5.32±0.11	4.82±0.11	4.75±0.34	4.59±0.24	4.77±0.47
Thr	2.59±0.12	2.54±0.26	2.53±0.16	2.53±0.15	2.41±0.18
Phe	2.51±0.04	2.99±0.16	2.95±0.21	3.00±0.22	2.88±0.23
His	1.48±0.03	1.59±0.23	1.62±0.08	1.48±0.04	1.52±0.05
Lysin	5.01±0.26	5.37±0.32	5.22±0.34	5.07±0.45	5.04±0.26
Arg	4.38±0.14	4.29±0.21	4.41±0.32	4.53±0.34	4.21±0.24
Val	2.74±0.10	2.87±0.16	2.64±0.12	2.69±0.15	2.68±0.18
NH_3	0.85±0.03	0.90±0.02	0.94±0.03	0.90±0.07	0.84±0.05

*FM, CVA, BM, HFeM and RM represent diet of Nile containing 100% of FM, or diets containing 50% FM and 50% of CVA, BM, HFeM or RM. Mean±SE

DISCUSSION

It is well known that protein sources which can replace FM in fish diets must be of low price, produced in sufficient quantities and contains proper protein levels with balanced amino acid and not have any adverse effects on growth or health of the fish[13,18].

Using BM to be partially replaced FM in diets of Nile tilapia did not affect the growth performance of fish. Using 50% BM to replace FM gave a good quality protein diet whereas, 100% replacement produces an almost poor diet[19]. Also, it was stated that a reduction in Nile tilapia performance was noted when depends on BM as the only protein source[20,21]. Similarly, it has been found that BM levels exceeding 50% of FM protein in diets fed to Nile tilapia fingerlings reared in cages for 120 days resulted in a significant reduction in fish growth, while 10% level was the most efficient[22]. However, in another study, growth and survival as well as feed conversion of *Clarias gariepinus* juvenile were not affected when FM was replaced completely (100%) by BM[23]. With the same concept, it has been found that the whole substitution of FM by BM improved effects on the growth performance of Nile tilapia fingerlings[24]. It has been stated that the variations among these results could be due to many factors as: Quality and source of protein, fish species and size and aquaculture systems[14]. Therefore, the varying results of performance of tilapia due to replace FM by BM indicate that the inclusion of BM in the feed should be limited.

Furthermore, partial replacement of FM by RM did not affect the FW and BWG of Nile tilapia. In another study, it has been found that 75% of RM could replace FM protein without significant effect on *O. mossambicus* growth[25]. On the other hand, it has been reported that because of presence of high content of glucosinolate (anti-nutrient) in rapeseed it was noted that only 15% RM could effectively substitute FM in *O. mossambicus* diets, while higher levels led to poor performance[26]. Also, it has been observed that tilapia growth is usually high when high quality source like FM is used but the reverse occur when low quality source such as plant protein is used[27].

On the same concept, it has been postulated that the growth of Nile tilapia did not affect with 50% replacement of FM by HFeM[28]. Also, it has been noted that substitution 50% of the FM in the control diet by HFeM did not have a significantly reduced growth performance in Nile tilapia[29]. However, it was stated that because of energy high need to destroy its keratinous material and increasing the availability of sulfur amino acid for growth and other metabolic processes the total replaced FM or SBM by HFeM decreased growth of fish and feed efficiency[12].

It has been reported a significant improvement in growth parameters and nutritional indices (BWG, SGR and FCR) of *M. rosenbergii* PL fed 50% FM replaced by *C. vulgaris* compared to other experimental groups (control, 100% FM, 75% *C. vulgaris*, 100% *C. vulgaris*)[30]. Similarly, it has been found an improvement in growth performance of Nile tilapia was obtained by addition of algae[31]. Moreover, it has been showed a significant improvement in survival, growth and body proximate composition of *O. niloticus* fed partial replacement of FM with dried microalgae *Chlorella* sp. and *Scenedesmus* sp.[32]. In addition, it was reported that increasing the level of algae in fish diet at levels less than 20% increased linearly with BWG of Nile tilapia[33]. With the same concept, it has been found that growth performance (BW, BWG and SGR) increased significantly ($p<0.05$) with increasing of *Chlorella sp*. replacement from zero to 50% algae[32]. Whole body composition shows no significant differences in ash, moisture and protein contents between experimental groups. Similarly, it was stated that the fish diet containing algae up to 45% did not cause a significant variation in carcass crude protein, ash and dry matter comparing with control diet for Nile tilapia[34]. However, it has been showed that the total protein, amino acid, carbohydrate and lipid content of *M. rosenbergii* PL fed FM replaced by 50% *C. vulgaris* were significantly ($p<0.05$) increased[30]. Also, it was reported a significant ($p<0.05$) higher dry matter and crude protein contents, but lower lipid content, in fish received algae at 50% level, compared to those fed 100% FM[32].

Also, the whole body fat content of fish fed different sources of dietary protein were higher than fat content of whole fish body fed 100% FM. Similarly, fish fed plant protein based diets obtained high fat compared to those fed FM based diet[35]. However, it has been showed a significant decrease of lipid % of the whole body Nile tilapia fed BM as source of protein instead of FM[20].

Moreover, no significant differences ($p>0.05$) in whole body composition of Nile tilapia fed 100% FM or FM replaced by 33 or 66% HFeM[36]. Also, it has been found that the substitution of FM by HfeM resulted in decrease of whole body EAAs. However, in this study, most of the EAAs compositions of whole fish fed 100% FM were similar to those fish fed different sources of protein (CVA, BM, HFeM and RM)[36].

CONCLUSION

The results showed that FM can be replaced by 50% of its level with other protein sources without any adverse effects on growth performance of Nile tilapia. Whole body composition shows no significant differences in ash, moisture and protein contents between experimental groups. However, the whole body fat content of fish fed different sources of dietary protein were higher than fat content of whole fish carcass fed 100% FM.

SIGNIFICANCE STATEMENT

This study focuses on describing the possibility of using alternative protein sources instead of fish meal in tilapia diets. This study will help farm management, researchers and policy maker to attain potential interest in using different protein sources instead of fish meal without any negative effects on growth or health of fish.

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