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DOI 10.26873/SVR-768-2019 **Original Research Article**

USING OF ANIMAL BY PRODUCTS AS AN ALTERNATIVE PROTEIN SOURCE IN NILE TILAPIA (*Oreochromis niloticus*) DIETS

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Abstract: Four diets were formulated to include different sources of animal by-products as a protein source in Nile tilapia (5.79 ± 0.05 g) diets. The first group fed diet containing fish meal (T1), while the 2nd, 3rd and 4th groups fed a mixture of poultry by-product meal (PBM), animal by-product meal (ABM) and kitchen waste meal (KWM) at 50:25:25 (T2); 25:50:25 (T3) and 25:25:50 (T4) ratio, respectively. All diets were formulated to be isonitrogenous and isolipidic. After 120 days, apparent digestibility and growth parameters were measured. The obtained results revealed that, the chemical composition of fish meal had the highest value of protein followed by PBM and KWM. Fish meal had higher essential amino acids content, except for the threonine and arginine amino acids, followed by PBM and ABM. The highest apparent digestibility of crude protein and ether extract were recorded in T1 and T2 followed by T4 and T3 groups. T1 and T2 groups exhibited significantly (*P*<0.05) the highest growth performance over fish fed T3 and T4 diets. It could be suggested that using of waste protein sources especially containing high PBM as a replacement for fish meal to reduce the feed cost per Kg fish Production.

Key words: animal by products; aigestibility; aish meal; arowth; Nile tilapia

Introduction

Nile tilapia (*Oreochromis niloticus*) has been widely cultured in Egypt due to its fast growth, efficient feed conversion and high market value (1, 2). In Egypt, the cost of feeding fish is about 70% from the total cost of farming (3). The high prices of the pelleted feeds resulted in decreasing the profit of tilapia farming (4). Also, the high prices and low availability of fish meal resulted in increasing the cost of pelleted feeds. Fish feed accounts for more than 50% of the total cost of fish farming.

For long time, fish meal has been served as major protein sources in manufacturing aquafeed due to its balanced amino acid profile and palatability (5). However, the resource shortages and rising price of fish meal seriously restrict the use of fish meal in aquaculture. In this light, much efforts have been conducted to seek the sustainable supplies of protein sources to substitute fish meal.

The plant protein sources have been limited used due to their relative low protein content and palatability, the presence of anti-nutritional factors and unbalanced amino acids profiles (6), and animal protein sources are also potential to replace fish meal in aquafeed because of their

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characterization in high protein content, total digestible dry matter and lack of anti-nutritional factors (7).

Animal by-products are used widely in aquafeed industry as an animal protein source due to its high nutritional value, palatability as well as the increased digestibility (8). Poultry wastes can be treated to preserve their nutrients and finally include them into fish diet which would reduce the cost of feeding as well as the level of environmental pollution (9).

This study was conducted to evaluate the possibility of replacing fish meal by mixture of poultry by-product, animal by-product and kitchen waste meals as an alternative protein sources and to study their chemical composition, as well as, their effects on digestibility coefficient, growth performance and nutrient utilization of Nile tilapia *(O. niloticus)*.

Materials and methods

Experimental waste by products

Experimental by products of poultry by-product meal (PBM), animal by-product meal (ABM) and kitchen waste meals (KWM) were collected from the poultry shops, animal slaughter houses and large restaurants, respectively. These wastes were boiled for 15 minutes to destroy any presence of *Escherichia coli* and *Salmonella sp.* by drying at temperature over of 60°C for 28 h, then, all ingredients were powdered in an electrical grinder, passed through a 0.5 mm sieve and mixed. All ingredients were processed into dry sinking pellet form with 1 mm diameter. Samples of ground waste were then taken to the laboratory for proximate composition analysis as shown in Table (1).

Experimental design

Four diets were formulated; the first treatment (T1) was kept as (Control) in which the fish was fed the basal diet with fish meal as a protein source, while in the other treatments, the fish meal (FM) in the basic diet was full replaced with a mixture of PBM, ABM and KWM in the following proportions; the second treatment (T2**)** 50, 25 and 25 %; the third treatment (T3), 25, 50 and 25 %; while the fourth treatment (T4), 25, 25 and 50 %, respectively and these ingredients were mixed after processing until obtaining a homogenous mixture (10). Each diet was fed to three replicates.

The diets were given according to their live body weight (BW) of the fish (3% of BW) and offered in two equal portions at 10.00 a.m. and 16.00 p.m. About 30 min after each feeding, rubber siphon was used to remove any feed residues from the aquarium tanks and maintain the water quality. The amount of feed given was constantly monitored so as not to over or under-feed them.

Experimental fish

A total number of 360 Nile tilapia (*O. niloticus*) fingerlings with an initial weight of 5.60-5.92 g were collected from private hatchery, Tollumbat No.7 in Riyad City, Kafrelsheikh Governorate, Egypt. Fish were randomly allocated into 12 glass aquaria (84 x 40 x 40 cm^3) (30 fish per aquarium) and acclimatized on the new environment (experimental conditions) for one week before the beginning of the experiment.

Growth parameters

Growth performance and feed utilization items were assessed by calculating average weight gain, average daily gain, specific growth rate, protein efficiency ratio, feed intake, feed conversion ratio, feed efficiency and survival rate.

Proximate chemical analysis

Chemical analysis of feed ingredients, experimental diets by following the standard methods, while amino acid analyzer was calibrated using a standard solution (AA-S-18, Sigma) according to (11). Apparent digestibility coefficients (ADC) of nutrients in the diets were determined using Cr_2O_3 as a marker (5g/kg) and was calculated according to (11).

Statistical analysis

Duncan multiple range test was used to detect the significant differences between the means of treatments (12). All analysis was performed using SAS (version 9.1 2004 SAS Institute, Cary, NC, USA) (13).

Results and discussion

As shown in Table (2), the CP; EE and ash contents were significantly varied ($P<0.05$) between different treatments. The FM had the highest value of CP followed by the PBM while the ABM had the lowest value. These results are supported by (14), who observed that, the values of FM protein were ranged between 64.31and 71.00 %. Conversely in the present study, the highest level of EE was in ABM followed by KWM while the FM had the lowest level of EE. The result agrees with (15) who observed that the EE of FM was ranged between 6.90 and 12.77%, respectively.

The results showed that, the KWM had the highest value of ash however, the lowest value was recorded in the FM. While, both PBM and ABM were intermediate. There were similar values for DM content in the fish fed for all dietary treatment ($P > 0.05$). Similar results were obtained by (16), who obtained that, the dry matter percentage was 93.16 and 94.23; crude protein was 70.44 and 52.12; crude fat was 7.36 and 23.47 and ash was 11.18 and 18.34 in FM and PBM, respectively. The opposite studies showed no significant variation in the case of crude protein between FM and PBM values (17). Moreover, (18) indicated that PBM is a suitable replacement of FM in practical formulation diets for tilapia.

Generally, the composition of PBM depends on processing conditions and the source of raw materials (19). So, there are some PBM have very high protein content (75-90%) with low contents of ash (less than 10%) and fat (less than 15%). Opposite values were reported by (20), who showed that the low quality of PBM had protein content (55-75%) higher levels of ash (up to 15%) and fat (more than 15% and up to 30%).

The present data revealed that, the essential amino acids (EAA) profile was significantly higher in the basal diet (control) except both threonine and arginine were higher in ABM and KWM, respectively. The lowest level of EAA found in KWM (Table 3). The chemical score of the free essential amino acid of the experimental FM, PBM, ABM and KWM were low for methionine, iso-leucine, and phenylalanine, while the highest value recorded for arginine and lysine. Similar results were obtained by (21). Additionally, (22) showed that the EAAs in FM and PBM were 5.97 and 6.09 for arginine; 2.06 and 1.98 for histidine; 3.73 and 3.89 for isoleucine, 6.78 and 7.04 for leucine; 12.78 and 12.17 for lysine; 2.92 and 2.74 for methionine; 4.05 and 4.17 for phenylalanine; 3.88 and 3.65 for threonine; 0.43 and 0.42 for tryptophan and 4.59 and 4.84 g /kg diet for valine, respectively.

However, PBM is deficient in one or more essential amino acids (16). But, the level of amino acid profiles of PBM is relatively like FM (23) making the ingredient a valuable protein source for many species. However, at replacing of FM with PBM may need to be supplemented with some essential amino acid such as lysine, methionine and threonine to make the best growth performance and body composition of fish.

The deficiency of EAAs results in poor utilization of dietary CP and reduces growth and feed efficiency. By estimating the level of EAAs in the tested ingredients, it was clear that, the level of all the essential amino acids was higher than the required level for fish feeding.

As shown in Table (3), the highest values of non-essential amino acids in tested different ingredients were recorded in FM except glycine and tyrosine. In contrast, the lowest value of non-essential amino acids was found in KWM and PBM had the highest value of tyrosine and aspartic acids while glycine showed the highest value in ABM. Similarly, (24) who found that amino acids of Nile tilapia fed FM, PBM or porcine by-product meal had similar amino acids profile.

The present results showed lower value of non-essential amino acid than those reported by (22), who reported that the non-essential amino acids value in FM and PBM were for alanine 5.98 and 5.85; aspartic 8.36 and 7.67; cystine 0.95 and 0.94; glycine 8.54 and 8.37; glutamate 13.37 and 13.53; proline 5.35 and 5.80; serine 3.75 and 3.68; tyrosine 2.80 and 2.70 g /Kg, respectively. In opposite, FM could only be replaced with PBM at a level which did not exceed 50%.

Table 1: Ingredients and chemical composition (%) of the experimental diets

Ingredients	Treatments			
	FM	PBM	ABM	KWM
Fish meal	10. 00	00.00	00.00	00.00
Poultry by-product meal	00.00	5.00	2.50	2.50
Animal by –product meal	00.00	2.50	5.00	2.50
Kitchen west meal	00.00	2.50	2.50	5.00
Soybean meal	50 .56	50.50	50.60	50.75
Yellow com	32.50	32.35	32.20	32.00
Com oil	4.38	4.50	4.43	4.38
Limestone, ground	1.52	1.60	1.71	1.80
Methionine	0.24	0.25	0.26	0.27
Premix*	0.30	0.30	0.30	0.30
Chromic oxide	0.50	0.50	0.50	0.50
Total	100	100	100	100
Proximate analysis of the experimental diets (% DM)				
Dry matter (DM)	91.57±0.11	91.69±0.07	91.60±0.07	91.48±0.12
Crude protein (CP)	35.20±0.15	35.03±0.14	35.16±0.25	34.67±0.45
Crude fat (EE)	10.17±0.21	10.33±0.14	9.85±0.08	10.57±0.65
Ash	12.18±0.16	12.54±0.55	12.70±0.28	12.48±0.21
Crude fiber (CF)	4.95±1.50	5.13±1.48	5.59±0.82	5.75±0.92
Nitrogen-free extract (NFE)	37.50±0.32	36.97±0.61	36.70±0.59	36.53±0.50

*Each 1 kg contains , Vitamin A, 200,00000 IU: Vitamin D3, 400,000 IU; Vitamin E, 5,00 mg; Vitamin C, 20,000 mg; Vitamin B1, 800 mg; Vitamin B2, 1,000 mg; Vitamin B6, 2,400 mg; Vitamin B 12, 40 mg; L-lysine, 3,000 mg; DL Methionine, 2,000 mg; Choline chloride, 5,000 mg; Niacinamide, 10,000 mg; Magnesium sulphate, 24,000 mg; Cobalt Sulphate, 80 mg; sodium selenite, 20 mg; potassium iodide, 240 mg; Calcium d pantothenate, 2,000 mg; Biotin, 150 mg, ferrous sulphate, 28,000 mg; Copper sulphate, 24,000 mg; Zinc sulphate, 24,000 mg; Manganese sulphate, 6,800 mg; Inositol, 5,000 mg.

Table 2: Chemical composition of ingredients waste meal used in experimental diets

Items	Treatments			
	FM	PBM	ABM	KWM
Dry matter	96.08± 0.15	95.68± 0.08	96.71± 0.85	96.11± 0.90
Crude protein	72.61± 2.35 ^{a}	58.31± 2.55 ^{b}	51.98± 3.05 ^{c}	52.98± 2.60 ^{c}
Crude Fat	9.61± 0.82^{c}	22.61± 1.05^{b}	25.06± 1.12 ^{a}	24.26± 0.90 ^{a}
Ash	12.09± 1.45 ^{c}	17.49± 2.15 ^{b}	19.28± 1.90^{ab}	21.63± 3.45^{a}

Means on the same row with different superscripts are significantly different (*P*<0.05).

Table 3: Essential and nonessential amino acids of FM, PBM, ABM and KWM used in experimental diets (%, wet/ wt.)

Items	Treatments			
	FM	PBM	ABM	KWM
Threonine	2.79±0.18^{b}	2.08±0.16 ^{c}	3.25± 0.21 ^{a}	2.00±0.21^{c}
Valine	3.72±0.21 ^{a}	2.80±0.21^{b}	3.06±0.30 ^{b}	2.56±0.19^{c}
Methionine	1.17±0.11 ^{a}	1.05± 0.01 ^{a}	0.52± 0.08 ^{b}	0.68±0.01 ^{b}
Isoleucine	2.71±0.23 ^{a}	1.97± 0.12^{b}	2.26±0.21 ^{b}	1.59±0.15 ^{c}
Leucine	5.53±0.31 ^{a}	3.74± 0.25^{c}	4.24±0.31^{b}	2.78±0.21 ^{d}
Phenylalanine	2.76±0.17 ^{a}	2.08± 0.14^{b}	2.62±0.15 ^{a}	1.67±0.14^{c}
Lysine	5.03±0.26 ^{a}	3.43± 0.22^{b}	3.87±0.23 ^{b}	3.67±0.31^{b}
Histidine	3.15± 0.20 ^{a}	2.92± 0.11 ^{a}	2.15±0.17 ^{c}	2.67±0.19 ^{b}
Arginine	3.56± 0.25^{b}	2.32±0.19 ^{c}	2.59±.020^{c}	4.08±0.31 ^{a}
Taurine	2.28± 0.14 ^{a}	1.95± 0.26^{b}	2.00± 0.16^{b}	1.61± 0.14 ^{c}
Aspartic	1.84± 0.21 ^{a}	1.96± 0.11 ^{a}	1.34± 0.08 ^{b}	1.05± 0.07 ^{b}
Serine	2.26± 0.14 ^{a}	1.82± 0.24^{b}	1.41± 0.10^{c}	1.68± 0.18 ^{b}
Glutamic	12.20± 0.41 ^{a}	8.98± 0.31 ^{b}	7.97± 0.31^{b}	11.07± 0.53 ^{a}
Glycine	2.37± 0.19^{b}	1.70± 0.17^{c}	3.90± 0.26 ^{a}	1.92± 0.24 ^{c}
Alanine	7.79± 0.31 ^{a}	5.29± 0.21^{c}	7.20± 0.34 ^{a}	6.31± 0.44^{b}
Cystine	0.12±0.01^{a}	0.07±0.002^{c}	0.09±0.001^{b}	0.07±0.001^{c}
Tyrosine	2.58± 0.24 ^{b}	2.93± 0.21 ^{a}	1.51± 0.22^{c}	2.12± 0.19^{b}
Proline	3.05± 0.31 ^{a}	2.65± 0.18^{b}	1.87± 0.13^{d}	2.24± 0.21^{c}

Means on the same row with different superscripts are significantly different (*P*<0.05).

Table 4: Apparent digestibility coefficients of the different diets

Items	Treatments			
	FM	PBM	ABM	KWM
Dry matter (%)	83.2±0.34^{a}	82.4±0.26^{a}	78.2±0.32^{c}	80.3±1.62^{b}
Organic matter (%)	85.3±0.14^{a}	85.2±0.35^{a}	80.9±1.27^{c}	83.1±1.24^{b}
Crude Protein (%)	89.8±0.24^{a}	88.9±0.52^{a}	81.2±0.65^{c}	84.3±1.52^{b}
Ether Extract (%)	96.6±0.45^{a}	96.5±0.23^{a}	87.2±0.65^{c}	93.4±0.65^{b}

Means on the same row with different superscripts are significantly different (*P*<0.05)

Table 5: Initial body weight, nutrient utilization and economical evaluation of Nile tilapia fed different diets (mean ± SD)

Items[1]	Treatments			
	FM	PBM	ABM	KWM
Initial body weight (g)	5.72±0.11[a]	5.92±0.22[a]	5.60±0.14[a]	5.92±0.22[a]
Final body weight (g)	99.25±0.07[a]	96.82±0.23 [a]	86.00±0.48[b]	83.32±0.06[c]
Body weight gain (g)	93.53±0.21[a]	90.90±0.32[b]	80.40±0.21[c]	77.40±0.10[d]
Gain in weight (g fish)	0.78±0.004[a]	0.76±0.010[a]	0.67±0.006[b]	0.65±0.004[b]
Specific growth rate	2.38±0.15[a]	2.33±0.05[a]	2.28±0.08[b]	2.20±0.05[b]
Total feed intake (g/fish)	180.9± 3.0 [a]	178.5±3.0 [b]	162.6 ±3.0[c]	157.2±3.0[d]
Av. Daily feed intake(g/fish)	1.51± 0.15 [a]	1.49± 0.15 [a]	1.36±0.15[b]	1.31± 0.15[c]
Feed conversion ratio	1.93 ±0.14[a]	1.96±0.07[a]	2.02±0.09[a]	2.03±0.31[a]
Crude protein (%)	32.23±0.15[a]	32.12±0.14 [a]	32.21±0.25 [a]	31.72±0.45 [a]
Protein intake (g/fish)	58.30±1.35 [a]	57.33±2.10 [a]	52.37±1.45[b]	49.86±2.15 [c]
Protein efficiency ratio	1.60±0.01[a]	1.59±0.05[a]	1.54±0.21[a]	1.55±0.07[a]
Survival ratio (SR %)	100 [a]	100 [a]	100 [a]	100 [a]
Economical evaluation[2]				
Costs (L.E)/ton	9000	7175	7275	7150
Relative to control (%)	100	79.72	80.83	79.44
Decrease in feed costs	00.00	20.28	19.17	20.56
Feed costs * (L.E)/kg WG	17.41	14.09	14.71	14.52
Relative to control (%)	100	80.93	84.25	83.16
Decrease in feed costs* (L.E)/kg WG	00.00	19.07	15.75	16.84
Kg Feed /kg weight	1.934	1.964	2.022	2.031
kg Weight / Kg feed	0.517	0.509	0.495	0.492

Values are the mean ± S.E. of triplicate groups of each treatment.
[1]Live body weight (LBW) in g of individual group of each experimental treatment was recorded every 2 weeks (14 days); Weight gain (WG) = final weight (g) – initial weight (g); Specific growth rate (SGR % /day) =100(lnW2−lnW1) / T; Average daily gain (ADG) = (W2 – W1) / t; Feed conversion ratio (FCR) = feed intake (g)/weight gain (g); Protein efficiency ratio (PER) = weight gain (g)/protein intake (g); Survival rate (SR %) = total number of fish at the end of the experiment × 100 / total number of fish at the start of the experiment. Where: W2 is the final weight, W1 initial weight and t is the time in days; ln = the natural log; T = period.
[2]Local price (L.E./Kg) for feed ingredients used FM (25); PBM (6); ABM (10) and KBW (5).

Apparent digestibility coefficients (ADC) of the different experimental tilapia fish diets are shown in (Table 4). The current data illustrated that the ADC of all studied parameters recorded the highest value in FM and PBM without any significant variation between them followed by ABM and KWM. These results are supported by (25), who demonstrated that, the ADC of commercial FM replacement diets with different level of PBM (0, 25, 50 and 100%) were organic matter 65.4, 67.5, 62.4 and 69.1 1; crude protein 97.2, 97.2, 96.7 and 97.2; lipid 85.3, 87.2, 87.0 and 89.9 %, respectively. In the same way, (26) reported that the ADC of DM, EE, CP and NFE were not altered by the inclusion of PBM.

The present results showed higher values than those reported by (27) where the ADC of diets containing mixture of ABM 25 (83.6% ± 1.15%), mixture of ABM 50 (79.21% ± 1.01%) and mixture of ABM 75 (78.7%± 1.57%) were not significantly different (*P*>0.05) compared with the control diet (83.4% ± 5.21%). The high ash content in PBM could reduce protein digestibility in fish diet (28, 29).

As shown in Table (5) the average initial weight of experimental Nile tilapia fish was ranged between 5.60 and 5.92 g/fish without any significant differences between the different treatments. The highest final weight was measured in FM (99.25±0.07 g) followed by PBM (96.82±0.23 g) and ABM (86.00±0.48 g), and KWM (83.32±0.06 g). This means that the final weight in FM increased about 2.45, 13.35 and 16.05 % when compared with PBM, APM and KWM, respectively. In the same trend, ADG was significantly different between different diets and take the same direction of previous results whereas the highest value of ADG noted in T1 followed by T2, T3 and T4, respectively. Similarly, (26) reported that the highest growth performance was recorded in tilapia fish fed PBM. (30) showed that the higher final growth weight and ADG of fish may be due to the type of diet and its composition, level of essential amino acids, the higher initial weight of the stocked fish or to higher rates of the supplemental food offered to the cultured fish. On the contrary, the present results of the ADG was lower than reported by (31) who found that the ADG values was between 1.6 and 3.04 g d-1 after feeding the cultured fish.

PBM is rich of protein (65%) with ridiculous the essential amino acids (EAA) profile which resulted in increased growth in fish (32,33. Improved growth rate and daily gain may be attributed to improve protein composition and essential nutrients in the test diets (34). Moreover, (16) reported that the growth performance increased in fish fed diets in which up to 50% of the FM was replaced by PBM, similar to results in *Tilapia zilli* (35).

As well as, average feed utilization in terms of total feed intake (TFI), daily feed intake (DFI), feed conversion ratio (FCR), total protein intake (TPI) and protein efficiency (PE) are presented in Table (5). The present results showed that DFI and TPI recorded the highest values in FM and PBM without significant differences between them and significantly higher when compared with APM and KWM. While, there are no significant difference in FCR and PE between treatments. Also, TFI recorded the highest quantity in FM followed by PBM, ABM and KWM. Similarly, (36) reported that the average daily intake of fish fed FM diet did not differ from those of fish fed diets FM with 20 and 40% PBM ($P > 0.05$) due to the protein source, fish species and size, experimental period and culture systems. The major problem in feeding PBM is limited content of essential amino acids especially methionine, phenylalanine, and lysine (37). (31) reported that, the FCR was ranged from 1.17 to 1.6 for sex-reversed tilapia fed on 30% crude protein. In contrary, high FCR value (5.56 -7.77) were obtained by (38) in Nile tilapia.

In the present study, the overall survival rate was 100% during the experimental period. These results harmonized with those reported by many researchers (25;39; and 22).

Moreover, the one ton feed cost in the present study was reduced in all replacing treatments of FM by 20.28; 19.71 and 20.56 % for PBM; ABM and KWM, respectively and decreased feed costs/kg weight gain by19.07; 15.75 and 16.84%, respectively . In this trend, (40) reported that, replacement of FM by a mixture of plant protein sources significantly reduced incidence costs, as well as being of immediate importance for feed production in Egypt.

Conclusion

From the present study, it could be concluded that using waste protein sources especially containing high PBM as a replacement of FM would be a helping tool to reduce feed cost per Kg fish production.

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