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An Evaluation of Fermented Silage Made from Fish By-Products as a Feed Ingredient for African Catfish (*Clarias gariepinus*)

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Abstract: An growth experiment was conducted to investigate the effect of partial or complete replacement of Fish Meal (FM) by dried Fermented Fish By-product Silage (FFS) in diets of African catfish(*Clarias gariepinus)*. Five dry pelleted diets containing 0, 25, 50, 75 and 100% FFS to replace the same percent of FM (on protein content basis) were prepared to be isonitrogenous (30% CP) and isocaloric (2700 kcal ME/kg diet) and tested in 3 months feeding experiment. Catfish (4.11-4.21 g) were fed the tested diets twice daily for six days a week in triplicate groups experiment. Results indicated that, dried FFS can successfully replace up to 50% of FM in catfish diets without any significant ($P<0.05$) loss in growth performance (body weight, body length, weight gain and specific growth rate) and feed utilization (feed conversion ratio and protein efficiency ratio). The higher levels of replacement of FM by FFS (75 or 100%) significantly ($P<0.05$) reduced growth performance, feed utilization parameters as well as significantly ($P<0.001$) effected fish body composition. Apparently, FFS can be used efficiently as a protein source in catfish diets to reduce feed costs. Moreover, from economic view, it was observed that replacing 50% of FM by FFS in diets did not significantly ($P<0.001$) adversed growth or feed utilization parameters of catfish and this replacement reduced feed costs/kg diet by 20.80 and feed costs/kg weight gain by 24.94%.

Key words: Fermented fish silage · fish meal replacement · growth · feed utilization · catfish

INTRODUCTION

Fish meal had an adequate concentration of these amino acids which are essential for normal growth but it is quit expensive and is in short supply. Thus, studies on the use of other efficient and cheaper sources of protein as substitutes for FM are necessary for aquaculture development.

Alternative resources such as meat and bone meal, hydrolyzed feather meal, fleshings-meal and blood meal [1, 2] dried fish and chicken viscera [3], poultry silage [4], cryfish meal [5] and shrimp meal [6] have been tried to replace fish meal either partially or fully, but even these pooled meals of various animal sources are not sufficient to meet the growing demands of fish raising industry.

Fish wastes can be advantageously upgraded into fish feed by fermentation with lactic acid bacteria. This procedure is safe, economically reasonable and environment echo-friendly [7]. The pH value of the fish pastes decreases below 4.5 during ensilage and this pH decrease is partially responsible for preservation [8].

In developing countries such as Egypt, fish silage is cheaper to produce, involves simple artisanal technology and possess good storage properties. It represents an alternative to FM in utilizing waste/trash fish (accounted for about 5% of annual farm production) as protein feedstuff for tilapia [9]. In Egypt, [10, 11] indicated the successful of partially replacement of FM by AFS in the diets of gilthead bream (*Sparus aurata*) and hybrid tilapia (*O. niloticus*×*O. aureus*), respectively. In another study, [9] found that dried FFS when used singly or mixed with soybean meal can replace half or three quarters of FM in 28% CP Nile tilapia diets without significant affect on growth performance, feed conversion, protein utilization or fish composition. [12] incorporated FFS in the diets of Nile tilapia, *Oreochromis niloticus* and African catfish, *Clarias garipinus* to replace 25, 50, 75 or 100% of FM and they found that, dried FFS can successfully replace

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up to 25 and 50% of FM in tilapia and catfish diets, respectively without any significant reduction in growth while the highest replacing levels (50, 75 or 100% for tilapia and 75 or 100% for catfish) significantly reduced growth performance for the two fish species and the same trend was obtained by [13] for Nile tilapia.

The present study aimed to evaluate the effect of replacing FM by FFS as protein source in the diets of African catfish.

MATERIALS AND METHODS

The practical work of the present study was carried out at Fish Nutrition Laboratory, Faculty of Agriculture, Benha University, Egypt, during the period from July to October, 2007.

Preparation of Fermented Fish Silage (FFS) and experimental diets: Fish by-products (non edible parts such as head, skin, fins and viscera) were obtained from El-Obour market. FFS was prepared by mixing the minced fish by-products (60%), orange peel (30%) as filler, molasses (5%) as a source of carbohydrate (energy) and 5% yogurt (as a source of *Lactobacillus spp* for lactic acid anaerobic fermentation process). Potassium sorbate solution (1%) as antimicrobial agent was sprayed and the mixture was packed in black polyethylene bags. All bags were incubated in tightly hard plastic container and stored at ambient temperature that ranged from 30 to 38°C. The ensilage process completed after 30 days and at the end, a liquid FFS of pH 4.5 was obtained and sun-dried for 3 days. The resultant dried FFS had brownish color and strong fish odor and contained 38.12% crude protein (CP) [12].

Five experimental diets were formulated (Table 1) to replace 0, 25, 50, 75 or 100% of FM by FFS (based on protein content). All diets were formulated to be isonitrogenous (30% protein) and isocaloric [2700 kcal ME/kg diet].

Fish and experimental system: Fingerlings of African catfish were obtained from World Fish Center located at Abbassa, Sharkia Governorate, Egypt. Fish were adapted to laboratory conditions and distributed randomly into 6 fiberglass tanks.

Fifteen circular fiberglass tanks (350 liter for each) were used (3 replicates for each diet) and each tank was stocked with 50 catfish fingerlings averaged 4.11 and 4.21 g in weight. All experimental tanks were aerated and fish in the experiment were fed on the pelleted diets (2 mm in diameter) at a daily rate of 10% (during the 1st month), then gradually reduced to 7% (2nd month) and 4% (3rd month) of total biomass. Fish were fed 6 day/week (twice daily at 9.00 am and 3.00 pm) and the amount of feed was bi-weekly adjusted according to the changes in body weight throughout the experimental period (90 days). About 25% of water volume in each fiberglass tank was daily replaced by aerated fresh water after cleaning and removing the accumulated excreta. Water temperature, pH and dissolved oxygen were measured daily at 2.00 pm while total ammonia was weekly measured. Water quality parameters measured were found to be within acceptable limits for fish growth and health [15]. Growth performance and feed utilization parameters were measured by using the following equations:

Table 1: Composition and proximate analysis of the experimental diets

	Diets				
Ingredients	D1	D2	D3	D4	D5
Fish meal (72%)	18.0	13.50	9.0	4.5	0.0
Fish silage (38% CP)	0.0	8.5	17.0	25.5	34.0
Soybean meal (48%)	28.0	28.0	28.0	28.0	28.0
Yellow corn	32.0	32.0	32.0	32.0	30.0
Wheat bran	14.5	10.5	6.5	2.5	0.0
Vegetable oil	4.0	4.0	4.0	4.0	4.5
Vit. & Min. Mixture[1]	2.7	2.7	2.7	2.7	2.7
Ascorbic acid	0.3	0.3	0.3	0.3	0.3
Cr_2O_3	0.5	0.5	0.5	0.5	0.5
Sum	100.0	100.0	100.0	100.0	100.0
Proximate analysis (determined on dry matter basis)					
Dry Matter (DM)	95.11	95.45	95.34	94.56	95.92
Crude Protein (CP)	30.45	30.00	29.85	29.51	29.18
Ether Extract (EE)	6.23	6.67	5.99	6.87	6.13
Crude Fiber (CF)	8.32	8.56	9.65	9.35	8.44
Ash	9.39	9.76	8.89	9.24	9.99
NFE[2]	45.61	45.01	45.62	45.03	46.26
ME[3] (Kcal/kg diet)	2754.00	2744.00	2734.00	2720.00	2704.00
P/E ratio[4]	110.6	109.3	109.2	108.5	107.91

[1]Vit & min mixture/kg premix: Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg

[2]Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)

[3]Metabolizable energy was calculated from ingredients based on [14] values for tilapia

[4]Protein to energy ratio in mg protein/kcal ME

Specific growth rate (SGR) = [LnW2 – LnW1/t] × 100

Where: Ln = the natural log, W1 = initial fish weight; W2 = the final fish weight in "grams" and t = period in days.

Weight gain (WG) = final weight (g) – initial weight (g).

Feed conversion ratio (FCR) = feed ingested (g)/weight gain (g).

Protein efficiency ratio (PER) = weight gain (g)/protein ingested (g).

Chemical analysis: At the end of the experiment, five catfish were chosen at random from each tank and subjected to the chemical analysis of whole fish body [16].

Statistical analysis: The statistical analysis of data was carried out by applying the computer program [17] by adopting the following model:-$Y_{ij} = \mu + \alpha_i + e_{ij}$ Where, Y_{ij} = the observation on the ijth fish eaten the ith diet; μ = overall mean, α_i = the effect of ith diet and e_{ij} = random error.

RESULTS

Proximate analysis of FFS: Proximate analysis of dry FFS used in the present study is shown in Table 2. As shown in this table, FFS contained 95.68, 38.12, 7.54 and 7.23% DM, CP, EE and ash, respectively. The high protein content of FFS indicated that FFS considered to be an excellent protein source in catfish diets.

Growth performance and feed utilization: Increasing replacing levels of FM by FFS up to 50% in catfish diets did not significantly affected the final Body Weight (BW) of catfish while the highest replacing levels (75 or 100%) significantly (P<0.001) reduced the final BW of catfish and the same trend was also observed for Weight Gain (WG) and Specific Growth Rate (SGR). Feed Intake (FI) was significantly (P<0.001) increased (Table 3) when 25% of FM was replaced by FFS while the higher replacing levels (50, 75 or 100%) significantly decreased with each increase in FFS content of catfish diets as a replacement of FM.

Table 2: Proximate analysis of FFS compared to FM and the indispensable amino acid requirements of Nile tilapia and catfish

	Proximate analysis (%), on dry matter basis			
FFS	DM	CP	EE	Ash
	95.68	38.12	7.54	7.23

	Amino acids profile (g/100 g protein)		
Amino acid	Requirements of catfish*	Fish Male (FM)	Fermented Fish Silage (FFS)
Lysine	5.10	5.80	4.11
Leucine	3.50	7.30	3.60
Isoleucine	2.60	4.30	2.07
Cystine	2.30	3.30	2.42
Methionine			
Phenylalanine	5.00	7.20	5.17
Tyrosine			
Threonine	2.00	3.60	2.28
Valine	3.00	5.40	3.27
Tryptophane	0.50	1.10	0.62
Histidine	1.50	2.50	4.81
Arginine	4.30	5.00	2.98
Total (%)		45.50	31.23

** [18]

Table 3: Growth performance, feed utilization and proximate analysis of catfish as affected by replacing FFS by FM in catfish diets

Item	Experimental diets T1	T2	T3	T4	T5	±SE	Prob.
Growth performance							
Body weight (g)							
Initial	4.21	4.11	4.17	4.15	4. 18	0.13	0.8761
Final	47.35^b	52.10^a	47.62^b	40.58^c	35.42^d	0.91	0.0001
WG (g/fish)	43.14^b	47.99^a	43.45^b	36.43^c	31.24^d	0.45	0.0001
SGR	2.69^b	2.82^a	2.71^b	2.53^c	2.37^d	0.03	0.0001
Feed utilization							
FI (g/fish)	59.67^b	63.67^a	57.00^c	54.00^d	47.00^e	0.55	0.0007
FCR	1.38^b	1.33^b	1.32^b	1.48^a	1.50^a	0.02	0.0003
PER	2.39^{ab}	2.51^a	2.54^a	2.23^b	2.21^b	0.04	0.0036

Means followed by the different letters in each row for each trait are significantly different (P<0.05)

Table 4: Proximate analysis of whole fish body as affected by incorporation of fermented fish silage in catfish diets

	Experimental diets						
Item	T1	T2	T3	T4	T5	±SE	Prob.
Dry mater	30.12	29.23	29.24	29.62	30.27	1.45	0.5624
Protein	66.12 [a]	64.66[b]	64.54[b]	64.43[b]	64.38[b]	1.56	0.0077
Ether extract	20.86 [a]	21.18[a]	20.03 [a]	18.41[b]	18.56[b]	0.14	0.0062
Ash	12.88	13.53	13.68	14.41	14.37	0.86	0.0637

Means followed by the different letters in each row for each trait are significantly different (P<0.05)

Table 5: Feed costs (L.E) for producing one kg weight gain by fish fed the experimental diets

Diets	Costs (L.E)/ ton	Relative to control (%)	Decrease in feed costs (%)	FCR	Feed costs* (L.E.)/kg weight gain	Relative to control (%)	Decrease in Feed costs * (L.E)/kg Weight gain
FFS0	2990.5	100	0	1.38	4.13	100	0
FFS25	2679.5	89.60	10.40	1.33	3.56	86.20	13.80
FFS50	2368.5	79.20	20.80	1.31	3.10	75.06	24.94
FFS75	2057.5	68.80	31.20	1.48	3.05	73.85	26.15
FFS100	1755.0	58.69	41.31	1.50	2.63	63.68	36.32

* Feed costs/kg weight gain = FCR × costs of kg feed, Local market price (L.E./ton) for feed ingredients used for formulating the experimental diets when the experiment was started; fish meal 8000 LE/ton, yellow corn 1250, soybean meal 2000, FFS 1000, wheat bran 900, corn oil 4000 and vit.& Min. Mixture 10000 LE/ton

Table 3 show also that, increasing the replacing levels of FM by FFS up to 50% did not significantly affected FCR and PER while the highest replacing levels (75 or 100%) significantly adversed FCR and PER of catfish. The best PER were recorded for fish fed the diet FFS50 where 50% of FM protein was replaced by FFS protein and the worst ones were recorded for fish groups fed the diets FFS75 and FFS100.

Proximate analysis of fish whole-body: Proximate analysis of catfish (Table 4) indicated that, Dry matter (DM) of fish bodies did not significantly affected by all replacing levels of FM by FFS and the same trend was also observed for ash content of fish whole-body. Compared to control fish group, all replacing levels of FM by FFS significantly (P<0.001) decreased protein and ether extract (EE) content whereas fish group fed the control diet (FFS0) gained the highest protein and EE contents.

Economical evaluation: Results of economic evaluation including feed costs, costs of one kg gain in weight and its ratio to that of control group fed the diet FFS0 are presented in Table 5. These results indicated that incorporation of FFS in catfish diets as a substitute of FM decreased feed costs by 10.40, 20.80, 31.20 and 41.31% for the diets FFS25, FFS50, FFS75 and FFS100 compared to the control diet (FFS0). Costs of one kg gain in weight were 4.13, 3.56, 3.10, 3.05 and 2.63 L.E for fish fed the diets FFS0, FFS25, FFS50, FFS75 and FFS100, respectively.

DISCUSSION

The high protein content of FFS make it a good source of animal protein in fish diets. [19] found that, dry FFS contained 50.07, 15.70 and 19.76% CP, EE and ash, respectively. [20] prepared FFS from three raw materials, commercial marine fish waste, commercial freshwater fish waste and tilapia filleting residue and he found that, protein contents of FFS were 77.67, 49.62 and 42.99 in FFS prepared from these wastes, respectively.

Results presented in Table 2 show that FM displayed better amino acid profile as it had higher levels of all indispensable amino acids except for histidine compared to those of FFS. Similar results were obtained by [9]. With regard to amino acid requirements, data of Table 2 indicated that, amino acids content of FFS covered all indispensable amino acid requirements for catfish except for lysine, isoleucine and arginine. However, [9] showed that, amino acids content of FFS did not covered all indispensable amino acid requirements for *O. niloticus* except for leucine.

Although it was not determined in the present study, a good apparent digestibilities for DM, CP and EE were reported during previous FFS feeding trials with Nile tilapia [21, 22]. They indicated that apparent protein digestibility decreased with the increase of FFS (from 85.5 to 80.6% for the 25 and 100% FM replacement levels, respectively). In another study, [23] showed that, apparent digestibility coefficient of DM, CP and gross energy for fermented shrimp head silage was high (>70%) for catfish fingerlings. In another study, [24] mixed FFS (2:1, w/w) with each of poultry by-products meal, soybean-hydrolyzed feather meal or menhaden fish meal and each mixture pelleted by cold extrusion method. They found that, Apparent Digestibility Coefficient (ADC) for dry matter, crude protein and gross energy of the pellets were high (>80%) and similar (P>0.05) among diets for O. *niloticus*.

In this study, results of growth performance revealed the possibility of replacing 50% of FM by FFS in catfish diets without negative effects on BW, WG and SGR and these results may be attributed to the Indispensable Amino Acid (IAA) content of FFS which covered the requirements of IAA for catfish except for lysine, isoleucine and arginine. Another reason for the good response of fish fed the FFS diets might be the presence of pre-hydrolyzed proteins, which facilitate digestion and therefore assimilation. In this hence, [25] reported similar behavior for fish fed dried fermented fish silage. It is known that fish can assimilate protein as amino acids and short peptides, so the protein breakdown during the treatment could have a positive effect on the digestibility and assimilation of this nutrient.

The superior performance of control fish group fed the diet FFS0 was referred to the fact that the nutritional value of FM-protein approximating almost exactly to the nutritional requirements of cultured finfish species [26]. When 25 or 50% of FM protein was replaced by FFS protein it did not followed by significant effect on all growth parameters (BW, WG and SGR) while the highest replacing levels (75 or 100%) significantly adversed these parameters. FM contained comparatively higher total indispensable amino acid (IAA) content (45.50%) than FFS (31.23%) and the IAA of FFS did not cover the requirements of catfish from these amino acids. Therefore, the highest replacing levels of FM by FFS (75 or 100%) significantly reduced all growth performance parameters of catfish.

It is important to remark that the lower substitution level of FM by FFS (25%) supported better growth rates in catfish in comparison with the highest levels (50, 75 and 100%). [27] reported a similar effect for low inclusion levels (15%) using fish silage in Atlantic salmon diets. Also[28] found that, replacing FM by shrimp head silage in Nile tilapia diets up to 15% showed the best response in growth performance while the highest replacing levels (20, 25 or 30%) resulted in the worst growth response. [29] reported that small amounts of silage improved the growth efficiency of Atlantic salmon fry. [23] concluded that dried FFS is suitable and has a potential as a protein feedstuff in catfish, *Clarias gariepinus* diets. [30] demonstrated that, ablone viscera silage can be used to replace FM in diets for ablone without significant differences in growth.

It was found that, replacing FM by shrimp head waste meal at 0, 15, 30, 45 and 60% in 30% protein diets reduced final BW, WG and SGR and the reduction was more pronounced at the highest replacement levels [31]. In another study, [9] found that replacement of 25, 50, 75 or 100% of FF by FFS alone or mixed with soybean meal (1:1) significantly (P<0.05) decreased the final BW of Nile tilapia fed 28% CP experimental diets while WG and SGR did not significantly affected by the partial or the complete replacement of FFS alone or when mixed with soybean meal.

The highest levels (50% FM replacement by FFS) were reported in earlier reports of [14] who found no differences in growth performance of Nile tilapia fed a formic acid preserved fish silage blended with FM (1:1) and growth performance was significantly reduced when the replacing levels increased up to 75%. Also, [25, 32] stated that, up to 75% of FM protein could be successfully replaced with tilapia silage and soybean meal (1:1) in 30% CP diets for all male *O. niloticus.*

Chemical analysis (DM, protein, EE and ash content) at the end of a feeding trial is frequently used to determine the influence of feed on fish composition. According to [18], endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish. It should be noted that within endogenous factors, the composition of the feed is only factor, which could have influenced the chemical composition of fish, as other endogenous factors were maintained uniform during the study. The present results indicated that, compared to control fish group, all replacing levels of FM by FFS significantly (P<0.001) decreased protein and EE content whereas fish group fed the control diet (FFS0) gained the highest protein and EE contents. On contrast with our results, [9] found that, replacing of FM by FFS up to 75% did not significantly affected protein content of tilapia bodies.

Results of the present study indicated that, replacement of 50% of FM by FFS in catfish diets did not significantly affected all growth and feed utilization parameters and reduced feed costs/kg diet and feed costs/kg weight gain by 20.80 and 24.94%, respectively. The higher replacing levels of FM by FFS (75 or 100%) in catfish diets significantly reduced all growth and feed utilization parameters and also reduced feed costs/kg diet and feed costs/kg weight gain.

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