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Research Article
Use of Locust Meal as Alternative Protein Source to Fish Meal in Practical Diets for Fingerling *Oreochromis niloticus*

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Abstract

Background and Objective: Reducing fishmeal levels is key to reducing feed costs for commercial fish farming and ensuring the sustainability of this enterprise. This study was aimed to evaluate the effects of replacing fish meal protein with locust meal protein in the feeds for fingerling *Oreochromis niloticus* (3.5±0.94 g). **Materials and Methods:** Seven practical diets (350 g kg^{-1} crude protein, 14.90 kJ g^{-1} gross energy) replacing 0, 10, 20, 30,40, 50 and 60% fish meal protein by locust meal protein were prepared. The diets were fed to triplicate groups of fish near to satiation for 8 weeks. **Results:** The live weight gain (LWG, 262.6-719.7%), feed conversion ratio (1.58-3.18), protein efficiency ratio (0.70-1.41), specific growth rate (2.30-3.76% day^{-1}), protein retention efficiency (3.62-9.42), hemoglobin (61.2-98.1 g dL^{-1}), hematocrit (16.37-31.35%) and RBCs counts (13.5-23.2×10^6 mm^{-3}) in fish fed diets with 0, 10,20 and 30% replacement of fish meal with locust meal did not show any significant differences. **Conclusion:** However, further replacement of fish meal by locust meal beyond 30% resulted in a significant fall ($p<0.05$) in the above parameters indicating that fish meal could be replaced by locust meal up to 30%. Based on the broken-line analysis of LWG, the optimum replacement level was found to be 29.8% which would be useful in formulating cost-effective commercial feeds for the intensive culture of this fish.

Key words: Replacement, fish meal, locust meal, fingerling *Oreochromis niloticus*

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

INTRODUCTION

Due to the ever-increasing demand for high-quality protein food, a need has been felt to meet this increasing demand through aquaculture as the wild stock has been exhausted due to overexploitation of the natural resources. Aquaculture is considered to be the answer to the current shortage of commercial fishery production and its intensification has led to the reliability of artificial feeding[1-3]. Protein is the most expensive item of feeds and is perhaps the crucial factor for the growth of cultured species[4-7]. Fish meal is the most alluring protein source for aquaculture diets as it is having a higher amount of protein, balanced amino acid and fatty acid profiles, more digestibility and is more palatable. Despite this, the more price of fish meals and low accessibility make it difficult to be used in most of the aquafeeds[8]. Therefore, the fish diet needs to be replaced with other conservation measures to promote sustainable aquaculture. Edible insects are important traditional food items in various parts of the world[9-12]. Orthoptera, especially locusts are often bred to feed on pets and zoo animals and have been inspected for animal feed[13]. The presence of large numbers of locusts that have died as a result of locust infestations makes them the ideal food for livestock, especially chickens. The expansion of aquaculture in Africa and Asia as well as the exploration for other protein sources, has resulted in the feeding of locusts and grasshopper in different trials and catfish and tilapia[14]. Locusts, an excellent source of protein, some species differ in their protein content i.e., from 50-60% of dry weight. It also contains sufficient amounts of iodine, phosphorus, iron, thiamine, riboflavin, niacin and carbohydrate levels are very low in locusts[15-17]. Several investigators are paying attention to replacing fish meals with locust meal[18-22].

Tilapia has become an eminent fish species in the world and plays a growing role in the global aquaculture trade. Therefore, the development of less expensive feeds using less expensive plant and animal protein sources will contribute to future sustainable aquaculture development[23-29]. Tilapia is a freshwater fish belonging to the Cichlidae family. It is currently recognized as the aquatic chicken owing to its rapid growth, adaptability to disease, high meat quality, capability to grow and reproduce in captivity and feed at low trophic levels[24,30,31]. Tilapia is currently cultivated in more than 100 countries worldwide[30,32-34]. Tilapia is farmed much faster and on a larger scale than any other group of fish. Rapid advances in livestock and a wide range of uses could mean that tilapia may eventually overtake carps to become the most important domestic fish. Tilapia has already become one of the most important farmed fish and is increasingly involved in international maritime trade[35,36]. There are about ten species of tilapia used to make aquaculture. Nile tilapia is the most important species of tilapia in the world. As the feed is the most expensive, it usually ranges from 30-60% of the total alterable price, affected by the strength of the cultural process[37-39]. The development of affordable feeds using cheap and locally available plant protein sources plays a major role in its future sustainable marine development. To this end, research was accomplished to replace the fishmeal protein with locust meal protein for Nile tilapia so that cost-effective nutritionally-balanced feeds could be made for its intensive culture.

MATERIALS AND METHODS

Study area: This study was conducted during the period of (12th January to 9th March, 2021), at the hatchery of the Department of Fisheries and Wildlife Science, Sudan University of Science and Technology, Khartoum, Sudan.

Preparation of experimental diets: Feasibility of replacing fishmeal with locust meal for Nile tilapia *O. niloticus* fingerling by were found out by preparing seven diets replacing 0% (D1), 10% (D2), 20% (D3), 30% (D4), 40% (D5), 50% (D6) and 60% (D7) fish meal protein by locust meal protein procured from (6 Market), Haj Yousif, Khartoum, Sudan in Table 1. All diets contained 350 g kg^{-1} crude protein[40] and 14.90 kJ g^{-1}gross energy. All the ingredients were weighed and blended thoroughly. These were then steam cooked at 80°C in hot water and oil, mineral and vitamin premixes were included in the bowl in isolation with continuous blending at 60°C. The final diet was cut as the small cubes and stored at -20°C for further utilization. The proximate compositions of the experimental diets used in this experiment were analyzed and are mentioned in Table 1. The amino acid content of experimental diets was analysed using automatic amino acid analyzer biochrom (UK). Recovery hydrolysis for analysis of tryptophan was performed in 4 N methanesulfonic acid and for sulphur amino acids in performic acid in Table 2. The fatty acid profiles of the experimental diets were analyzed gas-liquid chromatography (Biobase Bk-Gc112A High-Performance Gas Chromatograph, Biobase Bioindustry (Shandong) Co., Ltd., Shandong, China).

Experimental system and animals: Fingerling *O. niloticus* were got from Hussien Fadoul Fish Farm, Soba-Khartoum, Sudan. These were transported to the hatchery of the

Table 1: Ingredients composition of experimental diets

	Experimental diets						
Ingredients (g kg^{-1} dry diet)	D1	D2	D3	D4	D5	D6	D7
Fish meal†	147.1	132.4	117.6	102.9	88.2	73.5	58.8
Locust meal‡	00.0	19.1	38.2	57.3	76.4	95.4	114.5
Groundnut cake§	57.7	57.7	57.7	57.7	57.7	57.7	57.7
Mustard oil cake¶	162.2	162.2	162.2	162.2	162.2	162.2	162.2
Soybean meal††	311.1	311.1	311.1	311.1	311.1	311.1	311.1
Wheat middling‡§	142.9	142.9	142.9	142.9	142.9	142.9	142.9
Cod liver oil	50	46	4238	34	30.1	26.1	
Mineral premix§§	20	20	2020	20	20	20	
Vitamin premix§¶	10	10	1010	10	10	10	
Alpha cellulose	99.1	98.7	98.4	97.9	97.5	97.1	96.7
Total	1000	1000	1000	1000	1000	1000	1000
Proximate analysis (g kg^{-1} dry diet)							
Protein CP	350±1.3	350±2.0	350±1.7	350±6.0	350±5.1	350±2.7	350±4.5
Ether extract	90.1±4.0	90.2±6.0	90.4±8.0	90.5±2.0	90.6±4.0	90.8±2.0	90.9±1.0
Fiber content	58.9±3.0	60.5±1.0	62.1±2.0	63.7±5.0	65.4±3.0	67.0±3.0	68.6±2.0
Ash	55.9±1.0	55.7±1.0	55.5±3.0	55.2±6.0	55.0±4.0	54.7±2.0	54.5±4.0
Calculated GE (kJ g^{-1}, dry diet)	14.9±0.3	14.9±0.2	14.9±2.0	14.9±0.1	14.9±0.4	14.9±0.3	14.9±0.2

†Fishmeal 680 g kg^{-1} CP, ‡Locust meal 523.9 g kg^{-1} CP, §Groundnut cake 520 g kg^{-1} CP, ¶Mustard oil cake 370 g kg^{-1} CP, ††Soybean meal 450 g kg^{-1} CP, ‡§Wheat Middling 140 g kg^{-1} CP, §§Mineral mixture (g kg^{-1} dry diet) calcium biphosphate 135.7, calcium lactate 326.9, ferric citrate 29.7, magnesium sulphate 132.0, potassium phosphate (dibasic) 239.8, sodium biphosphate 87.2, sodium chloride 43.5, aluminium chloride.6H_2O 0.154, potassium iodide 0.15, cuprous chloride 0.10, manganous sulphate H_2O 0.80, cobalt chloride 6H_2O 1.0, zinc sulphate 7H_2O 4.0 (Halver, 2002)[96], §¶Vitamin mixture (g kg^{-1}dry diet) choline chloride 5.0, inositol 2.0, ascorbic acid 1.0, niacin 0.75, calcium pantothenate 0.5, riboflavin 0.2, menadione 0.04, pyridoxine hydrochloride 0.05, thiamine hydrochloride 0.05, folic acid 0.015, biotin 0.005, alpha-tocopherol 0.4, vitamin B_{12} 0.0001, Amipharma Laboratories Ltd, Khartoum North, Sudan (Halver, 2002)[96]

Table 2: Amino acid composition (g kg^{-1} dry matter) of the experimental diets

	Experimental diets						
	D1	D2	D3	D4	D5	D6	D7
Arginine	27.1±0.60	27.2±0.1	27.3±0.0	27.3±0.03	27.4±0.01	27.4±0.05	27.5±0.02
Histidine	9.9±0.1	9.9±0.4	9.9±0.1	9.9±0.2	9.9±0.3	9.8±0.3	9.9±0.1
Isoleucine	14.1±0.2	14.2±0.5	14.3±0.2	14.5±0.4	14.6±0.2	14.8±0.1	14.9±0.01
Leucine	24.6±0.5	24.6±0.3	24.6±0.1	24.6±0.0	24.6±0.2	24.6±0.1	24.7±0.01
Lysine	22.3±0.1	21.3±0.1	21.5±0.4	21.0±0.2	20.6±0.2	20.1±0.1	19.7±0.05
Methionine	6.9±0.2	6.9±0.3	6.9±0.5	6.7±0.1	6.9±0.5	6.9±0.2	6.8±0.03
Cystine	2.7±0.1	2.8±0.1	2.9±0.1	3.0±0.1	3.1±0.1	3.2±0.3	3.3±0.05
Phenylalanine	14.8±0.1	14.7±0.1	14.5±0.3	14.3±0.3	14.1±0.5	13.9±0.1	13.7±0.03
Tyrosine	14.0±0.2	14.0±0.4	14.1±0.1	14.1±0.1	14.2±0.2	14.2±0.3	14.3±0.03
Threonine	13.3±0.1	13.3±0.5	13.2±0.3	13.1±0.2	13.1±0.2	13.0±0.1	12.9±0.04
Tryptophan	2.4±0.1	2.5±0.3	2.7±0.1	2.8±0.1	3.0±0.2	3.2±0.1	3.3±0.03
Valine	17.5±0.5	17.5±0.2	17.5±0.2	17.5±0.1	17.4±0.4	17.4±0.3	17.4±0.03

[1]Mean values of 3 replicate ±SEM and [2]Not statistically significant ($p>0.05$)

Department of Fisheries and Wildlife Science, Sudan University of Science and Technology, Khartoum, Sudan and given a prophylactic dip in $KMnO_4$ solution (1:3000) and stocked and kept in oxygenated plastic containers (aquarium) in fisheries laboratory. At this time, the fish were fed to apparent satiation by feeding a diet consisting of mustard oil cake, soybean meal and wheat middling in the form of soft cake. For conducting the experiments, *Oreochromis niloticus* fingerlings (3.5±0.94 g) were separated from the above-acclimated lot and kept in triplicate groups in 80 L circular polyvinyl tanks (water volume 65 L) fitted with a continuous water flow-through (1-1.5 L min^{-1}) system at the rate of 20 fish per tank for each dietary treatment. Fish were supplied with experimental diets as soft cake to apparent satiation twice daily at 0900 and 1600 hrs. No feed was offered to the fish on the day they were weighed. Initial and weekly weights were recorded on a top-loading balance. The feeding trial lasted for 8 weeks. Unconsumed feed was strained on a screen, dried and weighed to assess the amount of feed eaten.

Water quality parameters: Water temperature, dissolved oxygen, free carbon dioxide, pH and total alkalinity during the feeding trial were recorded following Yousif *et al.*[41]. The average water temperature, dissolved oxygen, free carbon

dioxide, pH and total alkalinity over the 4 weeks feeding trial, based on daily measurements, were 25.0-28.5°C, 6.5-7.4 mg L^{-1}, 7-8.4 mg L^{-1}, 7.2-7.7 and 75-83.5 mg L^{-1}, respectively.

Proximate composition analyses: After starvation of 24 hrs, a randomized sample of 25 fish were killed by dipping in MS-222 solution (200 mg L^{-1}), minced and six subsamples of initial body composition analysed. At the end of the trial, nine fish from each replicate of dietary treatments were sampled, killed, pooled separately and frozen at -20°C for analyzing the biochemical composition. Six subsamples of the pooled samples of each replicate (n = 3×6) were subjected to proximate composition analysis. Proximate composition of the diets, initial and final carcass was worked out following Yousif *et al.*[42] as described in the previous investigation[43]. Biobase high automatic oxygen bomb calorimeter (Biobase Meihua Trading Co., Ltd. China) was used for estimating the gross energy content of experimental diets.

Blood collection and analysis: Blood from the caudal vein of 9 fish per replicate was randomly collected, pooled for analyzing haematological parameters. The procured blood was transferred to heparinized Eppendorf tubes. The three subsamples (n = 3×3) of pooled samples from the heparinized tube were utilized for the assessment of the RBCs and Hb following the methods of Vani *et al.*[44]. The Hct level was examined by centrifuging fresh blood at 3600 g for 6 min in a micro Hematocrit Centrifuge (SH-120 High-Speed Micro Hematocrit, China).

Estimation of liver superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities: Liver was taken off from five starved fish of each replicate tank, pooled and five subsamples (n = 3×5) were utilized for assessing the SOD, CAT and GPx activities following the procedures of Zehra and Khan[45], Khan and Khan[46] and Abdel-Hameid *et al.*[47], respectively. The procedure used by the researcher[48] was utilized to evaluate the tissue protein content for the calculation of enzyme-specific activities.

Diet performance evaluation: The effects of replacement of fish meal by locust meal in diets for fingerling *O. niloticus* during the present experiment was assessed by considering the growth and conversion efficiency indices[49] as follows:

$$\text{Live weight gain } (\%) = \frac{\text{Final individual bodyweight} - \text{Initial individual bodyweight}}{\text{Initial individual bodyweight}} \times 100$$

$$\text{Specific growth rate } (\%) = \frac{\text{In final bodyweight} - \text{In initial bodyweight}}{\text{Number of experimental days}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed fed}}{\text{Weight gain}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain}}{\text{Protein fed}}$$

$$\text{Protein deposition of whole fish } (\%) = \frac{\text{Final bodyweight (g)} \times \text{final body protein (\%)} - \text{Initial bodyweight (g)} \times \text{initial body protein (\%)}}{\text{Protein intake (g)}} \times 100$$

$$\text{Survival Rate } (\%) = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Statistical analysis: The growth data were subjected to one-way analysis of variance[50] before analysis, data were tested for normality using Shapiro-ilk test and the homogeneity of variance using Levene's test for equality of variances. When a significant treatment effect was observed, Tukey's honestly significant difference test was used for multiple mean comparisons at a $p<0.05$ level of significance. Quadratic regression analyses of live weight gain were done to evaluate the optimum replacement level of fish meal by locust meal[51]. Statistical analyses were done using origin (version 6.1, Origin Software, San Clemente, CA).

RESULTS

The amino acid composition of experimental diets was also affected by the substitution of fish meal by locust meal in Table 2. Arginine, histidine, isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine, tryptophan and valine contents of the experimental diets were not significantly affected among the varying replacement groups and found to be in the range of 27.1-27.5, 9.8-9.9, 14.1-14.9, 24.6-24.7, 19.7-22.3, 6.7-6.9, 2.7-3.3, 13.7-14.8, 14-14.3, 12.9-13.3, 2.4-3.3 and 17.4-17.5 g kg^{-1} dry matter. The fatty acid composition of the test diets was also not affected significantly except eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) which were found to decrease in D5, D6 and D7 significantly ($p>0.05$) were in 40, 50 and 60% fish meal protein in Table 3. The EPA values in fish fed D5, D6 and D7 diets were recorded to be 12.4, 11.3 and 10.1 g kg^{-1}, respectively. The fish fed diets D5, D6 and D7 exhibited the DPA content 3.9, 3.5

Table 3: Fatty acids profile of the experimental diets (g kg^{-1})

		Experimental diets						
Fatty acid		D1	D2	D3	D4	D5	D6	D7
Sat								
Myristic	14:0	9.3±0.2	9.0±0.1	8.6±0.2	8.2±0.1	7.8±0.1	7.5±0.1	7.1±0.1
Palmitic acid	16:0	65.7±1.0	69.3±5.0	72.8±7.0	76.4±4.0	80.0±2.0	83.5±5.0	87.1±7.0
Stearic acid	18:0	24.0±1.0	21.2±5.0	22.0±3.0	22.9±3.0	23.7±1.0	24.6±5.0	25.4±4.0
Mon								
Palmitoleic acid	16:1 n-7	16.2±2.0	15.4±2.0	14.6±1.0	13.8±3.0	13.0±4.0	12.2±1.0	11.4±3.0
Oleic acid	18:1 n-9	142.1±8.0	146.2±6.0	150.2±4.0	154.3±3.0	158.4±1.0	162.5±8.0	166.6±5.0
Gadoleic acid	20:1 n-11	15.7±2.0	14.6±3.0	13.6±5.0	12.5±3.0	11.5±3.0	10.5±3.0	9.4±0.1
Erucic acid	22:1 n-9	12.3±0.2	11.3±0.1	10.3±0.2	9.3±0.4	8.4±0.1	7.4±0.1	6.4±0.3
n-3 LC-PUFA								
Linoleic acid (LA)	18:2 n-6	189.4±7.0	189.6±6.0	189.7±3.0	189.8±3.0	189.9±3.0	190.0±8.0	190.2±5.0
Gamma-linolenic acid (GLA)	18:3 n-6	0.3±0.0	0.3±0.01	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.0	0.3±0.0
Arachidonic acid	20:4 n-6	1.8±0.2	1.8±0.1	1.8±0.1	1.8±0.3	1.8±0.2	1.8±0.3	1.7±0.5
Alpha-linolenic acid (ALA)	18:3 n-3	23.8±1.0^{e}	25.8±2.0^{de}	27.8±1.0^{d}	29.8±4.0^{d}	31.8±2.0^{c}	33.8±3.0^{b}	35.8±0.1^{a}
Stearidonic acid	18:4 n-3	3.2±0.1	3.0±0.1	2.7±0.3	2.5±0.1	2.2±0.1	2.0±0.4	1.7±0.2
Eicosapentaenoic acid (EPA)	20:5 n-3	16.9±2.0^{a}	15.8±1.0^{a}	14.7±2.0^{ab}	13.5±4.0^{ab}	12.4±5.0^{b}	11.3±1.0^{c}	10.1±3.0^{d}
Docosapentaenoic acid (DPA)	22:5 n-3	5.7±0.1^{a}	5.3±0.1^{a}	4.8±0.4^{a}	4.4±0.2^{ab}	3.9±0.1^{b}	3.5±0.2^{c}	3.0±0.2^{d}
Docosahexaenoic acid (DHA)	22:6 n-3	22.0±4.0^{a}	20.6±3.0^{a}	19.2±1.0^{a}	17.8±3.0^{ab}	16.3±1.0^{b}	14.9±1.0^{c}	13.5±5.0^{d}

[1]Mean values of 3 replicate ±SEM, [2]Not statistically significant (p>0.05) and alphabetical letters showed different significance level

Table 4: Growth performance and per cent survival of fingerling *Oreochromis niloticus* fed diets containing varying replacement levels of fish meal by locust meal[1,2]

	Varying replacement levels of fish meal by locust meal						
	D1	D2	D3	D4	D5	D6	D7
Initial weight (g fish^{-1})	3.6±0.03	3.5±0.04	3.6±0.03	3.6±0.01	3.4±0.01	3.4±0.05	3.5±0.04
Final weight (g fish^{-1})	29.22±0.4^{a}	28.34.±0.3^{a}	29.51±0.1^{a}	27.42±0.3^{b}	21.01±0.5^{c}	17.13±0.3^{d}	12.69±0.5^{e}
Absolute weight gain (g fish^{-1})	25.62±0.2^{a}	24.84±0.4^{a}	25.91±0.1^{a}	23.82±0.4^{b}	17.61±0.6^{c}	13.73±0.5^{d}	9.19±0.5^{e}
Live weight gain (%)[3]	711.7±1.6^{a}	709.7±1.1^{a}	719.7±2.4^{a}	661.6±3.6^{b}	517.9±4.3^{c}	403.8±3.2^{d}	262.6±2.4^{e}
Feed conversion ratio[4]	1.64±0.06^{d}	1.61±0.02^{d}	1.58±0.03^{d}	1.62±0.04^{d}	1.98±0.03^{c}	2.54±0.02^{b}	3.18±0.01^{a}
Specific growth rate (% day^{-1})	3.74±0.05^{a}	3.73±0.01^{a}	3.76±0.05^{a}	3.63±0.02^{b}	3.25±0.03^{c}	2.89±0.05^{d}	2.30±0.03^{e}
Protein efficiency ratio[5]	1.36±0.03^{b}	1.38±0.02^{b}	1.41±0.03^{a}	1.37±0.01^{c}	1.12±0.05^{c}	0.87±0.03^{d}	0.70±0.01^{d}
Protein deposition (%)[6]	20.37±0.2^{a}	20.14±0.2^{a}	20.94±0.1^{a}	20.30±0.3^{a}	15.22±0.1^{b}	11.07±0.2^{c}	8.05±0.2^{d}
Protein gain (g fish^{-1})	3.85±0.01^{a}	3.62±0.02^{b}	3.86±0.01^{a}	3.52±0.03^{b}	2.39±0.04^{c}	1.74±0.01^{d}	1.06±0.03^{d}
Protein retention efficiency (%)	9.17±0.5^{a}	9.06±0.3^{a}	9.42±0.2^{a}	9.13±0.1^{a}	6.85±0.5^{b}	4.98±0.4^{c}	3.62±0.2^{d}
Survival (%)	-	93±2.6	100	97±3.2	100	100	100

[1]Mean values of 3 replicate ±SEM, [2]Not statistically significant (p>0.05) and alphabetical letters showed different significance level

and 3.0 g kg^{-1}, respectively. Similarly lower values of DHA (16.3, 14.9 and 1350 g kg^{-1}) in groups fed on D5, D6 and D7 diets was noted.

Over the 8 weeks feeding trial. Replacement of fish meal by locust meal on protein to protein basis was found to be feasible up to 30% as evident by insignificant differences ($p<0.05$) among the live weight gain (661.6-719.7%), feed conversion ratio (1.58-1.64), the protein efficiency ratio (1.36-1.41), specific growth rate (3.63-3.76%) and protein deposition (20.14-20.94%) of fish fed diets D1, D2, D3 and D4 wherein 0, 10, 20 and 30% fish meal protein was replaced by locust meal protein in Table 4. However, further replacement of fish meal by locust meal protein (beyond 30%) resulted in a significant decrease ($p<0.05$) in growth and conversion efficiencies. Significantly ($p>0.05$) poorest LWG (262%), FCR (3.18), low PER (0.70), SGR (2.30%) and PD (8.05) were detected in fish fed diet D7 wherein 60% fish meal protein is replaced with locust meal protein. Based on the broken-line analysis of LWG, the optimum replacement level was found to be 29.8% in Fig. 1.

The body composition of the fish was significantly altered by the different replacement levels of locust meal in Table 5. No remarkable differences in moisture content (772-779 g kg^{-1} wet weight basis) were evident in fish fed diets D1, D2, D3 and D4. However, remarkable variations in body moisture were detected in fish fed diets D5 (753 g kg^{-1}), D6 (742 g kg^{-1}) and D7 (731 g kg^{-1}) compared to that of D1, D2, D3 and D4. Ash content differed insignificantly (21.6-22.1 g kg^{-1} wet weight basis) among the groups. No significant differences amongst the body protein

Table 5: Carcass composition (g kg^{-1} wet weight) of fingerling *Oreochromis niloticus* fed diets containing varying replacement levels of fish meal by locust meal[1,2]

		Varying replacement levels of fish meal by locust meal						
	Initial	D1	D2	D3	D4	D5	D6	D7
Moisture	748.0±22.0	779.4±5.6^{a}	771.1±8.3^{a}	774.3±9.7^{a}	772.1±7.5^{a}	753.6±5.4^{b}	742.1±7.2^{c}	731.1±7.8^{d}
Crude protein	108.0±3.0	145.1±5.2^{a}	141.2±0.5^{a}	143.9±0.1^{a}	142.7±0.9^{b}	131.1±0.8^{c}	122.8±0.6^{d}	113.2±0.4^{e}
Crude fat	43.0± 2.0	33.7±3.8^{e}	34.5±0.4^{d}	34.1±0.5^{de}	35.2±0.8^{d}	46.1±0.3^{c}	49.1±0.4^{b}	54.7±0.6^{a}
Ash	23.0± 0.2	21.6±1.8	22.1±0.5	21.7±0.3	21.9±0.6	22.1±0.6	21.5±0.4	21.6±0.2

[1]Mean values of 3 replicate ±SEM, [2]Not statistically significant (p>0.05) and alphabetical letters showed different significance level

Table 6: Hematological indices and antioxidant status of fingerling *Oreochromis niloticus* fed diets containing varying replacement levels of fish meal by locust meal[1,2]

	Varying replacement levels of fish meal by locust meal						
	D1	D2	D3	D4	D5	D6	D7
Hematocrit (%)	31.24±0.4^{a}	30.11±0.3^{a}	29.88±0.1^{a}	31.35±0.5^{a}	27.18±0.2^{b}	21.68±0.1^{c}	16.37±0.3^{d}
Red blood corpuscles (10^{6} mm^{-3})	2.18±0.2^{a}	2.32±0.1^{a}	2.27±0.1^{a}	2.19±0.3^{a}	1.82±0.2^{b}	1.63±0.1^{c}	1.35±0.4^{d}
Hemoglobin (g dL^{-1})	9.81±0.1^{a}	9.38±0.9^{a}	9.11±0.4^{a}	9.72±0.7^{a}	8.62±0.8^{b}	7.19±0.7^{c}	6.12±1.2^{d}
Superoxide dismutase (U mg^{-1} protein)	82.4±13.0^{a}	80.6±12.0^{a}	79.4±16.0^{a}	71.2±24.0^{a}	63.7±13.0^{b}	53.9±15.0^{c}	45.1±12.0^{d}
Catalase (U mg^{-1} protein)	34.1±4.0^{a}	31.5±5.0^{a}	35.2±3.0^{a}	28.3±9.0^{a}	24.8±4.0^{b}	19.3±4.0^{c}	13.6±5.0^{d}
Glutathione peroxidase activity (U mg^{-1} protein)	187.3±24.0^{a}	182.4±26.0^{a}	185.6±23.0^{a}	173.9±1.2^{a}	164.1±33.0^{b}	151.7±44.0^{c}	139.2±51.0^{d}

[1]Mean values of 3 replicate ±SEM, [2]Not statistically significant (p>0.05) and alphabetical letters showed different significance level

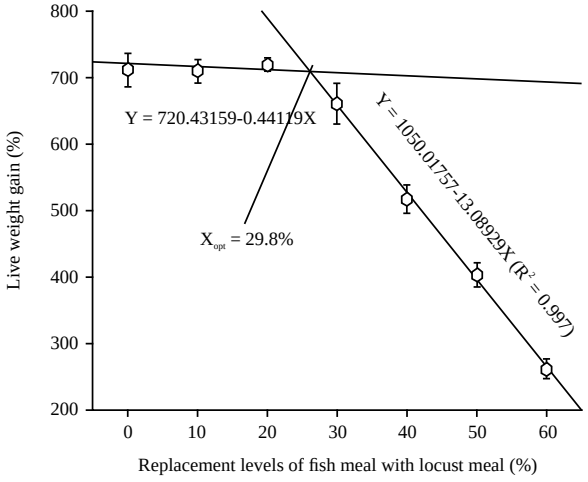

Fig. 1: Broken-line relationship of live weight gain to replacement levels of fish meal with locust meal for fingerling *Oreochromis niloticus*

(141-145 g kg^{-1} wet basis) of the fish fed diets of D1, D2, D3 and D4 replacing 0, 10, 20 and 30%fish meal protein was evident. Whereas in fish fed diet D7 replacing 60% fish meal showed a sharp ($p<0.05$) decline (113 g kg^{-1} wet basis) in body protein. No remarkable differences ($p>0.05$) among body fat were evident in fish fed diets D1, D2, D3 and D4 (33.7-34.5 g kg^{-1} wet basis). However, a significant increase ($p<0.05$) in body fat was noted in fish fed dietsD5 (46.1 g kg^{-1}), D6 (49.1 g kg^{-1}) and D7 (54.7 g kg^{-1}) replacing 40, 50 and 60% fish meal protein. In the current investigation, survival was not significantly affected among the variable treatments and was found to be in the range of 93-100% (Table 1).

The effects of different replacing levels of fish meal by locust meal on liver SOD, CAT, GPx activities and haematological (Hct, RBCs and Hb) tools in fingerling *O. niloticus* is mentioned in Table 6. The almost constancy ($p<0.05$) of liver SOD, CAT and GPx activities with the hiking replacing values of fish meal by locust meal up to 30% was recorded and, thereafter, a declining trend was noted in fish fed diets D5, D6 and D7 wherein 40, 50 and 60% fish meal protein was replaced by locust meal protein. The poor values of SOD (45.1 U mg^{-1} protein), CAT (13.6 U mg^{-1} protein) and GPx activities (139.2 U mg^{-1} protein)was recorded in fish fed D7 diet.The stable response of Hct, RBCs and Hb were noted in fish fed diets from 10 to 30% replacement of fish meal by locust meal. While the declining trend for blood tools was recorded on further replacement of fish meal by locust meal. The lowest values of Hct (16.3%), RBCs (1.35 ×10^{6} mm^{-3}) and Hb (6.12 g dL^{-1}) were recorded in fish fed diet D7.

DISCUSSION

Choosing feed ingredients is a crucial parameter in the design and making of supplemented quality feed products of any aquatic species[51-56]. Fish meal is an important ingredient that is widely used as a source of animal protein has the high protein and essential amino acids but a higher price than available sources of plant protein[57-61]. Besides this, the accessibility of fish meal is falling day by day due to its high requirement in other than aquaculture industry like livestock, poultry etc. The reduced stock of fish meal in future will intensely affect fish production. Considering this, it is essential to partially reduce or eliminate fish meal in the fish diet.

The method to reduce fish meal from fish diets is to replace it with alternate protein which should be less expensive and easily accessible, allowing for continued expansion of aquaculture. Because of this, various plant and animal protein sources have been assessed for the replacement of fish meal[4,9,54,59,61-65]. Locust meal is a good source of protein, amino acids and fatty acids profile[21,66-68]. Several workers have attempted to replace a fish meal with a locust meal in the past[19-22]. Amino acids, because they influenced fish growth and price, have found great value in fish nutrition studies[69]. Fish in the first months of life grow rapidly and that is why the supply of amino acids at this time is essential for body protein and energy[70]. Amino acids are the crucial substrates for protein synthesis and essential flow regulators in several metabolic pathways[71]. An imbalance of the amino acid diet pattern can lead to a fall in dietary intake and protein consumption[72,73]. Also, essential fatty acids in the diet must be taken into account when determining the nutritional value of the ingredients and feeds[74]. The need for essential fatty acids can only be met by providing long chain (LC) Polyunsaturated fatty acids (PUFA) in the diet, especially α-linolenic acid (LNA, 18: 3ω3) and linoleic acid (LA, 18: 2ω6) and various eicosapentaenoic acid (EPA, 20: 5ω3) and docosahexaenoic acid (DHA, 22: 6ω3) depending on the species[72,75]. In this study, however, the amino acid composition was not significantly affected among the varying replacement groups. The diets D1, D2 and D3 with 10, 20 and 30% replacing fish meal with locust meal showed the balanced fatty acid profile of the experimental diets in these groups. Whereas diets D5, D6 and D7 significantly ($p>0.05$) wherein 40, 50 and 60% fish meal protein are replaced by locust meal protein have the lower values of EPA, DHA and DPA leading to an imbalanced fatty acid profile for the groups which fed on these diets. Data of the present study revealed that 30% of the fish meal protein could be replaced by locust meal without hampering the growth of the *O. Niloticus* suggesting that the amino acids and fatty acid profile of the diets up to 30% replacement of fish meal protein with locust meal protein was as per the need of the fish. However, the further substitution of fish meal with locust meal protein in diet D5, D6 and D7 depressed growth and conversion efficiencies. This reduced performance at higher replacement of fish meal by locust meal (D5, D6 and D7) may be due to the lower amounts of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)in the D5, D6 and D7 diets. In the present study, fish showed a relatively small but significant decrease ($p<0.05$) in SGR with increasing replacement of fish meal with locust meal beyond 30%. This may be because of the nutritional imbalance particularly the shortage of the essential fatty acids at higher levels of locust meal inclusion in the diet. High FCR noted at higher inclusion of locust meal in D7 (60% replacement) maybe because of the low nutrient utilization from locust meal at higher inclusion level. A similar observation on replacing fish meal with locust meal was also found for other aqua species[20-22]. Related outcomes were also detected by Brah *et al.*[76], where in body weight and weight gain significantly fall with increasing higher substitution rates of fish meal with grasshopper meal. Deng *et al.*[77] have also documented that decreased final body weight, AWG and SGR were noted with the increasing levels of replacement of fish meal by locust meal in broiler diet. Tran *et al.*[67] conveyed that locust meals may be utilized to replace part of fish meal in fish diets.

Antioxidant power is important for maintaining complex fish antibodies, which include enzymatic and non-enzymatic antioxidant activities. Antioxidant enzymes including SOD, catalase and GPx form the first line of enzymatic defence against free radicals in organisms[43,77,78]. GPx involves in the exclusion of hydroxyl free radicals (OH°) and promotes the decomposition of hydrogen peroxide in water and oxygen and restores lipid hydroperoxide[79]. The SOD and GPx and catalase (CAT) are the main antioxidant enzyme group that promotes the conversion of reactive oxygen species to less reactive species[80,81]. Superoxide dismutase is considered to be the first line of protection against oxygen[82,83]. Cao *et al.*[84] reported that excessive replacement of fish meal by maggot meal would make shrimp in a state of oxidative stress. Using more than 4.9% yellow mealworm *Tenebrio molitor* meal as a replacement for a fish meal might affect antioxidant enzyme activities of pearl gentian grouper, *Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀[85]. Dietary replacement of FM by 3% yeast hydrolysate could improve the antioxidant capability of juvenile Jian carp[86]. In the current research, an almost constancy ($p<0.05$) of liver SOD, CAT and GPx activities with the enhancing replacing values of fish meal by locust meal up to 30% was recorded and, thereafter, the declining trend was noted in fish fed diets D5, D6 and D7 wherein 40, 50 and 60% fish meal protein was replaced by locust meal protein.

Haematological analyses may indicate the health status of fish[73,87]. A haematological examination of fish demonstrated the stable response of Hct, RBCs and Hb in fish fed diets D1, D2, D3 and D4 with 0-30% replacement of fish meal by locust meal. While the declining trend for blood tools was recorded on further replacement of fish meal by locust meal (D5, D6 and D7). Feeding of dried locusts for 91 days did not harm haematological parameters but resulted in a slight reduction in the number and diminished ovarian

steroidogenesis, which could reduce fertility[88-90]. Migratory locust food (*Locusta migratoria*) can replace up to 25% of the fishmeal in the isoproteic diet of Nile tilapia fingerlings without harmful influences on digestive function, growth function and haematological tools[91,92].

Except for growth fall at higher amounts of locust inclusion in fish fed D5, D6 and D7, no diet linked mortality or any other nutritional pathologies were perceived in this research. The reverse relationship between growth parameters and higher replacement levels of fish meal by locust in fish diets has been noted in several previous researches[18,20-22,93]. Replacement of fish meal with locust meal of Nile tilapia may be affected by several biological factors such as species, life stage, sex, feeding habits, diet composition, resembles studies reported of replacing fish meal with locust meal in several other studies in broilers[67,76,94,95].

CONCLUSION

The current experiment showed that a 30% inclusion level of locust meal produced the best result in terms of growth. It is, therefore, recommended that locust meal can be incorporated up to 30% as a replacement for fish meal without compromising the growth of fish. There are a variety of protein sources that can be used in aquaculture without affecting growth function, feed performance and body composition, given the need for amino acids, locust meal can be used as another protein ingredient in fish food.

SIGNIFICANCE STATEMENT

This study discovered that locust meal could be beneficial for the Nile tilapia fingerlings *Oreochromis niloticus* as an alternative protein source to fish meal in the limit of 30% in the diet, to formulate cost-effective commercial feeds for *Oreochromis niloticus.*

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