Effect of adding different levels of soaked pigeon pea seeds (*Cajanus cajan* L) on the Growth and Feed Utilization Efficiency of Nile tilapia (*Oreochromis niloticus*) fingerlings.

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Abstract

This study was conducted to evaluate the effect of socked pigeon pea seed meal on Growth and Food Utilization Efficiency of Nile tilapia (*Oreochromis niloticus*) fingerlings. 120 fish the weight and length average (3.4±0.04g and 3.2±0.00cm) were randomly distributed in 12 plastic Tanks (Aquarium) 36L according to the complete randomization, allocated to a represent four treatments (T0, T1, T2, and T3) of Nile tilapia (*Oreochromis niloticus*) fingerlings. four feeds were prepared in pellet form which consisted of 0%, 25%, 50% and 75% pigeon pea seed meal as replacement of fish meal. All diets contents 30% protein. Nile tilapia (*Oreochromis niloticus*) fingerlings were fed at the rate of 5% of their body weight. The result showed significant variation ($P < 0.05$) in final body weight, weight gain (WG), daily weight growth (DWG), Specific Growth Rate (SGR), and Food Conversion Ratio (FCR) among treatments. But, high SGR in T0 (control) and the best FCR were obtained at T 0% pigeon pea seed meal. Over all, best growth performance was observed in fish fed diet T1 (25%). The best performing fish's final weight, DWG, SGR and FCR were 9.46±0.33g, 0.13±0.01g/day, 2.93±0.73% /day and 1.49±0.30 respectively. On the other hand, there was high significant variation ($P < 0.05$) in survival rate were obtained at T2 (50%). this study recommend that a diet containing 25% Pigeon pea seed meal for juvenile Nile tilapia is optimum in terms of growth performance and profitability, and does not impact water quality and plankton abundance in plastic Tanks (Aquarium) culture system.

Introduction

Food security is a pressing global issue as the human population is projected to reach between 7.5 and 10.5 billion by 2050 [34]. With capture fisheries becoming increasingly unsustainable due to overfishing, aquaculture is expected to overtake capture 2 fisheries in supplying the world's protein requirements in the future [17]. Indeed, aquaculture is the fastest growing food production sector in the world, with an average annual growth rate of 6.3% since the year 2000 (average 8.8% per year between1980 and 2010) and currently accounts for approximately 47% of the world's fish supply [17]. During the same period, total aquaculture production in Africa increased at a rate of 11% per annum. Ponds remain the most common

tilapia production system in Africa, contributing about 38– 93% of total tilapia production from aquaculture in many Africa countries [14]. Tilapia is a hardy prolific, fast growing tropical fish, and it can survive on a diversity of food. Algae are probably their most common food in the wild. On fish farms they are fed a highprotein pelleted feed. They can be fed by hand or with sprinkler mechanism, and generally fed twice per day [18]. Sudan has often been described as the largest country in Africa and at present the population is about 40 million people. The Sudanese consume a substantial amount of meat in their diet, but the country demand for fish is not yet satisfied the present per capita consumption at 1.38 kg/year [36].This level is low when compared to the neighboring countries. Aquaculture is the fastest growing sector of world human food production and has an annual increase of about 10% [15]. To sustain such a high rate of growth a matching increase in fish feed production is imperative [2]. The feed is the most expensive component in the intensive aquaculture where it represents over 50% of the operating cost, moreover protein itself represents about 50% of feed cost , therefore the selection o f proper quality of dietary protein is a necessity for successful fish culture practices [12] . Fish meal is considered the most desirable animal protein ingredient in aquaculture feeds because of its high protein content, balance amino acid profile, high digestibility and palatability, and as a source of essential fatty acid [20]. Fish nutritionists have made several attempts to partially or totally replace fish meal with less expensive and locally available protein sources. In this aspect, several feed ingredients have been investigated in an attempt to find substitutes for fish meal in the diets of tilapia. These include animal protein sources such as, the fishery byproducts, shrimps meal, and feather, bone meal and blood meal. Plant protein sources including soy bean meal cotton seed meal, ground nut meal, wheat bran meal, sunflower cake and Pigeon peas plants [31, 10, 13]. Cotton seed, groundnut and sun flower cakes are one of the best plant protein sources for tilapia in developing countries due to its high availability, relatively low price, good protein content not less than 26.54% depending on processing methods and amino acid 3 profile [16]. Replacement of fishmeal by cheaper ingredients of either animal or vegetable origin in aquatic animal feed is necessary because of the rising cost and uncertain availability of fishmeal [25, 21].These feeds are not only considerably cheaper than fish meal but also enjoy high availability and accessibility in certain regions of the world. Soy bean meal and wheat bran meal has been used as a protein source in diets of various fish species [22, 36]. Nevertheless the use of Pigeon peas is limited by the presence of ant- nutritional factors such as protease inhibitors, Amylase inhibitors, Oligosaccharides and poly phenol; the current study was designed to assess the growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) fed diets containing Pigeon pea [4] as replacer of fishmeal.

Material and Methods

Fish collection

Total number of (200) Juveniles of Nile tilapia (*Oreochromis niloticus*) with an average initial body weight of (3.4±0.01 g), and total length (3.2 ± 0.04 cm) were purchased from private fish farm of Hussein Fadol, Soba West Agriculture Scheme, and transferred to the fisheries laboratory in Sudan University of Science and Technology, College of Animal Production Science and Technology, Department of Fisheries and Wildlife using suitable gillnet and distributed into experimental plastic Tanks (Aquarium). Aerators were used for aerating the water of experimental aquaria. The pH, Dissolved Oxygen and Temperature were recorded every day/week, using pH electronic meter (model, No. 201) and thermometer.

Collection of ingredients

Pigeon peas, Wheat bran, Groundnut Cake, sorghum and fishmeal were acquired from halt kuku Market

and were manually sorted to remove impurities. The premix (Mineral Mix) was obtained from livestock shop. Manufactured by using electrical balance (Model; 2003, Max; 200g cl; 0.001g, AC; 220W/50HZ, S/N; 11g, SF; 400).

Processing of ingredients

In attempting to remove anti- nutritional factors, seeds were soaked by dipping them in water in plastic container for 24 hours with soaking ratio of 100 g/300 ml (1:3w/v) at room temperature. After soaking, the seeds were oven dried at 60°C overnight. Pigeon peas were milled by using milling machine and stored in name labelled containers prior to analysis.

Chemical composition

Chemical composition to determine composition of raw and soaked Pigeon pea seeds was carried out according to standard method adopted by Association of Official Analytical Chemists [5]

DM= Dry Matter. CP= Crude Protein. EE= Ether Extract. CF= Crude Fibre.NFE= Nitrogen Free Extract.

Table 1. Proximate composition of Pigeon peas (*Cajanus Cajan* L) DM%

Type	DM	CP%	EE%	CF%	Ash	NFE
raw	91.63	20.13	2.42	10.50	3.47	55.11
Soaked	79.22	18.40	1.99	3.98	2.43	35.66

Moisture content was determined by drying samples in an oven at 70°C for 48 hours.

Crude protein was determined according to Kjeldah method. Samples were digested in concentrated sulphuric acid by a digester, distillate and titrated to obtained nitrogen. Crude protein was obtained by multiplying nitrogen content with conversion factor of 6.25.

Crude fibre was determined by digesting a sample with weak base preceded by weak acid. Ankom 220 Fibre analyzer was used to determine the crude fibre.

Crude lipid was determined after soxhlet extraction of dried samples with 1.25% H2SO and 1.25% NaOH Ash content was determined by calcinations method. Samples which were previously oven dried were put in crucible and heat in a muffle furnace at 550°C for 3 hours and then cooled before weighing.

Experimental diet formulation

Diets were formulated to replace 0% (control), 25%, 50%, and 75% of fishmeal. And give up to 30% crude protein. During compounding of diets, the weighed proportions of dry milled ingredients were carefully mixed by hand before adding water. The mixture was then passed through meat machine with 0.5 mm sieve size to make pellets. Pellets were then dried by passing in the oven for 6 hours at 40°C and then spread on the floor under room temperature for one week prior to packaging. Composition of experimental Diets (g/100 g diet) is shown in Table 2.

Experimental design and feeding regime

The experimental design was a completely randomized design (CRD). 120 Nile tilapia fingerlings with initial average body weight and length (3.4±0.01g) and 3.2±0.00 respectively, were randomly distributed in plastic Tanks (Aquarium) with a capacity of 20 litres at a stocking density of 10 Nile tilapia per bucket. Fish were fed four times a day according their appetite; diets were given at periods (8:30, 11:00, 14:00 and 17:00 o clock), at 5% of body weight throughout the experimental period. The quantity of feed was adjusted weekly based on the weight of fish to avoid feed wastage and deterioration of water quality parameters (dissolved oxygen, ammonia, temperature and PH). A Remaining feeds at the end of the

Table 2. Composition of the Experimental Diets /100g.

Ingredients / Treatments	T (0%)	T (25%)	T (50%)	T (75%)
Fish Meal %	24	18	12	6
Pigeon pea %	0.0	6	12	18
Groundnut Cake %	30.4	35	39	43.4
Wheat Bran %	28	27	25	22
Dora fitrita %	17	13.4	11.4	10
Mineral Mix %	0.3	0.3	0.3	0.3
Vegetable Oil %	0.2	0.2	0.2	0.2
Vitamin mixture %	0.1	0.1	0.1	0.1
Total	**100**	**100**	**100**	**100**
Total crude protein intake	**30.0±0.01**	**30.2±0.07**	**30.1±0.05**	**30.0±0.01**

1-Fishmeal 49% CP; Pigeon pea 18.4% CP; Groundnut Cake 44.8% CP; Dora fitrita 11.5% CP and Wheat bran 15.7%. 2-Mineral mixture (g/100g dry diet) calcium biphosphate 13.57; calcium lactate 32.69; ferric citrate 02.97; magnesium sulphate 13.20; potassium phosphate (dibasic) 23.98; sodium biphosphate 08.72; sodium chloride 04.35; almunium chloride.6H2O 0.0154; potassium iodide 0.015; cuprous chloride 0.010; mangnous sulphate H20 0.080; cobalt chloride. 6H2O 0.100; zinc sulphate. 7H2O 0.40 [19] 3-Vitamin mixture (g/100 dry diet) choline chloride 0.500;inositol 0.200; ascorbic acid 0.100; niacin 0.075; calcium pantothenate 0.05; riboflavin 0.02; menadione 0.004; pyridoxine hydrochloride 0.005; thiamin hydrochloride 0.005; folic acid 0.0015; biotin 0.000 5; alpha-tocopherol 0.04; vitamin B12 0.00001; LobaChemie, India [19].

week were weighed and used to estimate feed intake.

Handling of experimental fish

Fish were weighed prior to the start of each experiment to obtain fish of consistent size and weight. A top pan balance was used in weighing procedures. Fish were acclimated to 6 Experimental environment for 10 days and fed control diet. After the acclimation period fish were netted from each plastic bucket using a fine mesh hand net and total weighed. The dietary treatments were at random assigned to the plastic buckets. Total weighing of experimental fish was done weekly throughout the experiment.

Data collection and computation

The study lasted for 60 days during which fish were fed the experimental diets containing Pigeon pea seed meal. Parameters measured were bulk initial weight, bulk final weight and feed intake. Etch 10 days weight gain was measured to monitor growth while other parameters were calculated as

described below:

Specific Growth

Specific growth rate (SGR) (% day (SGR) (% day-1) % = {In Wt_2- In $Wt_1/t_2 – t_1$} × 100

Average Daily weight gain

Average Daily weight gain (ADWG) (day-1) = ($Wt_2 – Wt_1$)/ t.

Where:

Wt_2= final weight (g) at time t_2 (end of experiment)

Wt_1= Initial weight (g) at time t_1 (beginning of experiment) and In = Natural logarithm.

t= time (day)

Feed utilization

Feed conversion ratio (FCR) = Weight of food fed (g) /increased weight (g) Feed intake (FI) (g day-1)) = Total feed intake /number of days

Protein productive value (PPV %) = (P2 – PI) ×100 / Protein intake (g)

Where:

P2: Protein content in fish carcass at the end; PI: Protein content at the start.

Survival rate (SR) = {N_2/N_1} × 100

Where:

N_2-Number of fish at the end of the experiment

N_1- Number of fish at the beginning of the experiment .

Data analysis

Data collected from this experiment were subjected to one - way analysis of variance (ANOVA). Comparisons of treatment means were done by LSD Honest at 5% level of significance. Analyses were performed using SPSS software version16.

Results and Discussion

Growth performance Pigeon pea

Table 3. Growth performance of Nile tilapia (O. niloticus) fed diets containing different levels of Pigeon pea seed meal.

Parameters Treats	Initial weight	final weight	WG	DWG g/d	SGR%
0%	3.4±0.01^{a}	8.93±0.44^{a}	6.63±0.73^{a}	0.13±0.01^{a}	2.93±0.73^{a}
25%	3.4±0.03^{a}	9.46±0.33^{a}	5.70±0.53^{a}	0.11±0.01^{a}	2.40±0.53^{a}
50%	3.3±0.05^{a}	8.10±0.28^{ab}	4.08±0.08^{ab}	0.08±0.00^{b}	0.78±0.08^{b}
75%	3.3±0.06^{a}	7.33±0.44^{b}	3.83±0.03^{b}	0.07±0.00^{b}	0.53±0.03^{b}
Sig.	Ns	*	*	*	*

WG = weight gain, DWG = daily weight growth and SGR%= specific growth rateData are re resented as mean of three samples replicates ± standard error.

Sig= Significant Ns= no Significant

^{a,b,c} Means in the same Column with the same letter are Significantly different at ($p \leq 0.05$).

The study demonstrated that there were significant differences ($p \leq 0.05$) in the (WG g/Fish), (DWG g/Fish) and (SGR) of mono-sex Nile tilapia (*Oreochromis Niloticus*) fed on diets with partially replaced by Pigeon pea. The growth parameters were analyzed and presented in Table 3.

The increment weight

Results in Table 4: show that the result of mean ± SD of increment weight of Nile tilapia (*Oreochromis niloticus*) fed on the experimental diet T0, T1, T2 and T3 throughout 60 days the increase in T0 (3.4±0.01 to 8.83±0.44), T1(3.3±0.03 to 9.46±0.33),T2 (3.3±0.05 to 8.10±0.28) and T3 (3.3±0.06 to 7.33±0.44).

Table 4. The Increment Weight of Mono-sex Nile tilapia (*Oreochromis niloticus*) (g/Fish) as affected with Pigeon pea Incorporation in Diets/Days.

Diets %	Weight of fish (g) at different times						
	0 times	10 days	20 days	30 days	40 days	50 days	Increment (g)
T 0.0	3.4±0.01^{a}	4.37±0.29^{ab}	5.60±0.26^{a}	7.35±0.27^{b}	8.82±0.24^{a}	8.83±0.44^{a}	5.79±0.41
T 25	3.4±0.03^{a}	4.74±0.33^{a}	6.36±0.27^{a}	8.06±0.23^{a}	9.04±0.12^{a}	9.46±0.33^{a}	6.06±0.30
T 50	3.3±0.05^{a}	4.14±0.04^{b}	4.60±0.27^{b}	7.38±0.26^{c}	7.60±0.26^{b}	8.10±0.28^{ab}	4.80±0.23
T 75	3.3±0.06^{a}	4.06±0.02^{b}	4.78±0.08^{b}	5.68±0.36^{d}	5.91±0.41^{c}	7.33±0.44^{b}	4.03±0.38

Data are represented as mean of three samples replicates ± standard error.
Means in the same row with the same letter are not significant difference ($P>0.05$)

Feed Efficiency

Results in Table 5. Indicate that the mean ± SD of feed intake, feed conversion ratio, protein productive value, condition factor (K %) and survival rate. The lower significant value of feed intake (The death fish is not lower values of the mortality rate excluded) was (5.88±0.00 and 6.79±0.00) was obtained by fish diet T0 (giving low FCR), and T2 (which gives high FCR). The high ($p<0.05$) value

Table 5. Growth and Feed Utilization Efficiency of Mono-sex Nile tilapia (*Oreochromis Niloticus*) Fingerlings Fed the Experimental Diets.

Treats parameters	T0 (0.0%)	T1 (25%)	T2 (50%)	T3 (75%)	Sig
Feed intake (g)	55.84±0.00^{c}	65.29±0.00^{a}	59.79±0.00^{b}	53.15±0.00^{b}	*
FCR (g)	0.86±0.85^{c}	1.20±0.19^{b}	1.49±0.30^{a}	1.12±0.10^{b}	*
PPV (g)	39.33±0.19^{a}	34.33±0.00^{a}	33.66±0.00^{b}	29.00±0.19^{c}	*
K (%)	1.26±0.16^{a}	1.19±0.16^{a}	1.21±0.11^{a}	1.29±0.36^{a}	NS
SR (%)	98.27±2.9^{a}	95.10±3.8 ^{b}	90.12±3.6^{c}	91.59±6.6 ^{c}	*

FCR =feed conversion ratio, PPV = Protein productive value, K (%) = condition factor, SR (%) = survival rate.
Data are represented as mean of three samples replicates ± standard error.
a,b,c Means in the same Column with the same letter are Significant different at ($p \leq 0.05$) NS: No Significant different at ($p \geq 0.05$)

of the K was (1.89 and 1.46) in T3and T0. The high survival rate was in T2 and T3.

Feeding Rate (Regime %)

Results in Table 6: indicate that the mean ± SD of feeding regime % for Mono-sex Nile tilapia (*Oreochromis niloticus*) fed the experimental diets/ Sample decreasing of feeding intake as feed rate decrease, however the initial highest feed intake was in T2 (13.80 ±0.58) and T0 (13.78 ±1.16), Although they showed the lower percentage at the final feeding

Table 6. Feeding Rate (Regime %) Intake by Mono-sex Nile tilapia (*Oreochromis Niloticus*) / Sample.

Diets	Feed intake (%) at different sample				
	1(15%)	2(12%)	3(9%)	4(6%)	5(3%)
T 0(0.0)	13.78^{c}	14.15^{a}	12.25^{b}	9.78^{c}	5.88^{c}
T1 (25%)	12.80 ^{d}	13.86^{b}	15.39^{a}	15.40^{a}	7.84^{a}
T2 (50%)	13.80^{a}	14.17^{a}	12.63^{c}	12.63^{b}	6.56^{b}
T3 (75%)	12.99 ^{b}	13.98^{b}	10.48^{d}	10.20^{d}	5.50^{d}

Data are represented as mean of three samples replicates ± standard error
a,b,c Means in the same row with the same letter are not significantly different (P>0.05)

regime T3 (2.50±0.40) and T0 (4.88±0.95) respectively (the differences was due to electricity problems, losing their appetite and at the end the mortality rate increased).

Length during Experiment

Table 7. Length of Nile tilapia (*Oreochromis niloticus*) During the Experiment .

Treats parameters	T0 (0.0%)	T1 (25%)	T2 (50%)	T3 (75%)	Sig
IL (cm)	3.4±0.00^{b}	3.5±0.00^{a}	3.3±0.00^{c}	3.4±0.00^{b}	NS
FL (Cm)	8.83±0.44^{a}	9.16±0.33^{a}	8.50±0.28^{ab}	7.33±0.44^{b}	*

Data are represented as mean of three samples replicates ± standard deviation.
a,b,c Means in the same row with the same letter are Significant different at (p≤0.05) NS: NO Significant different at (p≥0.05)
IL = initial length, FL = final length.

Results in Table 7: indicated that mean ± SD of the initial length (cm) and final length (cm) of Mono-sex Nile tilapia (*Oreochromis niloticus*) fed 0%, 25%, 50% and 75% diets showed no significant differences (P>0.05) initial length and showed the significance (P<0.05) in final length.

Discussion

The study was conducted to evaluate the effect of adding pigeon pea seeds of four experimental diets (0%, 25%, 50% and 75%) containing socked pigeon peas seed meal, as replacement of fish meal on the growth Performance and carcass composition of *Oreochromis niloticus* under culture conditions. The

different physic-chemical parameters of the surface water were within the accepted range where tilapia can achieve maximum growth [9]. They were also comparable with the previous study [18] in Lake Kuriftu. Moreover, no significant variation was detected between the two sites. This suggested that the dietary crude protein does not negatively affect the water quality at experimental scale [23, 26]. most tilapia utilize starch efficiently from 22 to 46% dietary starch while 22% considered as optimum level for juvenile tilapia [34].Processing of Pigeon peas using cold water led to the reduction of nutrients due to Nutritional change which caused losses of water soluble components such as carbohydrates and nitrogen. [33] Detailed decrease in dry matter of beans after soaking. The present result showed the Low crude protein in soaked Pigeon peas than in raw Pigeon peas with agree that was reported by [24] due to leaching during soaking. but contrasting these results is [3] who detailed that protein content of Jack Bean Carnavalia ensiformis Seeds increased from 88-92% after 96 hours of soaking in water. Badifu attributed increased protein when kernels were soaked in cold water for 48 hours. However crude protein obtained in this study was not contrary to results reported by [31] who reported decreased crude protein as a result of soaking sorghum cultivars in cold water. Moreover, processing of Pigeon peas by using cold water led to the reduction of crude lipid. Nevertheless it is within the value reported by [30] when two groups (A and B) of Mucuna seeds were soaked in water for 12 and 24 hours. Low content a of ash in the soaked Pigeon peas could be explained by leaching of minerals despite the fact that it is still within the values reported by [28, 4] studies Pigeon peas are known to contain mineral such Iron, Calcium, Manganese, magnesium and Zinc which tend to dissolve and hence leaching into soaking medium during the whole process of soaking. Results in Table 4.shaw Growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) Juvenile fed diets containing soaked Pigeon peas seed meal as replacement of fish meal. The lower significant value of feed intake (5.88±0.00 and 6.79±0.00) was obtained by fish diet T0 (gave low FCR), and T2 (which gave high FCR). The highest ($p<0.01$) value of the specific growth was (2.93±0.73 and 2.40±0.53) in T0 and T1. The highest survival rate was in T2 and T1. But generally the growth was not optimum in T2 and T3 fed diets containing (50% and 75%) soaked Pigeon peas seed meal. Poor palatability accounts for feed rejection and or poor feed intake. [7, 27] explained that Poor feed intake led to starvation of Chinook salmon fed diets reached in ant nutritional. [1, 8] reported poor intake in Nile tilapia (*Oreochromis niloticus*) fed diets containing tannin while [6] reported similar phenomenon, which has occurred in common, carps. Results in Table 5. indicated that mean ± SD of the initial length (cm) and final length (cm) of Mono-sex Nile tilapia (*Oreochromis niloticus*) fed control, 25%, 50% and 75% diets showed no significant differences ($P>0.05$) initial length and showed the significance ($P<0.05$) in final length. Fish fed soaked Pigeon peas seed meal 25% diets recorded high growth and feed intake next to those fed the control diet. And significantly different from those fed soaked Pigeon peas seed meal 50% and 75% diets. fish fed diets containing soaked 25% conferred better growth and feed utilization (feed intake) than those fed diets the control diet.

Conclusion

Based on the results obtained from replacement of fish meal, by processing of Pigeon peas in cold water (1:3w/v) for 24 hours under room temperature has significant influence on the nutritive value of Pigeon peas. However slight reduction in dry matter, crude lipid and ash content has occurred. The results of this study show that processed Pigeon peas seed can be included in Nile tilapia diets at 25% level without harmful effect in terms of growth performance and feed utilization of Nile tilapia fingerlings. It is therefore concluded that whenever the cost of Pigeon peas is than fish meal, fish farmers can use soaked

Pigeon peas to replace up to 25% fish meal in Nile tilapia diets as a way of reducing the current demand pressure on fish meal.

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