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Research Article

Effect of Different Sources of Plant Protein on the Growth of Tilapia Fry (*Oreochromis niloticus*)

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Abstract

Background and Objective: In aquaculture, lower feed costs are generally desired, this may be achieved without impacting growth by lowering the level or replacing less expensive feed ingredients for more expensive ones. According to the study's objective, cheaper plant protein sources must be used for Nile tilapia fry growth and performance, encouraging the use of a variety of locally available plant protein sources. **Materials and Methods:** For 6 weeks, Nile tilapia fry (*Oreochromis niloticus*) weighing an average of 2.821.02 g was kept in glass aquaria (75×35×30 cm). Fish were fed a 35 percent protein diet consisting of peanut meal (PM), sesame meal (SM), cottonseed meal (CSM), wheat bran (WB) and sorghum (S) as energy sources during culture. Every day, the fish were fed 5% of their body weight (3 doses). **Results:** The fish on a diet containing peanut meal (PM), cottonseed meal (CSM) and wheat bran (WB) grew the fastest in this study, with a growth rate of 108.2±0.96%, followed by diets containing peanut meal (PM) and wheat bran (WB) at 81.1±1.32%. Diets with PM, CSM, SM and WB showed the least growth 61.7±2.01% and followed by diets with PM, SM and WB 60.9±2.01%. The fish's weight increased in all treatments indicates that the experimental diets were well-accepted by the fish. **Conclusion:** After the experiment, no significant differences ($p \geq 0.05$) between the diets were found. Diet consisting of PM, CSM and WB caused the most weight increase (108.2±0.96%) and the highest food conversion ratio (2.27±0.39). A diet containing PM, CSM and WB was the best diet in terms of both cost and FCR, with the lowest feed cost per kilogram of fish produced (2.84 SP kg⁻¹ fish).

Key words: Plant protein sources, tilapia fry, growth performance, aquaculture, diet cost, feeding, food conversion ratio

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

INTRODUCTION

Aquaculture is one of the world's fastest-growing food-producing industries and it helps to preserve wild fisheries while also providing food and nutritional security¹. The increasing need for fish for human consumption has required a quick expansion of fish farming, which has improved aquaculture feed production significantly. Aquaculture is the quick food production sector in the world, with continuous growth over the previous three decades². Progress and developments in current technology and farming practices in terms of fingerling production, culture systems, culture methods and the manufacturing of high-quality fish feed have all contributed to the growth of the fish business³. There Aquaculture has increasingly filled the growing demand for seafood, now producing over half of all fish and seafood for human consumption.

For a long time, many species of fish have been a part of the diet of some ethnic groups throughout all continents⁴. Tilapias are tropical cichlid teleosts native to Africa, the Mediterranean and the Middle East. Tilapia species are one of the first cultivated fish as evidenced by line drawings discovered in Egyptian tombs dating back to 2000 BC⁵. Due to its genetic improvement, ease of culture, rapid growth, high adaptability to survive even in difficult environmental conditions, stronger resistance to a specific disease and high protein composition in meat, tilapia is known as "aquatic chicken"^{6,7}. Tilapia is one of the most widely cultivated fish species and it is the world's second most farmed fish after carps. Today, tilapia has grown in importance in aquaculture and its consumption is on the rise all over the world. The annual global production of farmed tilapia has been steadily increasing in recent years⁸.

The high-quality feed can be fulfilled through ingredients selection with balanced nutritional content. Protein is one of the nutritional aspects that are especially important to have in the feed ingredient because it directly affects the cost⁹. The optimal dietary protein level promotes the efficiency of aquaculture and reduces feed costs¹⁰. Protein quality is determined by several parameters, including amino acid compositions, digestion, absorption and metabolic usage¹¹. Protein levels in the feed can be used to assess feed quality as protein concentration has a direct impact on the fish's growth, physiological and biochemical characteristics. Feeds with a higher protein content resulted in increased fish growth, weight gain and specific growth rate (SGR) with lower feed conversion value. Due to its conversion into energy, a high level of protein in the feed may harm fish growth as well as an

increase in nitrogen excretion in the water¹². As a result, optimizing feed protein consumption in industrial feed formulations is a top priority to provide the best performance for fish growth at the lowest cost¹³.

With the continued growth of tilapia production, the demand for appropriate diets based on locally produced resources has become a necessity¹⁴. The replacement of fishmeal with locally accessible, less expensive plant feedstuffs is important for aquaculture's future development. Using local vegetable protein to feed tilapia fish and the influence it has on their growth. Plant proteins are extremely similar to Fish Meals in terms of protein content and amino acid digestibility¹⁵. However, the use of plant-based protein sources as an alternative element in the fish diet is commonly hampered by antinutritional compounds. The amount of antinutritional substances in the body has a direct impact on nutrient intake and digestion^{16,17}. Plant protein concentrates have an advantage over whole meals as their non-digestible fibre is removed, allowing for higher concentrations in aqua diets¹⁸. Nile tilapia is an omnivore feeder fish that feeds mostly on plants, phytoplankton and benthic algae and has been introduced to ponds to help control aquatic weeds¹⁹. Therefore, the present study was based on the diets containing different cheaper plant protein sources that must be used for Nile tilapia fry growth and performance, encouraging the use of multiple plant protein feedstuff sources that are locally and readily available.

MATERIALS AND METHODS

Fish sample and experimental procedures: The study was carried out at the Faculty of Natural Resources and Environmental Studies, Fisheries Laboratory, Sudan from 15/6/2018-31/7/2018. Elseleit Hatchery in Khartoum provided fry *Oreochromis niloticus* with an initial weight of 2.3 ± 1.02 g. Before being stocked, the fish were given a week to acclimate to aquarium conditions. The fish were randomly assigned to five experimental groups with three replicates in five glass aquaria (75×35×30 cm) containing about 68 L of water. As a control treatment, fish in one aquarium were given no food, while the other four groups of fish were given one of the four experimental diets. Different sources of plant proteins, such as peanut meal (PM), sesame meal (SM), cottonseed meal (CSM) and wheat bran (WB) and sorghum (S), were used to formulate diets. Diet 1 had only PM as a source of protein, whereas diet 2 had equal amounts of both PM and SM. Diet 3 had the same quantity of PM and CSM but diet 4 had the same

amount of PM, SM and CSM in a 1:1:1 ratio. Based on NRC recommendations for Nile tilapia, all four diets contained the same amount of protein (35%).

Diet formulation: The nutrition laboratory of the Animal Production Research Center in Khartoum conducted a proximate study of the constituent feeds. Standard procedures were used to determine the proximate composition of the diets and diet constituents²⁰. The Kjeldahl technique was used to determine the crude protein concentration ($N \times 6.25$). Soxhlet extraction was used to determine the crude lipid content. Moisture content was determined by oven drying to a consistent weight, total ash was determined by muffle furnace burning and carbohydrate was determined by subtracting moisture, total organic nitrogen (protein), ether extract and ash from 100% (Table 1). Except for wheat bran, all feed materials were processed to fine powders in a commercial grain grinding mill. Each diet was made by thoroughly mixing the ingredients in the proper proportions. Then, using an electric meat grinder, water was gradually added until a moist mash (dough) was created (2 mm). Diets were then let to dry in the sun during the day and then dried indoors overnight under ventilation ceiling fans. To make the dry pellets more easily ingested by the fry, they were broken into smaller crumbles (1.5 mm sieve). The ingredient contents of the four diets show in (Table 2).

Feeding and growth performance analysis: Feeding took place three times a day, at 8:00 am, 12:00 pm and 3:00 pm. Each week, the daily feed ratios were changed depending on growth and the feeding response. For 6 weeks, fish were fed at a rate of 5% of their body weight per day. Fish were counted and weighed in groups at the end of the growth trial to evaluate weight gain, survival rate, feed conversion ratio and food cost per diet. The following formulae²¹ were used to determine various performance criteria:

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

$$\text{Mean weight gain (\%)} = \frac{\text{Mean weight gain (g)}}{\text{Mean initial weight (g)}} \times 100$$

$$\text{Daily growth rate (g day}^{-1}\text{)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Number of experimental days}}$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Food intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at harvest}}{\text{Number of fish stocked}} \times 100$$

$$\text{Feed cost kg}^{-1} \text{ fish} = \text{Feed price kg}^{-1} \times \text{FCR}$$

$$\text{Economic efficiency (\%)} = \frac{\text{Return}}{\text{Feed cost}} \times 100$$

Statistical analysis: The data was analyzed using the Statistical Package for Social Science (SPSS) Software (version 21) and the means were tested for significance using (ANOVA, two way) and the *post hoc* test, using the Least Significant Difference (LSD) test for the mean separation, to significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Fish require protein in their diets, protein levels can affect the growth, gut microbial composition, nutrient metabolism and various physiological reactions in fish²². In the present study, ingredients were selected to consider their nutritional quality and also their cost-effectiveness. The results obtained showed that the fish accepted the prepared diets, so the fish weight increased in all the treatments (Table 3). The control

Table 1: Proximate composition (%) of the ingredient used for diet formulation

Feedstuff	Dry matter	Crude protein	Crude lipid	Ash	Carbohydrates
Peanut meal	91.8	42.0	8.0	5.4	36.4
Sesame meal	95.2	41.3	11.9	17.6	24.4
Cotton seed meal	95.9	32.1	8.2	5.7	49.9
Wheat bran	92.3	11.8	2.5	3.9	74.1
Sorghum	91.7	10.7	3.4	2.8	74.8

Table 2: Composition of the experimental diets (%)

Ingredients	Diet 1 (35% CP)	Diet 2 (35% CP)	Diet 3 (35% CP)	Diet 4 (35% CP)
Peanut meal (42% protein)	77.00	38.95	46.00	29.04
Sesame meal (41.3% protein)	-	38.95	-	29.04
Cotton seed meal (32.1% protein)	-	-	46.00	29.04
Wheat bran (11.8% protein)	17.25	16.58	6.00	9.66
Sorghum (10.7% protein)	5.75	5.52	2.00	3.22
Total	100	100	100	100

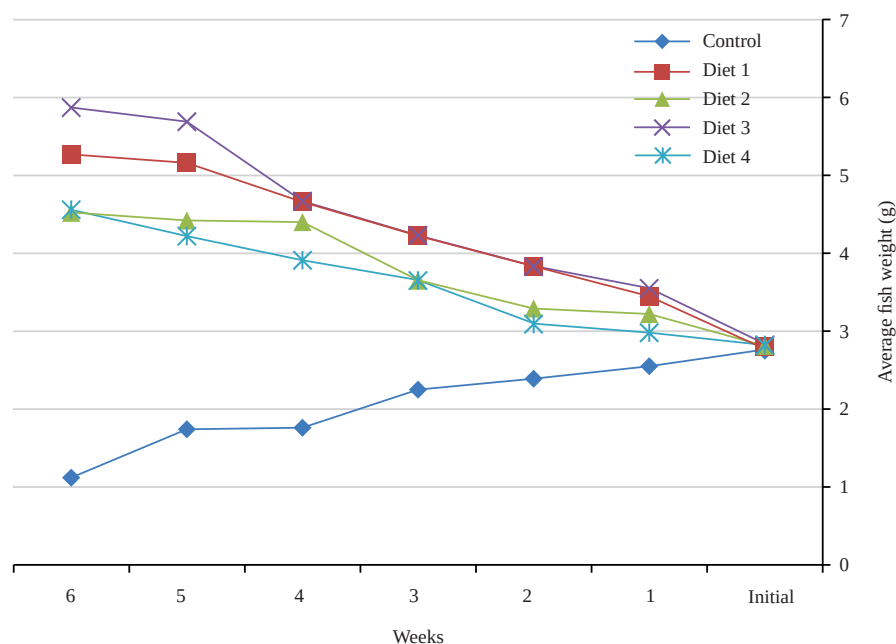


Fig. 1: Weekly increase in body weight of fry fish fed different experimental diets

Table 3: Growth performance of fry fish in a 6 weeks

Parameters	Control	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g)	2.75±1.45	2.80±1.02	2.81±1.02	2.82±1.02	2.82±1.02
Final weight (g)	1.71±1.89	5.07±1.56	4.52±1.12	5.891.67	4.56±1.12
Weight Gain (g)	-1.04±4.45	2.27±0.39	1.71±0.45	3.05±0.87	1.74±1.21
Weight gain (%)	-37.8±4.67	81.1±1.32	60.9±2.01	108.2±0.9	61.7±2.01
Daily weight gain (g)	-0.025±0.22	0.054±0.12	0.041±0.09	0.073±0.02	0.041±0.09
FCR	-	3.18±0.44	3.74±0.78	2.27±0.65	3.63±0.78
Survival (%)	46.7±2.12	80.0±2.22	60.0±2.44	63.3±2.24	60.0±2.44

treatment which was left without food lost some weight (Fig. 1). The mobilization and use of the fish's stored fats resulted in weight loss.

The fish given diet 3 grew the fastest in this experiment, with a growth rate of $108.2 \pm 0.96\%$, followed by diet 1 at $81.1 \pm 1.32\%$. Diets 4 and 2 produced the least growth ($61.7 \pm 2.01\%$ and $60.9 \pm 2.01\%$, respectively) (Table 3). Bodyweight gain of fish fed diet 3 was 34.4% higher than those fed diet 1 through both diets have the same protein level of 35% (Fig. 2). This can be explained by the higher nutritional quality of the diet due to the addition of cottonseed meals which augmented the essential amino acid contents.

Cottonseed meal is also known to be high in vitamins, particularly B vitamins, which interact as co-enzymes in protein, lipid and carbohydrate metabolism. Previous study²³ revealed that Nile tilapia was grown in earthen ponds when given only CSM (42% CP). CSM can be utilized as a source of fertilizer in semi-intensive tilapia culture to improve natural food production within fish ponds, in addition to being used

as a protein source in commercial pelleted diets for tilapia²⁴. In most regions of the world, CSM is a commonly available and inexpensive plant protein source.

As a protein concentration source, sesame meal was added to peanut meal but did not increase fish performance as much as cottonseed meal did. The growth of the fish in this condition (diet 2) was lower than that of fish given only peanut meal (diet 1). The latter increased by $81.1 \pm 1.32\%$ whereas diet 2 fish (fed peanut meal and sesame meal) increased by only $60.9 \pm 2.01\%$ (Table 3). This is a surprising finding given that sesame meal is known for having a much better balance of essential amino acids than other oilseed meals, including peanut meal. Antinutritional Factors (ANFs) in plant protein meals decrease growth performance and feed efficiency²⁵ as well as affect digestive enzyme activity and animal digestion/absorption capacity²⁴. This observation was consistent with the study²⁵ suggest that sesame oil cake can be a suitable protein source for a carnivorous fish when replacing at least half of the fishmeal protein (without amino acid supplementation) and decrease feed efficiency in

Table 4: Cost of the experimental diets (SDG kg⁻¹)

Ingredients	Cost/kg ingredient	Diet 1		Diet 2		Diet 3		Diet 4	
		Percentage	Cost/kg diet	Percentage	Cost/kg diet	Percentage	Cost/kg diet	Percentage	Cost/kg diet
Peanut meal	1.00	77.00	0.77	38.95	0.389	46.00	0.460	29.04	0.29
Sesame meal	0.75	-	-	38.95	0.292	-	-	29.04	0.218
Cottonseed meal	1.603	-	-	-	-	46.00	0.736	29.04	0.465
Wheat bran	0.6	17.25	0.104	16.58	0.099	6.00	0.036	9.66	0.058
Sorghum	1.07	5.75	0.062	5.52	0.059	2.00	0.021	3.22	0.034
Cost/kg diet			0.936		0.839		1.253		1.065

Table 5: Feed cost for producing 1 kg of fish

Diets	Cost/kg	FCR	Diet cost/kg fish
1	0.936	3.18	2.98
2	0.839	3.74	3.14
3	1.253	2.27	2.84
4	1.065	3.63	3.87

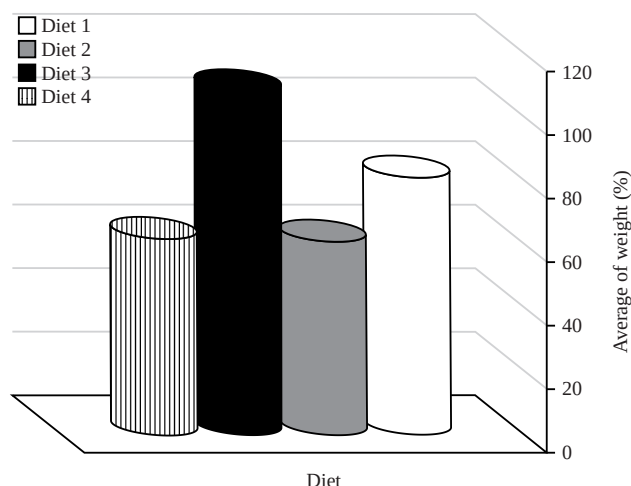


Fig. 2: Final weight (%) of fry fish fed different experimental diets

rainbow trout fry (*Oncorhynchus mykiss*). Several and Dernekba and Cel²⁶ stated that Nile tilapia (*Oreochromis niloticus*) had low growth and feed utilization due to high participation levels of particular oilseed meals.

When added to peanut meal and cottonseed meal (diet 4) in this study, sesame meal slowed the growth of fish, resulting in a bodyweight gain of $61.7 \pm 2.01\%$ as compared to $108.2 \pm 0.96\%$ with diet 2 (Table 3). As a result of this research, it is clear that sesame meal harms fish growth. This is most likely owing to exceptionally high tannin levels in sesame meals, which are increased by poor storage conditions. Tannin levels in sesame meals have been reported to exceed 20 g kg^{-1} , or 2%. Tannins are considered "nutritional inhibitors" by binding to consumed plant proteins. Studies^{27,28} found low tannin uptake in Nile tilapia (*Oreochromis niloticus*) fed tannin-rich diets.

Food conversion ratio (FCR) was defined as the amount of food consumed (kg) per kilogram of fish produced, when dietary protein levels increase, the feed conversion ratio decreases. Diet 3 had an FCR of 2.27 ± 0.65 as compared to 3.18 ± 0.44 , 3.63 ± 0.78 and 3.74 ± 0.78 , for diets (1, 4 and 2), respectively (Table 3). Fish fed diet 3 were better than the other diets and there was no significant effect ($p > 0.05$) on FCR among the four experimental diets fed by tilapia fish. Several studies argued that low food conversion ratio (FCR) value is an indicator of feed utilization efficiency of formulated feed. Poor performance in terms of feed conversion ratio for fish-fed diets that contain vegetable protein, refer to Protease inhibitors such as trypsin and chymotrypsin inhibitors and the diet contained less lysine than this fish needs. In the present study, high feed conversion ratios were observed in three groups. This reduction in the growth could be related, not only to dietary amino acid profile but also to the presence of ANFs. For African catfish (*Clarias gariepinus*) fed a sesame seed meal-based diet²⁹ conforms to this observation. Dongmeza *et al.*²⁸ reported that Nile tilapia (*Oreochromis niloticus*) had low growth and feed utilization due to high participation levels of various oilseed meals.

One of the nutritional aspects that are specifically important to be in the feed ingredient and directly determine the cost is protein⁹. For the cost, a relatively low price is still on the top of the list and sustainability is needed to be maintained³⁰. Based on the recent prices of the different ingredient feeds used in this study, it is evident that diet 3 contained PM and CSM is the most expensive diet having a price of $1.253 \text{ SDG kg}^{-1}$, followed by diet 4 consisting of PM, SM and CSM with a price of $1.065 \text{ SDG kg}^{-1}$. Diet 1 contain PM only having a price of $0.936 \text{ SDG kg}^{-1}$, while diet 2 consists of PM and SM being the least expensive diet having a price of $0.839 \text{ SDG kg}^{-1}$. The high prices of the diets were due to their contents of cottonseed meal which has an abnormally high price in the market (Table 4). On the other hand, when taking into consideration both diet cost and food conversion ratio (Table 5) it is clear that diet (3) is the best diet with the lowest feed cost per kilogram of fish produced ($2.84 \text{ SDG kg}^{-1} \text{ fish}$), followed by diet 1 ($2.98 \text{ SDG kg}^{-1} \text{ fish}$),

then diet 2 (3.14 SDG kg⁻¹ fish) and finally diet 4 (3.87 SDG kg⁻¹ fish). According to this study, the best diet was the one that contained both peanut meal and cottonseed meal as protein concentrates.

Due to enhanced dietary protein quality, this study found that using both peanut meal and cottonseed meal (diet 3) as protein concentrates in diets for Nile tilapia fry resulted in the best growing and food conversion. Diet 3 was also the most cost-effective in terms of feed per kilogram of fish produced. Sesame meal did not increase fish performance more than cottonseed meal in this study. If a fresh stock of sesame meal had been used, better results could have been attained.

CONCLUSION

This study discovers that utilizing both peanut meal and cottonseed meal (diet 3) as protein sources in diets for Nile tilapia fry (*Oreochromis niloticus*) can be favourable for the optimal development and food conversion ratio due to improved dietary protein quality. Diet 3 was also the most cost-effective in terms of feed per kilogram of fish produced. This research will assist the researcher in determining the best varieties of economical plant protein that may be used to replace all or part of the animal protein in the Nile tilapia fry diet.

SIGNIFICANCE STATEMENT

Diets for Nile tilapia fry should include other protein concentrates besides peanut meal, which is the most common concentrate used in Sudanese fish farms, for best performance. More studies should be conducted to formulate the lowest-priced diets from locally available feedstuffs for feeding various stages of Nile tilapia.

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