

Replacement of Fish Meal with Fermented Fish Offal Silage in the Diet of the Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

This study evaluated the potential of fermented fish offal silage as a protein source for the Nile tilapia (*Oreochromis niloticus*) fry. The silage was prepared from minced fish offal, 15% molasses, and 5% yogurt, and was stored for 35 days. During storage, the pH was stabilized at 4.30, maintaining good organoleptic characteristics, with a dark brown color and a pleasant malty aroma, showing no signs of spoilage. The dried silage contained $13.03 \pm 0.89\%$ protein, $41.9 \pm 0.35\%$ fat, $3.99 \pm 0.43\%$ ash, and $6.92 \pm 0.53\%$ moisture. Levels of non-protein nitrogen, total volatile base nitrogen, peroxide value, and free fatty acids were within acceptable limits. The silage was deemed microbially safe, with pathogens either below risk levels or absent. Three isoproteinous and isocaloric diets were formulated with 60, 80, and 100% of fermented fish offal silage replacing fish meal and were fed to *O. niloticus* fry for 27 days in a feeding trial. The best growth of fish occurred at the lowest inclusion level (60%) of fermented fish offal silage diet (FFO 60) yielding weight of 4.53g and length of 6.01cm. The length-weight relationship revealed a positive allometric growth. The 80% replacement of fish meal resulted the lowest mean weight gain ($4.12 \pm 0.11\text{g}$) compared to 60 and 100% replacements even less than the reference diet, i.e., $4.27 \pm 0.48\text{g}$, $4.23 \pm 0.25\text{g}$ and 4.50 ± 0.12 , respectively. The cost of the reference diet for 1kg of weight gain was the highest at Tk 102.80/kg, followed by the lowest inclusion level of fermented fish offal silage diet (FFO 60) at Tk 72.16/kg. This study suggested that fish offal silage can replace up to 60% of fish meal in the diet of *O. niloticus* fry without any retardation of growth, no significant changes in carcass protein, and at a reduced cost.

INTRODUCTION

Aquaculture is the fastest-growing food production sector in the world, contributing significantly to food security and economic development. The Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed fish species due to its adaptability, high growth rate, and ability to thrive in diverse environmental conditions (FAO, 2014). However, the rapid expansion of tilapia farming has led to increased demand for high-quality protein sources in fish feed, primarily fish meal, which is the most nutritionally balanced and digestible ingredient for aquafeeds (Tacon & Metian, 2015). The reliance on fish meal

presents several challenges, including rising costs, limited availability, and concerns over environmental sustainability (**Oliveira et al., 2020**). These challenges have driven researchers and feed manufacturers to explore alternative protein sources that are both cost-effective and nutritionally adequate (**Goda et al., 2007**).

One of the promising alternatives of fish meals is fermented fish offal silage (FFO) is a protein-rich product derived from the bioconversion of fish processing wastes. Fish offal, which includes viscera, gills, and other processing waste, constitutes a significant portion of fishery discards and is often underutilized or disposed of improperly, contributing to environmental pollution (**Fagbenro & Jauncey, 1994; Abdullah Al et al., 2020**). By converting fish offal into silage, valuable nutrients can be preserved and repurposed for aquafeed formulations, thus reducing waste and promoting circular economy principles (**Asefa et al., 2021**).

Fermentation, a widely used method for producing fish silage, enhances nutrient availability by breaking down proteins into peptides and free amino acids, which improves digestibility and feed efficiency (**Mahendrakar et al., 1995**). Unlike acid-based silage, which requires chemical preservatives, microbial fermentation naturally lowers pH through lactic acid production, inhibiting spoilage microorganisms and preserving essential nutrients (**Özyurt et al., 2017**). Additionally, fermentation reduces anti-nutritional factors and enhances the palatability of fish silage, making it a viable alternative protein source in fish diets (**Goda et al., 2007**).

Several studies have investigated the potential of fish silage as a replacement for fish meal in aquafeeds, with mixed results. **Fagbenro et al. (1994)** reported that partial replacement of fish meal with fish silage (30–75%) in tilapia diets resulted in comparable growth performance, while complete replacement led to reduced feed efficiency due to imbalanced amino acid profiles. Similarly, **El-Sayed (2004)** highlighted that fish silage can replace up to 75% of fish meal in tilapia diets, depending on factors such as species, diet formulation, and processing methods. In contrast, **Asefa et al. (2021)** recommended a much lower inclusion level (20%) for optimal growth, citing potential limitations in nutrient digestibility at higher inclusion levels. These variations suggest that the efficacy of fish silage as a fish meal replacer depends on multiple factors, including feed formulation, processing techniques, and species-specific dietary requirements (**Tacon & Metian, 2015**).

One of the main challenges of using fish silage in aquafeeds is its high moisture content, which can affect diet stability and pellet durability. However, drying techniques and the use of binding agents can improve the physical quality of fish silage-based feeds (**Oliveira et al., 2020**). Additionally, lipid oxidation is a concern in stored fish silage, but antioxidants such as butylated hydroxytoluene (BHT) can help mitigate this issue (**Mahendrakar et al., 1995**). These findings emphasize the need for further research to optimize fish silage formulations and inclusion levels in commercial aquafeeds.

This study investigated the effects of replacing fish meal with fermented fish offal silage at different inclusion levels (60, 80, and 100%) in the diet of *O. niloticus* fry. The main objectives of this study were to evaluate the impact on growth performance, feed utilization, carcass composition, and economic feasibility. By assessing FFO as a sustainable

protein source, this research aimed to contribute to reducing aquaculture's reliance on fish meal while promoting waste valorization in fish processing industries.

MATERIALS AND METHODS

1. Production of silage

Fresh fish offal, comprised mix of intestinal parts, gill, air bladder etc. (without scale and bones) were collected from the local markets of Chattogram and transported to the Nutrition and Feed Technology Laboratory (NFT) at the Institute of Marine Sciences, University of Chittagong. Styrofoam boxes with ice were used to collect and transport the raw material. In the lab, the raw materials were washed properly with tap water, kept in freezer at -20°C until use. The frozen raw material was chopped and minced using an electric mincer (Model: OMG-3250R). One portion of minced raw material (RM) was kept at ambient temperature (30°C) for 3 days and monitored pH and organoleptic changes before discarding.

Before fermentation, the minced raw material was cooked at 50°C for 20-30 minutes to inhibit hetero-fermentative bacteria (**Batista, 1987**) and cooled to ambient temperature. The cooked RM was then mixed with 15% molasses, 5% yogurt, 2.2g/ kg BHT (Butylated hydroxy toluene), and 0.25g/ kg PS (Potassium sorbate) (**Palkar, 2017**).

The silage mixture was placed in polythene bags, sealed, and stored in airtight containers for anaerobic fermentation over 5 days. The produced silage was heated at 80°C to halt enzymatic hydrolysis. After fermentation, the silage was stored at 30°C for 35 days. Treatments were labeled as FFO I, FFO II, and FFO III, with one part was stored for biochemical analysis and another part was dried and stored at -20°C for feeding trials.

2. Organoleptic changes and biochemical analysis

Organoleptic properties (liquefaction, color, smell, putrefaction) were observed from days 0 to 5 and on day 35. pH was measured daily (days 0 to 14) and at the end of storage (day 35) using a digital pH meter (HANNA HI98107). Proximate analyses (protein, fat, ash, moisture) were done following **AOAC (2016)** methods. Essential amino acids (EAAs) were determined using chromatographic techniques (**Snyder & Berger, 2016**). NPN, TVBN, and PV were measured on days 0, 5, 14, and 35, while free fatty acids (FFAs) were analyzed until day 14.

3. Microbial analysis

Total plate counts and tests for pathogenic bacteria (*E. coli*, *Vibrio* spp., *Salmonella* spp., and *Staphylococcus* spp.) were conducted on the raw material (day 0) and silage (day 5) using standard methods (BDS ISO 4833: 2009, ISO 16649-3: 2005, etc.) in the Fish and Quality Control Laboratory, Department of Fisheries, University of Chittagong

4. Diet formulation with silage

One reference and three experimental diets were formulated including 14 feed ingredients, replacing fish meal by 60, 80, and 100% silage. All diets were isoproteinous and isocaloric, meeting the nutritional requirements of *O. niloticus* fry. Proximate composition of the prepared diets was analyzed according to **AOAC (2016)** methods.

5. Experimental design

The feeding trial was carried out adopting hapa-in-pond system at Meridian Hatchery Ltd., Muhuri Project, Mirsarai, Chattogram. The duration of the experiment was 27 days from 17 September to 13 October 2021. A total of 12 hapa (3 experimental diets with three replicates = $3 \times 3 = 9$ and 1 reference diet with three replicates = $1 \times 3 = 3$; total = $9 + 3 = 12$) were used to conduct the feeding experiment. The dietary treatments were randomly assigned to the hapa to avoid bias. The dimension of each hapa was 1m^3 ($1\text{m} \times 1\text{m} \times 1\text{m}$) and made of synthetic net (nursery size mesh). The Nile tilapia fry (*O. niloticus*) of 26 days old (mean weight and length of 0.25 ± 0.02 g and 2.38 ± 0.04 cm) were stocked to 12 hapa at the rate of 120 fry/ m^2 . The fries were fed four times from 8 am to 5 pm at three hours intervals. The given amount of feed was adjusted based on the weight of fish, as obtained on every sample.

6. Water quality management

Water quality parameters (i.e., temperature, dissolved oxygen, pH, and ammonia) were recorded using an appropriate equipment. Partial change of water was performed once a week and aerations were provided at night and during high heat at midday.

7. Growth performance

Growth metrics, such as length-weight relationship was analyzed according to **Cren (1951)** and **Froese (2006)**. Mean weight gain (MWG), average daily weight gain (ADG), mean weight (MW) and mean length (ML), specific growth rate (SGR), condition factor (K) and survival rate (SR) were measured following methods by **Sedgwick (1979)**, **De Silva (1995)**, **Htun-Han (1978)**, and **Venkatachalam et al. (2018)**.

8. Feed utilization

Feed utilization was assessed through average feed intake (AFI), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein intake (PI), protein efficiency ratio (PER), protein productive value (PPV), and energy ratio (ER), based on methods described by **Nose (1971)** and **Castell and Tiews (1980)**.

9. Carcass composition analysis

Carcass composition (i.e., protein, fat, ash, and moisture) at stocking and trial end was determined using standard methods (**AOAC, 2016**).

10. Cost estimation

The cost per unit growth of the Nile tilapia fry was calculated by determining production costs of fish offal silage, experimental diets and reference diet at first. Then, this cost was used to calculate the cost of diets per kg weight gain.

11. Statistical analysis

Data were processed using MS Excel, SPSS v. 22, and Sigma Plot v. 12.5. Results were expressed as means $\pm$ SD. One-way ANOVA was used to identify significant differences among diets, followed by Tukey's test at $P < 0.05$.

RESULTS & DISCUSSION

1. pH during ensilation and storage

The pH of fish offal silage varied over the fermentation and storage period (Fig. 1). RM had an initial pH of 7.91, which dropped to 6.52 and 6.16 on days 1 and 2, before slightly increasing to 6.90 by day 3. In contrast, FFO showed a consistent decline in pH over time with an initial pH of 6.50, dropping to 5.50 by day 2 and 4.50 by day 4. However, pH showed slight fluctuations after day 6, ranging from 4.23 to 4.44, with a final pH of 4.30 on day 35. The observed decrease in pH indicates successful fermentation, as acidification is crucial for silage preservation. **Fagbenro and Jauncey (1994)**, who reported that an ideal pH for fish silage preservation is below 4.5, ensuring microbial stability and inhibiting spoilage by bacteria. The stabilization of pH after day 11 suggests that fermentation reached equilibrium, consistent with the findings of **Asefa *et al.* (2021)**, who observed a similar trend in acidified fish silages. Variations in pH of FFO during storage may be due to differences in buffering capacity and microbial activity, as previously observed by **Goda *et al.* (2007)**. Together all, our results confirm that fermentation effectively reduces pH, ensuring the stability and preservation of fish offal silage over time.

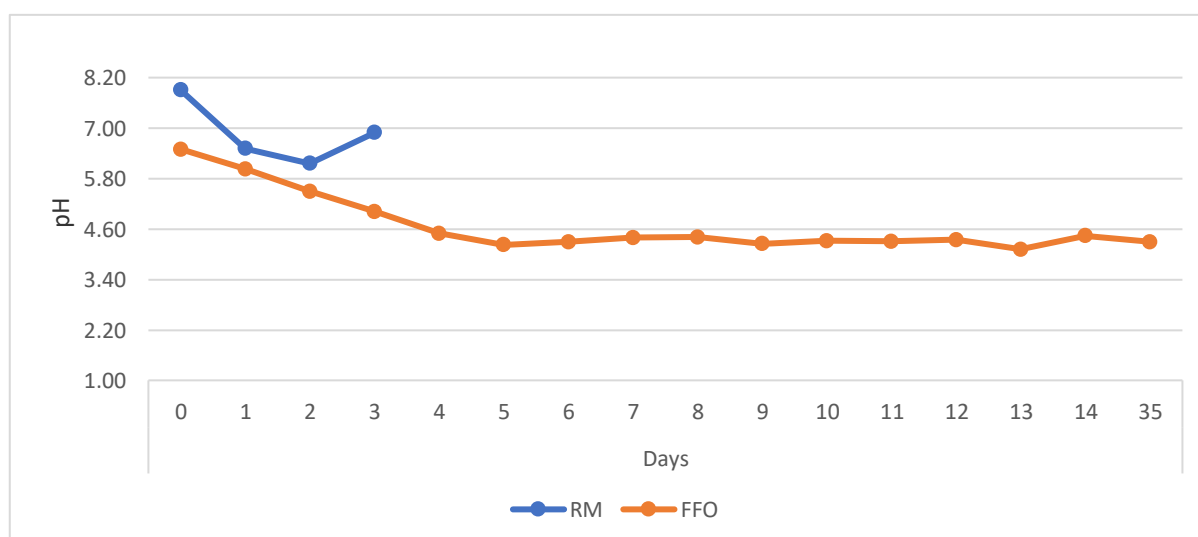


Fig. 1. The changes of pH for raw material (RM) and fermented fish offal silage (FFO) during ensilation and storage

2. Ensilation and organoleptic properties

In order to assess the behavior of the raw material toward fermentative agents during production and storage, some organoleptic parameters such as gradual changes in thickness, color and smell of both raw material and silage were observed. On day 0, the

ensilage mixture of fish offal was thick, light grey in color, and with raw fish smell. Even on day 2, the mixture remained unchanged. No changes in consistency or color were noted in untreated raw material after 3 days at room temperature (30°C) and was discarded. On the other hand, by day 5, the silage mixture was completely liquified with dark brown color, pleasant malty aroma, and contained an oil layer on top indicating successful ensilation (Table 1). Consistently, **Kung et al. (2018)**, who reported that well-fermented silage should have minimal odor due to lactic acid production. **Russel et al. (1991)**, following fermentation of 48h, fish offal changed greatly in appearance and physical properties i.e., the initial appearance of coarsely ground meat liquified with a clear layer of oil on top which is consistent with the current findings. In this research, at the end of 35 days, silage was found to retain same organoleptic characteristics as the initial condition and no putrefaction was observed.

Table 1. Changes in organoleptic properties of raw material and fish offal silage during ensilation and storage

Day	¹ RM	² FFO	Remarks
D0	Thick, light grey color, raw fish smell	--	No ensilation
D1	Same	Thick, light grey color, raw fish smell	Ensilage in progress
D2	Same	Same	Ensilage in progress
D3	Putrid smell *Discarded	Same	Ensilage in progress
D5	--	Liquefied, dark-brown color, pleasant malty aroma	Fermentation done
D35	--	- Same - No putrefaction or growth of mould	All silages retained organoleptic properties similar to that of the production phase.
Picture			

Legend: 1= Raw material, 2= Fermented fish offal silage.

3. Nutritional quality of silage

The RM contained $16.74 \pm 0.71\%$ protein, $35.74 \pm 0.91\%$ fat, $4.13 \pm 0.10\%$ ash, and $58.6 \pm 1.38\%$ moisture (Table 2). During fermentation, protein content decreased to $14.33 \pm 0.83\%$, likely due to the breakdown of protein into soluble peptides and amino acids. However, as fermentation progressed, the protein level increased to $15.32 \pm 0.88\%$ after

drying and further stable at $12.58 \pm 1.31\%$ after 35 days of storage, suggesting a slower release of protein through microbial activity.

Fat content showed an increasing trend from $42.32 \pm 1.49\%$ at production to $42.75 \pm 0.91\%$ after drying but slightly declined to $39.65 \pm 1.39\%$ by the end of storage (Table 2). This reduction could be attributed to lipid oxidation and microbial metabolism, as reported by **Asefa *et al.* (2021)**.

Ash content remained relatively stable, increased slightly from $4.27 \pm 0.87\%$ at production to $4.77 \pm 0.36\%$ after 35 days, possibly due to the de-solubilization of some minerals in the fermentation liquid.

Moisture content of the produced silage stable at $72.01 \pm 1.60\%$, while it was slightly lower at storage condition i.e., $61.67 \pm 1.56\%$. The moisture content was significantly dropped to $11.31 \pm 0.68\%$ after drying (Table 2). The dried silage remained sticky and could not be ground into powder, likely due to the presence of molasses and the absence of filler substances, as previously observed by **Fagbenro and Jauncey (1994)**.

In this study, the observed protein slightly decreased over time. **Goda *et al.* (2007)**, who reported that prolonged fermentation can improve protein solubility and availability by breaking down complex proteins into free amino acids. The reduction in protein and fat at the end of storage could be linked to lipid oxidation, which is a common challenge in fermented silages (**Oliveira *et al.*, 2020**). Additionally, moisture retention in fermented fish silage is known to contribute to its semi-liquid nature, which can impact handling and storage (**Mabesi *et al.*, 2022**).

Table 2. Proximate composition of raw material and fermented fish offal silage at different phases

Composition (%)	Raw material	Production	Dried	End of storage
Protein	16.74 ± 0.71	14.33 ± 0.83	15.32 ± 0.88	12.58 ± 1.31
Fat	35.74 ± 0.91	42.32 ± 1.49	42.75 ± 0.91	39.65 ± 1.39
Ash	4.13 ± 0.10	4.27 ± 0.87	3.99 ± 0.43	4.77 ± 0.36
Moisture	58.6 ± 1.38	72.01 ± 1.60	11.31 ± 0.68	61.67 ± 1.56

4. Essential amino acids composition

The composition of total essential amino acids (EAAs) was found to be 9.34% in raw fish offal, 9.46% in dried silage, and 3.23% after 35 days of storage (Table 3). The reduction in EAAs during storage may be attributed to the enzymatic breakdown of free amino acids. Lysine was the most abundant at 2.97%, while arginine was the least abundant at 0.35% in dried silage. **Tazim *et al.* (2021)** reported amino acids in relatively high concentrations, except for tryptophan in formic acid and fermented silages, which are comparable to the current findings. Additionally, they identified higher concentrations of lysine, leucine, isoleucine, and phenylalanine, which align closely with the present study (notably, phenylalanine was not measured). **Fagbenro and Jauncey (1994)** analyzed the amino acid compositions of fermented tilapia silage stored for 30 days, noting a significant decline (>8% difference) in tryptophan content within the first 7 days of fermentation,

followed by an additional decrease (up to 11%) after 30 days, with the loss stabilizing during prolonged storage of 180 days. The reduction of tryptophan could not be linked to the current analysis, as it was not measured. **James (1966)** suggested that the losses of amino acids in fermented fish silages are generally associated with their interaction with sugars present in unutilized molasses. Conversely, **Jonsson *et al.* (1983)** and **Gauthankar *et al.* (2021)** attributed the loss of amino acids to their use as a nitrogen source by certain microbes.

Table 3. Essential amino acids composition of raw material and fermented fish offal silage

¹ EAA	² RM (%)	³ FFO	
		Dried silage	Silage at D35
1. Arginine	0.50	0.35	0.12
2. Histidine	0.51	0.40	0.13
3. Isoleucine	2.31	1.65	0.57
4. Leucine	0.60	0.46	0.16
5. Lysine	4.15	2.97	1.03
6. Methionine	1.46	1.04	0.34
7. Threonine	2.55	1.81	0.63
8. Valine	1.02	0.78	0.25
Total EAAs	13.10	9.46	3.23

Legend:1= Essential Amino Acids, 2= Raw material, 3= Fermented fish offal silage.

5. Chemical quality of silage

The chemical quality of fish offal silage was assessed through non-protein nitrogen (NPN), total volatile basic nitrogen (TVBN), peroxide value (PV), and free fatty acid (FFA) levels during ensilation and storage (Table 4). NPN content increased from an initial 0.45% in raw material to 0.69% on day 5, indicating protein breakdown during early fermentation. However, by day 14, a significant decrease to 0.49% was observed, followed by a subsequent increase to 0.61% by day 35, suggesting continued microbial activity and protein hydrolysis. This trend is consistent with previous findings by **Mahendrakar *et al.* (1995)**, who reported similar NPN fluctuations during fish silage fermentation.

TVBN levels, an indicator of protein degradation and microbial activity, were initially measured at 13.5mg N/100 g in RM. It increased sharply to 22.5mg N/100g on day 5 before declining to 18mg N/100g on day 14. By day 35, TVBN rose again to 20mg N/100g, likely due to ongoing protein degradation. This pattern is aligned with **Disney (1978)**, who found similar TVBN trends in fish silage, with fluctuations influenced by fermentation conditions.

PV values, reflecting lipid oxidation, increased significantly from 0.45 meq/kg in RM to 19.59 meq/kg on day 5 and peaked at 20.21 meq/kg on day 14. By day 35, PV declined to 17.6 meq/kg, indicating some reduction in oxidative activity over time. These results are comparable to those reported by **Mahendrakar *et al.* (1995)**, who found that PV levels initially increased but later declined in silages without antioxidants. However, **Özyurt *et al.* (2017)** reported lower PV values (1.11–4.54 meq O₂/kg oil in Klunzinger's pony fish

silage and 1.76–3.39 meq O₂/kg oil in gibel carp silage), likely due to differences in raw materials and oil extraction methods. FFA content, initially high in RM (31.57%), showed an abrupt decline to 3.41% on day 5 due to enzymatic hydrolysis of lipids. It slightly increased to 3.83% by day 7 and remained stable until day 14. By day 35, FFA increased marginally to 4.34%, likely due to microbial lipase activity, as reported in a previous study (Özyurt *et al.*, 2017).

Table 4. Chemical quality of the fermented fish offal silage

Treatment	Day	¹ NPN (%)	² TVBN (mg N/100g)	³ PV (meq/kg)	⁴ FFA (%)
FFO	D0	0.45	13.50	0.45	31.57
	D5	0.57	24.00	6.44	8.06
	D14	0.45	21.50	15.59	6.34
	D35	0.49	15.00	12.21	6.02

Legend: FFO= Fermented fish offal silage, 1= Non-protein nitrogen, 2= Total volatile base nitrogen, 3= Peroxide value, 4= Free fatty acid.

6. Microbial safety

The total bacterial count of raw fish offal was 1.3×10^5 cfu/g and FFO reaching 1.7×10^5 cfu/g (Table 5). *E. coli* and *S. aureus* were found at 4.3 cfu/g and <10 cfu/g while other pathogens namely *Vibrio cholerae* and *V. parahaemolyticus*, *Salmonella* spp., and *Listeria monocytogenes* were absent. Özyurt *et al.* (2017) analyzed the microbial quality of fish silage of seabass (*Dicentrarchus labrax*) processing wastes using formic acid and fermentation method. In their investigation, *E. coli*, *Salmonella* spp., *Staph. aureus* and *Listeria* spp. were not detected in any silage samples, a result which agrees with current results to some extent. Though *E. coli* and *S. aureus* were found in this experiment, the counts were quite low or below risk level. Additionally, they reported initial TVC (total viable count) of seabass processing waste as 4.3 cfu/g, which was comparatively higher than FFO in this study.

Table 5. Total viable bacteria and pathogens in raw material and fermented fish offal silage

Microbial count	¹ RM	² FFO
1. Aerobic plate count (cfu)/g	1.3×10^5	1.7×10^5
2. <i>Escherichia coli</i> (MPN)/g	4.30	<0.30
3. <i>Vibrio cholerae</i> & <i>V. parahaemolyticus</i> (in 25g)	Absent	--
4. <i>Salmonella</i> spp. (in 25g)	Absent	Absent
5. <i>Staphylococcus aureus</i> (cfu)/g	<10	<10
6. <i>Listeria monocytogenes</i> (in 25g)	--	--

Legend: 1= raw material, 2= Fermented fish offal silage.

7. Experimental diets

Feed ingredients for the formulated reference and experimental diets are outlined in Table (6). All diets were of equal weight and the ingredients were either same or equivalent. Fish meal was the only animal protein source in the reference diet (RD); while, in experimental diets, fish meal was replaced with fermented fish offal silages at the rate of 60, 80, and 100%. The proximate compositions of the diets are shown in Table (7).

Table 6. Composition of reference diet and experimental diets replacing fish meal at different levels

Ingredient (kg)	¹ RD	Experimental diets		
		² FFO 60	³ FFO 80	⁴ FFO 100
Fish meal	200	80	40	0
Fish offal silage	0	120	160	200
Soybean meal	372	448	468	487
Rapeseed meal	91	62	23	5
Rice polish	80	0	0	0
Wheat flour	84	75	61	33
Corn	20	0	0	0
⁵ DORB	0	0	0	0
⁶ CGM	99	150	184	210
Vitamin premix	1	1	1	1
⁷ MCP	5	5	5	5
Vegetable oil	23	29	28	29
Fish oil	24	29	29	29
Potassium sorbate	0.8	0.8	0.8	0.8
⁸ BHT	0.2	0.2	0.2	0.2
Total	1000	1000	1000	1000

Legend: 1= Reference diet, 2,3,4= Fermented fish offal silage diets replaced fish meal at 60, 80, and 100% level; 5 = De-oiled rice bran, 6= Corn gluten meal, 7= Mono-calcium phosphate, 8= Butylated hydroxy toluene.

Table 7. Proximate compositions of reference and experimental diets

Nutrients (%)	¹ RD	² FFO 60	³ FFO 80	⁴ FFO 100
Protein	40.00	40.00	40.00	40.00
Fat	9.63	9.31	9.11	9.10
Fiber	3.67	3.17	2.87	2.77
Ash	8.42	6.63	6.05	5.57
⁵ NFE	3300	3300	3300	3300
⁶ DE (kcal/kg)	1.24	1.02	0.93	0.85
Ca	1.16	0.97	0.92	0.89
P	40.00	40.00	40.00	40.00

Legend: 1= Reference diet, 2,3,4 = Experimental diets replaced fish meal at 60, 80, and 100% level; 5= Nitrogen free extract, 6= Digestible energy.

8. Growth curve

Reference diet exhibited comparatively higher growth (weight 4.75g and length 6.16cm) than fermented dietary treatments, FFO 60 (weight 4.53g and length 6.01cm), FFO 100 (weight 4.47g and length 5.93cm), and FFO 80 (weight 4.37g and length 6.0cm), respectively, as shown in Fig. (2a, b). However, variations in growth based on weight and length between RD and all the experimental treatments were found insignificant ($P > 0.05$). The growth curve for weight showed a minimum value in the FFO 80 group (4.37g), while the lowest length was observed in the FFO 100 group (5.93cm). These findings contrast with the commonly accepted correlation between weight and length gain in fish, as noted by **Cavalheiro *et al.* (2021)**.

In this study, it is evident that the lower inclusion level of fish offal silage i.e., 60% substituting fish meal in experimental diets achieved better weight gain which agrees with **Asefa *et al.* (2021)**. They found 20% inclusion of fish silage in the diets of *O. niloticus* fries with better growth and recommended further study with different inclusion levels. **El- Sayed (2004)** in his review, highlighted the efficacy of fish silage as fish meal replacer, and indicated that 30 to 75% fish silage can be successfully incorporated in tilapia diet depending on fish species and size, silage source, and diet composition (**Fagbenro *et al.*, 1994; Fagbenro & Jauncey, 1994**). However, complete replacement of fish meal by fish silage (i.e., 100%) as dietary protein source reported to give poor growth in *O. niloticus* (**Hernandez, 1983; Adejumo, 1987**) which matches the present findings. Among the fermented fish offal treatments, however, no significant variations of growth were found except for SAFO 80 with significantly lower growth than the reference group.

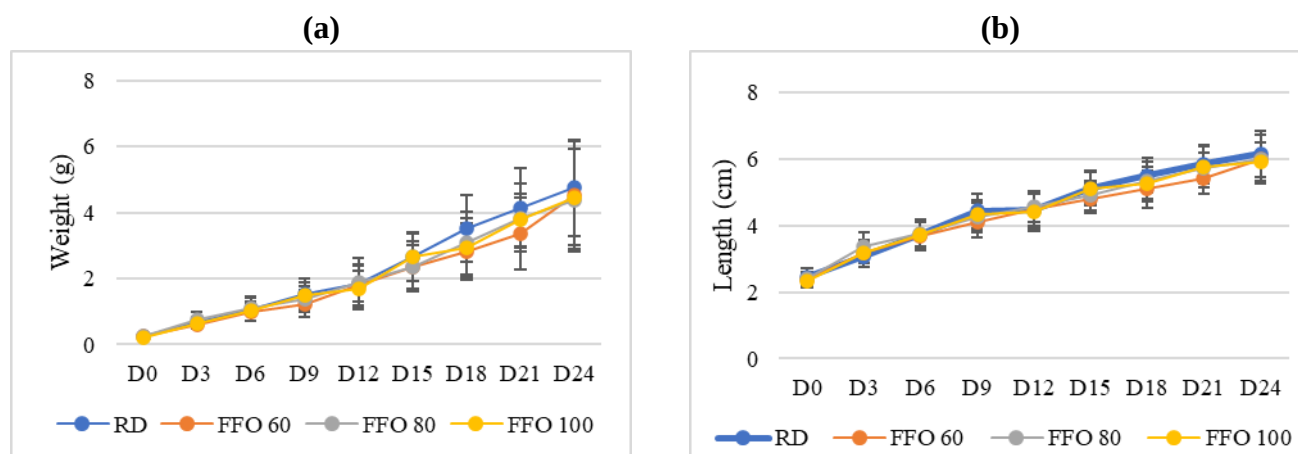


Fig. 2. Growth curve based on (a) weight and (b) length of *O. niloticus* fry fed experimental diets (FFO 60, FFO 80, FFO 100) and reference diet (RD)

9. Length-weight relationship

Length-weight relationship is an important tool to assess the growth pattern of fish, and it varies depending upon the condition of the aquatic environment (**Anani & Nunoo, 2016**). The scatter plot of $\text{Log } W = \text{Log } a + b \text{ Log } L$ provided Y axis intercept 'a' -1.788, -1.721, -1.746 and -1.739 with their corresponding slope 'b' 3.108, 3.009, 3.039 and 3.031 for

RD, FFO 60, FFO 80 and FFO 100, respectively (Fig. 3). In this study, the 'b' values for all fermented treatments, including the reference diet (RD), were slightly above 3, indicating that the growth of the experimental fish was positively allometric—reflecting optimal growth conditions. This aligns with the cube law described by **Cren (1951)**, and the regression coefficients are also consistent with the findings of **Anani and Nunoo (2016)**, who studied the cultured Nile tilapia. Some other researchers recorded the values of b 2.7–3.0 for *O. urolepis* and 2.7–3.2 in *T. zillii*, respectively, which are comparable with the findings of this study (**Imam et al., 2010; Nehemia et al., 2012**). According to **Raghavan et al. (2007)**, values of b above 3 are possible in a suitable environment such as stress-free condition. It should be noted that b can vary seasonally, even daily and between habitats (**Thulasitha & Sivashanthini, 2012**). A strong correlation between the weight and length of *O. niloticus* fry is established from the values of R^2 found as 0.984, 0.97, 0.978 and 0.979 for RD and the experimental treatments FFO 60, FFO 80 and FFO 100, respectively. The length-weight relationship of *O. niloticus* fry in all dietary treatments were found to be linear conforming to the general formula of length and weight of fishes.

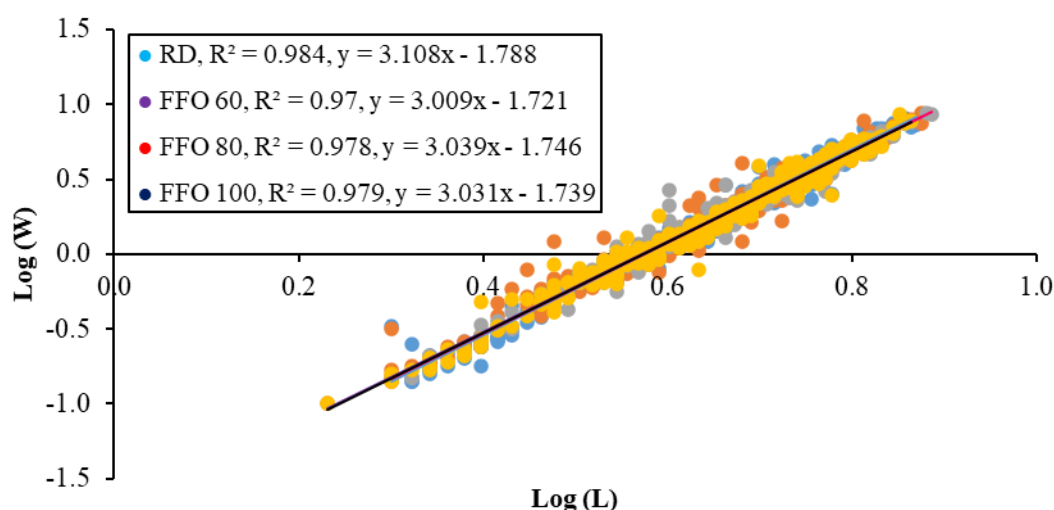


Fig. 3. Length-weight relationship of *O. niloticus* fry fed experimental diets (FFO 60, FFO 80, FFO 100) and reference diet (RD)

10. Growth response

The growth performance of *O. niloticus* fry varied across diets (Table 8). Initial weight (IW) of the fry did not significantly differ across treatments, ranging from 0.24 ± 0.02 g (FFO 100) to 0.26 ± 0.01 g (FFO 60). Final weight (FW) was highest in the RD group (4.75 ± 0.13 g) and lowest in FFO 80 (4.37 ± 0.11 g). Mean weight gain (MWG) followed a similar trend, with RD (4.50 ± 0.12 g) being significantly higher ($P < 0.05$) than FFO 80 (4.12 ± 0.11 g). Specific growth rate (SGR) was highest in RD ($12.35 \pm 0.02\%$ /day) and greater than FFO 60 ($11.93 \pm 0.56\%$ /day) and FFO 80 ($11.95 \pm 0.12\%$ /day), while FFO 100 ($12.27 \pm 0.45\%$ /day) was comparable to RD. Condition factor (K) and survival rate (SR) remained similar across treatments (Table 8).

Fagbenro *et al.* (1994), who reported that fish meals can be partially replaced with fish silage up to a certain level, while total replacement resulted in inferior growth compared to the control diet. This study also attributed the lower growth response to the absence of fish meals, which may have reduced diet palatability or appetite stimulation. Notably, **Fagbenro *et al.* (1994)** observed better growth at a higher level (75%) than in this study (60%). In contrast, **Asefa *et al.* (2021)** recommended a lower inclusion level of 20% fish silage for *O. niloticus* fry, reporting improved growth. These differences may be attributed to variations in culture conditions, fish size, silage preparation methods, and raw materials used. However, based on species, size, and diet composition, previous studies have suggested that fish silage can be successfully incorporated into tilapia feed at levels ranging from 30 to 75% (**Fagbenro *et al.*, 1994; Fagbenro & Jauncey, 1994**).

Table 8. Growth responses of *O. niloticus* fry fed experimental diets and reference diet

Treatment	¹ IW (g)	² FW (g)	³ MWG (g)	⁴ ADG (g/day)	⁵ SGR (%/day)	⁶ K (%)	⁷ SR (%)
⁸ RD	0.25 ± 0.01	4.75 ± 0.13	4.5 ± 0.12	0.19 ± 0.03	12.35 ± 0.02	2.03 ± 0.03	99.17 ± 0.68
⁹ FFO 60	0.26 ± 0.01	4.53 ± 0.47	4.27 ± 0.48	0.18 ± 0.02	11.93 ± 0.56	2.08 ± 0.05	98.33 ± 1.36
¹⁰ FFO 80	0.25 ± 0.01	4.37 ± 0.11	4.12 ± 0.11	0.19 ± 0.05	11.95 ± 0.12	2.02 ± 0.02	97.5 ± 0.68
¹¹ FFO 100	0.24 ± 0.02	4.47 ± 0.24	4.23 ± 0.25	0.18 ± 0.01	12.27 ± 0.45	2.1 ± 0.03	98.54 ± 0.16

Legend: 1= Initial weight, 2=Final weight, 3=Mean weight gain, 4=Average daily weight gain, 5=Specific growth rate, 6= Condition factor, 7= Survival rate, 8= Reference diet; 9, 10, 11= experimental diets replaced fish meal at 60, 80, and 100% level.

11. Feed utilization efficiency

The feed utilization efficiency varied across treatments, with the FFO 60 diet showing the best performance. It had the lowest feed conversion ratio (FCR) (1.18 ± 0.05) and highest feed conversion efficiency (FCE) ($85.01 \pm 3.36\%$), significantly ($P < 0.05$) better than FFO 80 (FCE: $74.93 \pm 4.59\%$). Feed intake (FI) was the highest in the RD group (5.78 ± 0.15 g/ fish) and lowest in FFO 60 (5.06 ± 0.76 g/ fish) (Table 9). The improved feed efficiency in FFO 60 suggests that partial replacement of fish meals with fermented fish offal silage enhances nutrient digestibility, supporting findings by **Fagbenro *et al.* (1994)**, who reported that fish silage can effectively replace fish meal up to a certain level without compromising feed efficiency. This could be attributed to fermentation, which improves protein digestibility and amino acid availability, as previously observed by **Asefa *et al.* (2021)**.

However, at higher inclusion levels (FFO 80 and FFO 100), feed efficiency declined, with FFO 80 exhibiting the lowest FCE ($74.93 \pm 4.59\%$) and the highest FCR (1.34 ± 0.08) (Table 9). This may be due to imbalanced nutrient composition or reduced palatability, as reported by **Fagbenro and Jauncey (1994)**, where excessive replacement of fish meal with fish silage led to reduced feed intake and growth. The lower FI in these groups suggests potential acceptance issues, possibly linked to changes in diet texture or the presence of anti-nutritional factors (**Goda *et al.*, 2007**). Despite this, protein efficiency ratio (PER) and protein productive value (PPV) remained comparable among diets, with PER ranging from

1.59 ± 0.17 (FFO 100) to 1.74 ± 0.07 (FFO 60), indicating that fish silage can serve as an effective protein source at optimal levels (Table 9). Overall, the results suggest that 60% inclusion of fermented fish offal silage is the most effective level for improving feed utilization in *O. niloticus* fry, while higher levels may reduce diet efficiency.

Table 9. Feed utilization efficiency of *O. niloticus* fry fed experimental diets and reference diet

Treatment	¹ FI (g/fish)	² FCR	³ FCE (%)	⁴ PI (g/fish)	⁵ PER	⁶ PPV (%)	⁷ ER
⁸ RD	5.78 ± 0.15	1.29 ± 0.05	77.85 ± 0.08	2.63 ± 0.07	1.72 ± 0.03	1.29 ± 0.06	1.09 ± 0.01
⁹ FFO 60	5.06 ± 0.76	1.18 ± 0.05	85.01 ± 3.36	2.47 ± 0.38	1.74 ± 0.07	1.05 ± 0.04	1.06 ± 0.04
¹⁰ FFO 80	5.51 ± 0.19	1.34 ± 0.08	74.93 ± 4.59	2.41 ± 0.08	1.71 ± 0.1	1.04 ± 0.06	1.17 ± 0.07
¹¹ FFO 100	5.39 ± 0.26	1.28 ± 0.14	78.89 ± 8.46	2.68 ± 0.13	1.59 ± 0.17	1.00 ± 0.11	1.08 ± 0.13

Legend: 1=Feed intake, 2=Feed conversion ratio, 3=Feed conversion efficiency, 4=Protein intake, 5=Protein efficiency ratio, 6=Protein productive value, 7=Energy ratio, 8=Reference diet, 9,10,11= experimental diets replaced fish meal at 60,80, and 100% level.

12. Carcass composition

The carcass composition of *O. niloticus* fry varied among treatments (Table 10). Protein content was at its highest value in the RD group (75.58 ± 0.62%) and the lowest in FFO 60 (60.7 ± 0.45%), while FFO 100 (62.69 ± 0.37%) showed a slight improvement compared to other FFO groups. Fat content increased with higher fish offal silage inclusion, with FFO 100 exhibiting the highest fat accumulation (27.87 ± 0.36%) compared to RD (22.58 ± 0.35%). Ash content ranged from 12.56 ± 0.09% (FFO 60) to 14.39 ± 0.11% (FFO 80), while moisture content remained relatively stable across treatments, with the lowest observed in FFO 60 (77.37 ± 0.11%) and the highest in RD (78.57 ± 0.09%). Crude fiber content showed minor variation, with no significant differences among diets (Table 10).

The decrease in carcass protein content in FFO-fed fish, particularly in FFO 60, may be attributed to differences in protein digestibility and amino acid balance compared to the RD diet, which contained fish meal. **Fagbenro et al. (1994)** reported that while fish silage is a viable protein source, its digestibility can be influenced by processing methods and inclusion levels. The increase in carcass fat with higher FFO inclusion suggests enhanced lipid deposition, likely due to the high oil content in fish offal silage, as observed in previous studies (**Asefa et al., 2021**). Similarly, **Goda et al. (2007)** found that replacing fish meal with fish silage could increase body fat accumulation due to variations in lipid metabolism.

The variations in ash content may reflect differences in mineral composition among diets, as previously reported by **Fagbenro and Jauncey (1994)**. While the RD group had the highest carcass protein content, FFO 100 showed a balance between protein retention and lipid deposition, suggesting that higher inclusion levels of fermented fish offal silage can enhance energy storage without severely compromising protein composition. However, optimizing the inclusion level is essential to prevent excessive fat accumulation, which could impact fillet quality and consumer preference.

Table 10. Carcass composition of *O. niloticus* fry fed experimental diets and reference diet

Treatment	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Moisture (%)
Initial content	60 ± 0.43	7.22 ± 0.61	20 ± 0.14	0.36 ± 0.04	81.22 ± 0.18
¹ RD	75.58 ± 0.62	22.58 ± 0.35	13.45 ± 0.16	0.30 ± 0.10	78.57 ± 0.09
² FFO 60	60.70 ± 0.45	18.80 ± 0.27	12.56 ± 0.09	0.41 ± 0.09	77.37 ± 0.11
³ FFO 80	60.62 ± 0.52	24.62 ± 0.21	14.39 ± 0.11	0.36 ± 0.08	77.70 ± 0.11
⁴ FFO 100	62.69 ± 0.37	27.87 ± 0.36	13.72 ± 0.14	0.27 ± 0.06	78.16 ± 0.09

Legend:1= Reference diet; 2,3,4= Fermented fish offal silage diets replaced fish meal at 60,80, and 100% level.

13. Cost per unit growth

The economic viability of using silage substituting fish meal is a vital issue. For 1kg weight gain of *O. niloticus* fry, the cost of reference diet was the highest (Taka 102.80/kg), followed by FFO 80 (Taka 78.96/kg), FFO 100 (Taka 72.80 /kg), and FFO 60 (Taka 72.16/kg), respectively (Fig. 4). It indicates that cost wise diet FFO 60 was the lowest while yielding growth equivalent to RD, highlighting the efficiency of lower-level dietary inclusion of silage. This result agrees with **El-Hakim *et al.* (2007)**, who reported highest cost/kg gain for control diet, however, they obtained the highest cost/kg gain for the lowest inclusion (50%) of fish silage which differs from the present finding.

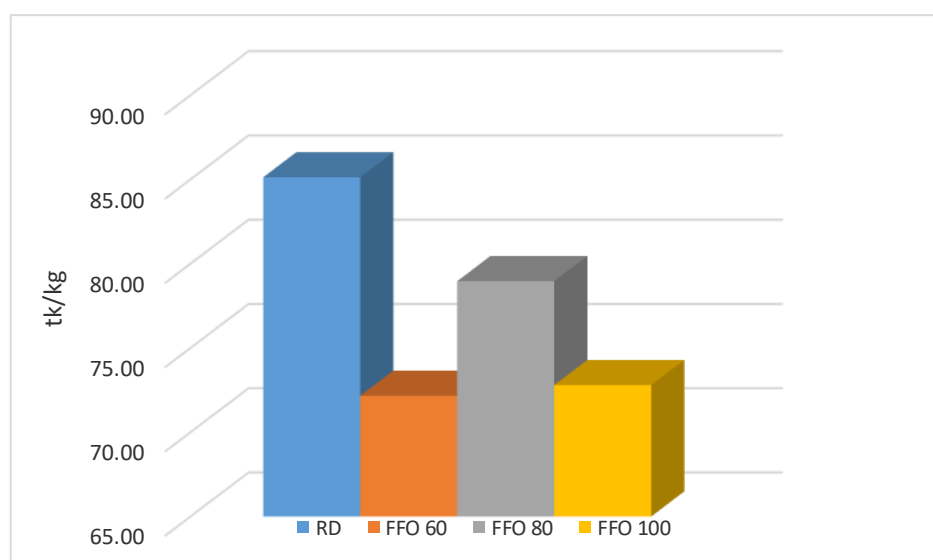


Fig. 4. Cost per unit growth of *O. niloticus* fry fed experimental diets (FFO 60, FFO 80, FFO 100) and reference diet (RD)

CONCLUSION

In this study, we found the contents of protein, fat and moisture in the fermented fish offal silage were comparably higher than reference diet. Furthermore, the levels of non-protein nitrogen, total volatile base nitrogen, peroxide value, and free fatty acids were within acceptable limits. Importantly, the silage was microbially safe and free from pathogens either below risk levels or absent which shows the potential alternative feed resource for *O. niloticus*, as it demonstrated comparable growth at a reduced cost substituting fish meal. However, further in-depth studies are recommended to justify and validate this finding with different fish species. It is also recommended to conduct long-time experiment to observe notable changes of gut microbiomes and fish health.

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DECLARATION OF INTEREST

There is no conflict of interest regarding financial, personal or other relationships with other people or organizations that could inappropriately influence this work.

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