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Effect of replacing marine protein with hydrolyzed feather meal on growth, apparent digestibility and body composition of juvenile tilapias; *Oreochromis mossambicus* (peters, 1852)

Jacob Abwao, Musa Safina, Robert Ondiba, Erick Ogello and Kevin Obiero

Abstract
The effects of substituting hydrolyzed feather meal for fresh water shrimp meal on growth, apparent digestibility and body composition in tilapia *Oreochromis mossambicus* were evaluated under laboratory condition. Five hundred fish were distributed in a completely randomized design with five treatments in quadruplicates with 25 fish (average weight 3.42±1.02g) per tank. The fish were fed isoproteinous (give the % protein level here) diet with increasing inclusion levels of hydrolyzed feather meal (HFM) (0%, 4%, 8%, 10% and 12%) designated as HFM0, HFM4, HFM8, HFM10 and HFM12 respectively. All the fish were fed at *ad libitum* for 30 days. Results indicated that fish fed the diet containing 10% inclusion level of HFM exhibited a significantly higher growth and nutritional parameters ($P<0.05$) in terms of mean final weight (8.05± 2.56), specific growth rate (3.67±0.29 g), food conversion ratio (1.97±0.11 g) and mean weight gain (4.9±0.33), compared to the other diets. Final body composition was influenced significantly by increasing the level of HFM through decreasing carcass moisture and lipids. Diet containing 12% HFM had significantly lower protein (11.75±0.05%) and ash (8.43±0.51) compared to diet HFM0. The study recommends at most 10% substitution of HFM for FSM for *O. mossambicus* culture under laboratory conditions.

Keywords: Feather meal, *O. mossambicus*, growth, digestibility

1. Introduction
Fish feed take up between 40-60% of the fish farm's production costs and is a major constraint to fish farming in resource poor regions [1]. Protein usually is the most expensive nutrient and its level and quality determine the cost of fish feeds. The majority of fish feeds contains fish or shrimp meal as the main sources of dietary animal protein. Major source of fishmeal being the ever diminishing capture fishery, the sustainability of the aquaculture sector is questioned. The sustainability of the aquaculture industry cannot be achieved unless progressive reduction of wild fish inputs into fish feed is addressed [2]. Consequently, the need for alternative dietary animal protein source to reduce cost cannot be overemphasized

The key animal protein sources in formulated fish feeds in Kenya are the *dagaa* (*R. argentea)* and fresh water shrimp (FWS) *Caridina nilotica (Roux).* However, dagaa is used for human consumption while the supply of fresh water shrimp is not reliable since it is low in supply during the *dagaa* closure seasons in Lake Victoria. The cost of transporting these raw materials to other areas is very high considering that many fish farms are located over 1000 kilometers from Lake Victoria. This has made the cost of these ingredients very high and perhaps for this reason only two companies in Kenya have met the standard protein requirement for farmed tilapia [3]. Therefore, the replacement of FSM by cheaper and available animal protein feedstuffs is likely to contribute to reduce the costs of *Oreochromis mossambicus* feeds.

Previous research have indicated that feather meal contains high protein level [2] hence could be incorporated in fish feed. Hydrolyzed feather meal (HFM) is a product from poultry feathers and has been recommended by many nutritional experts as a possible replacement for the more expensive fish meal (FM) and shrimp meal [4].

This is because of its high protein level, commonly in the range of 70- 82%, high lipid level in the range of (8.3-15%) and low fiber (0.68%) [5] Hydrolyzed feather meal is deficient in lysine and methionine but is adequate in cystine and arginine which are important in tilapia nutrition [6]. Despite proven applicability of HFM in aquaculture it has not been incorporated as an ingredient in tilapia diet in Kenya. This is perhaps due to lack of knowledge on available hydrolyzing process, its effects on apparent digestibility and its impact on growth and survival of tilapia. The current study was conducted to assess the effects of replacing marine protein with hydrolyzed feather meal on growth, apparent digestibility and body composition of juvenile tilapias; *Oreochromis mossambicus*

2. Materials and Methods

2.1 Experimental site

The experiment was carried out for a period of 30 days at the Verid laboratory in Saudarkrokur, Iceland between January and February 2016. The protein and fat proximate analysis was done in Matis laboratories located in Reykjavik following the methods described in (AOAC, 1995) [7].

2.2 Experimental diets

The different ingredients were chosen in consideration of their similarities to the ingredients commonly used as tilapia feeds in Kenya. Shrimp meal, rapeseed meal, soya meal, fish meal, yttrium oxide and the premix was sourced from Laxa feed mill Ltd, Iceland, wheat-bran from Lifland Ltd, while wheat and plant oil bought from the local stores in Saudarkrokur.

Poultry feather was procured from ISfugl harvesting factory and transported to the MATIS laboratory located in Reykjavik. The feather was washed in running tap water and pressure cooked in an autoclave at 220 Kpa at 121ºC for 35 minutes. The hydrolyzed feather was then dried by spreading a thin layer in trays for 24 hours at 30ºC. The feather was then blended and oven dried at 75ºC for 12 hours and milled to make the meal.

Winmix software was used to derive the formula for the test diets as provided in Table 3. Five isoprotein (36% CP) diets (HFM0, HFM4, HFM8, HFM10 and HFM12) were formulated with increasing inclusion levels of feather meal partially replacing Shrimp shell meal (SSM). The inclusion level of fish meal and soya meal was kept constant but inclusion ratio of other ingredients was varying for keeping good amino acid balance in the diets for tilapia.

All the ingredients were ground into fine powder using a laboratory milling machine and mixed as per the formulation for each treatment until homogenous. Water was added to the mixture to produce dough and pelletized into 1.5mm pellets using laboratory pelletizer then oven dried for 24 hours at 75 ºC.

Table 1: Formulation of ingredients composition in the experimental diets (g/100g)

Ingredient	HFM 0	HFM 4	HFM 8	HFM 10	HFM 12
Fish meal	7	7	7	7	7
Shrimp shell meal	60	49	39	16	0
Hydrolyzed feather meal	0	4	8	10	12
Soya meal	10	10	10	10	10
Rapeseed meal	0	0	0	25	43
Wheat Bran	8	18	19	0	0
Fish oil	3	3	7	0	1
Wheat	10	8	8	30	25
Laxa premix	1	1	1	1	1
Yttrium oxide	1	1	1	1	1
Total	100	100	100	100	100
Estimated composition (g/kg)					
Crude protein	360.0	360.0	360.0	360.0	360.0
Crude fat	60.0	60.0	100.0	60.0	95.4
Crude ash	197.4	166.7	141.3	91.3	55.7
Crude fiber	17.9	29.2	31.2	45.5	68.0
NFE – fiber**	266.1	284.7	271.8	333.1	312.9
Dry Matter	900.9	901.3	904.6	889.6	892.3
Calculated gross energy(MJ/kg) in DM*	16.3	16.9	18.2	18.2	19.7

*Gross energy value is calculated according to gross energy constants in nutrients: fat= 39,5MJ/kg; protein= 23,6MJ/kg; NFE= 17,3MJ/kg.

**The NFE values are calculated estimates of CHO with fibers excluded.

Table 2: Estimated Amino acid composition (%) of the ingredients.

Amino acid (%)	HFM0	HFM4	HFM8	HFM10	HFM12
Lysine	1.8	1.7	1.6	1.6	1.6
Methionine	0.6	0.6	0.6	0.6	0.7
Arginine	2.2	2.8	3.0	2.1	2.2
Isoleucine	1.7	2.1	2.1	1.6	1.0
Histidine	0.9	1.1	1.1	0.8	0.9
Threonine	1.5	2.0	1.9	1.4	1.5
Phenylalanine	2.0	2.4	2.4	1.7	1.7
Tryptophane	0.9	1.3	1.3	0.7	0.8
Leucine	2.4	2.6	2.7	2.7	1.9
Valine	2.0	2.5	2.7	2.1	2.0
Met+Cyst	1.0	1.1	1.2	1.4	1.5

2.3 Proximate Analysis

The chemical composition of experimental diets and feces samples were determined in triplicates based on the methods of (AOAC, 1995) [7]. Protein was analyzed by micro-Kjeldahl method where the sample was digested in sulphuric acid then put into a distillation unit, 2400 Kjeltec auto sampler system. The acid solution was made alkaline by NaOH and ammonia distilled into boric acid and titrated with H_2SO_4.The nitrogen was multiplied by a factor of 6.25 to obtain the crude protein content of the sample. Crude fat was extracted by soxhlet method by boiling samples in petroleum ether at temperature range of 40-60ºC. Moisture was analyzed by drying 2 g of diet samples in an oven at 105 ºC for 4 hours, cooled in a desiccator and reweighed. The moisture content was calculated as:

$$\text{Moisture content, \%} = \frac{\text{Sample weight (g)} - \text{Dry sample weight (g)}}{\text{Sample weight}} \times 100$$

Ash content of the diets were analyzed by burning 2g samples of each diet in a muffle furnace (Griffin and George ltd) at a temperature of 550 ºC for 4 hours then cooled in a desiccator and reweighed. Ash content was calculated as:

$$\text{Ash, \%} = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \times 100$$

Gross energy of the diets and feces were determined with the help of oxygen bomb calorimeter (IKA C 200 model). 0.5g of dried sample was put into a crucible and then a cotton string was tied to connect the firing wire and the food sample in the crucible. The calorimeter vessel was filled up with oxygen and placed into the water jacket filled with water of 25 ºC. The Gross energy of the diet samples and the feces was recorded after 13 minutes, after detecting the heat created in total combustion of the sample.

2.4 Experimental Design

Tilapia *O. mossambicus* mixed sex juveniles were obtained from a private fish farm south of Reykjavik and acclimatized at the Verid Laboratory for 14 days before commencement of the experiment. During acclimation they were fed a commercial diet (40% crude protein). 25 juveniles of average weight 3.4±0.01 g and length 5.84±0.03 g were randomly stocked in 20 buckets, each of capacity 17 liters and supplied with aerated fresh water (flow rate 1 liter min^{-1}). Five isoprotein (36% CP) *diets* were fed to the fry in quadruplicates to satiation for 30 days through an automatic feeder set to dispense the feeds every 10 minutes for 25 seconds, during constant light period (24L: 0D). Water temperature was maintained at 26.4 ºC ±0.67. The estimated ingredient and amino acid composition of the diet is shown in Table 2 and 3 respectively.

2.5 Determination of growth performance

The weight and length of the tilapia fingerlings were recorded at the commencement of the experiment and at the end. The specific growth rate (SGR), condition factor (K-Factor), feed conversion ratio (FCR) and mean weight gain (MWG) were used as the growth parameters and were calculated using the formula:

- Mean weight gain (g) = (mean final weight – mean initial weight)
- Specific growth rate (SGR); %/day $=100\times \ln(W_2) - \ln(W_1)/\Delta T$. Where W1 and W_2 are the initial and final body mass and ΔT is the time between measurements.

$$\text{Survival} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

- Condition factor (CF), $K= 100W/L^3$. Where, K is the condition factor, L is the total length of fish in cm while W is the weight of fish in grams.
- FCR = net feed intake / increase in body mass

2.6 *In vivo* digestibility evaluation of ingredients.

Fecal collection began seven days after fish had begun feeding experimental diet. Feces were collected from each experimental tank every morning by siphoning through a 100µm mesh material. The Feces were dried for 4 hours in an oven set at 50 ºC then frozen at -26 ºC [8]. The Feces samples from each diet treatment were pooled together in the course of the experimental period until sufficient quantity was obtained for digestibility determination.

Apparent digestibility coefficient of each diet was calculated thus:

ADC (%) = 100-[100($F/D\times YO_d / YO_f$)], where; ADC is the apparent digestibility, F is the percent of nutrient or energy in the feaces, D is the percent of nutrient or energy in the diet, YO is the percent of yttrium oxide in the diet while YO_f is the percent of yttrium oxide in the feaces [8].

2.7 Evaluation of degree of hydrolysis of proteins in the diets

The degree of hydrolysis was carried out in two steps: 0.1g of the sample of each diet was dissolved in 10ml of distilled water and pH was adjusted to 2.0 using 2N hydrochloric acid (HCl). 0.0029g of pepsin was added to the mixture and shaken in an incubator for 1hour at 37 ºC. The pH of the mixture was again adjusted to 5.3 using $NaHCO_3$ and finally to 7.5 using 2N NaOH. In the mixture was added 0.004g of pancreatic enzyme and shaken in an incubator for 2hours at 37 ºC. The digestion was terminated by submerging the samples in boiling water for 10 minutes. The samples were kept in the refrigerator until determination of the DH. The degree of hydrolysis assay was determined by O-phthaldialdehyde (OPA) method as outlined in the procedure by (Nielsen, 2001). (OPA) reagent was prepared by dissolving 1.905g of di-Na-tetraborate decahydrate and 50mg of SDS (Na-Dodecyl-sulfate) in 35ml of distilled water and stirred until completely dissolved before adding 40mg of OPA dissolved in 1ml of ethanol and 44mg of DDT(Dithiothreitol 99%) dissolved in 50ml of Distilled water. A standard solution was also prepared by dissolving 5mg of serine in 50 ml of water and adding 30mls of OPA reagent. A blank solution was prepared deionized water using the same procedure as the standard. 30µl of the sample from enzymatic digestion was added into the microplate and mixed with the same quantity of OPA reagent and allowed to stand for two minutes before spectrophotometer reading performed at 340nm. The calculation for DH was determined according to the formula of (Nielsen, 2001 [9].

2.8 Evaluation of carcass composition

Samples of 10 fish were taken from each treatment at the beginning and the end of the study to evaluate the initial and final proximate body composition respectively. The Samples were ground using a blender. Each content was put in plate and placed inside FOSS scan Near Infrared spectrophotometer

(Foss Hillerod, Denmark). The parameters analyzed for included: moisture, fat, protein and ash.

2.9 Statistical Analysis
Statistical analyses was done using Sigmaplot version 13 programme. Shapiro-Wilk test indicated no deviation from normality (P>0.05) for replicate SGR, FCR, CF and survival values. One-way analysis of variance was used to test for significant different at α=0.05 between the means of the treatments. The results were considered significantly different at p<0.05 and where there was a significant difference, Tukey multiple comparison test was used to compare the variance amongst the means.

3. Results
3.1 Proximate analysis of the feed
Proximate analysis of the diet treatments are shown in Table 3. HFM was also analyzed and the biochemical composition was; 3.6±0.1 and 1.2±0.1% for moisture and ash respectively while protein and lipid were 72.8 and 18.1% respectively. The analyzed protein content in the diets was in general below the approximated 36% CP level, with minimal fluctuations between diets. The lipid level did fluctuate more from the approximated 6% CF level, where the HMF0 diet had lowest value (4.5%) but HFM12 the highest value (9.3%). The analyzed lipid level of HFM was higher than expected, but that fact does not explain the whole variance between diet types. The ash content varied significantly (p<0.05) between diets, in the range of 20.2%-4.6. The calculated content of Nitrogen free extracts (fibers + other carbohydrates) is high in general and in the range of 37.3-48.2%. The HMF10 and HMF-12 have the highest NFE value. The inclusion of wheat is high in these two diets. The calculated gross energy content is reflected in measured GE content, but with some aberrance in diet HFM0 and HFM4.

Table 3: Proximate composition of the diets (% as fed basis).

Ingredient (%)	HFM 0	HFM 4	HFM 8	HFM 10	HFM 12	HFM
Protein	32.9	35.2	33.8	34.9	34.4	72.8
Lipid	4.5±0.8	6.1±0.8	7.3±0.8	5.6±0.8	9.3±0.8	18.1±0.8
Ash	20.2 ±6.9^{a}	17.9±2.0^{a}	15.3±0.1^{a}	9.0±0.2^{b}	4.6±0.2^{b}	1.6±0.6
Moisture	1.8 ±0.8^{a}	3.5±0.3^{b}	3.4±0.1^{b}	2.3±0.2^{b}	3.6±0.6^{b}	4.4±0.4
NFE*	40.6	37.3	40.2	48.2	48.1	40.6
GE-calculated**	16.3	16.6	17.2	18.4	19.4	23.8
Gross Energy (KJg^{-1}) measured	13.70±0.11^{a}	15.42±0.03^{b}	17.05±0.04^{ab}	18.13±0.03^{c}	19.13±0.10^{bc}	23. 7±0.13

*Nitrogen free extracts (NFE): calculated= 100-(%CP+CF+%ash+%moisture)

**Gross energy values in dry matter (DM) are calculated according to gross energy constants in nutrients: fat= 39.5MJ/kg; protein= 23.6MJ/kg; NFE= 17.3MJ/kg.

Effect of hydrolyzed feather meal on growth and survival of *O. mossambicus.*

Data on fish growth performance fed increasing inclusion levels of hydrolyzed feather meal during the 30 days experimental period are presented in table 4. The initial weight of the fish did not differ significantly (P>0.05). There were significant differences in the final mean weight, SGR and mean weight gain of the fish amongst the dietary treatments (P<0.05). Fish fed diet containing 10% and 12% hydrolyzed feather meal (HFM10 and HFM12) exhibited significantly higher final mean weight (8.05± 2.56 and 7.61±2.14 respectively) and specific growth rate (3.67±0.57 and 3.36±0.14) respectively, (P<0.05) compared with those fed diets HFM0, HFM4 and HFM8. The groups fed diet HFM10 and HFM12 (P=0.697) were not significantly different in terms of SGR. Fish fed diet HFM0, HFM4 and HFM8 showed similar response in SGR and final mean weight (FMW) (P>0.05). Mean weight gain increased with increasing levels of HFM from 3.6±0.22g for diet HFM0 and HFM8 to 4.9±1.18 g for diet HFM10.

FCR was significantly lower (P<0.05) in diet HFM10 and HFM12 than the other diets. FCR was affected by increasing levels of HFM. The diet containing 10% and 12% HFM (HFM10 and HFM12) had a significantly lower FCR (P<0.05), as shown in figure 4. Survival rate was not significantly affected by the dietary treatments (P>0.05). In all the treatments, survival was above 75%.

Table 4: Growth performance, survival and condition factor of *O. mossambicus* fed diets with increasing inclusion levels of hydrolyzed feather meal (Mean ± SEM).

Parameter	HFM 0	HFM 4	HFM 8	HFM 10	HFM 12	P-Value
Number of fish stocked	100 (4 x 25)	100 (4 x 25)	100 (4 x 25)	100 (4 x 25)	100 (4 x 25)	P = 1.000
Initial length (cm fish^{-1})	5.9±0.61^{a}	5.8±0.63^{a}	5.8±0.58^{a}	5.8±0.61^{a}	5.8±0.57^{a}	P = 0.791
Final length (cm fish^{-1})	7.26±0.68	7.21±0.65	7.22±0.65	7.51±0.79	7.29±0.74	P = 0.008
Initial mean wt.(g)	3.43±1.02^{a}	3.43±1.02^{a}	3.42±0.95^{a}	3.42±0.97^{a}	3.42±0.95^{a}	P = 0.875
Mean final wt.(g)	6.97±1.91^{a}	7.19±1.95^{a}	7.06±1.74^{a}	8.05± 2.56^{b}	7.61±2.14^{a}	P = 0.006
SGR (% day^{-1})	2.97±0.07^{a}	3.08±0.06^{a}	2.99±0.16^{a}	3.67±0.29^{b}	3.36±0.14^{b}	P = 0.042
Condition Factor	1.77±0.45	1.82±0.58	1.83±0.61	1.88±0.57	1.91±0.63	P = 0.674.
Survival (%)	94±0.5	77±1.8	85±1.0	87±2.4	83±2.8	P = 0.488

Values are Mean ± S.E of four replicates. Means having the same letter in the same row are not significantly different at P<0.05

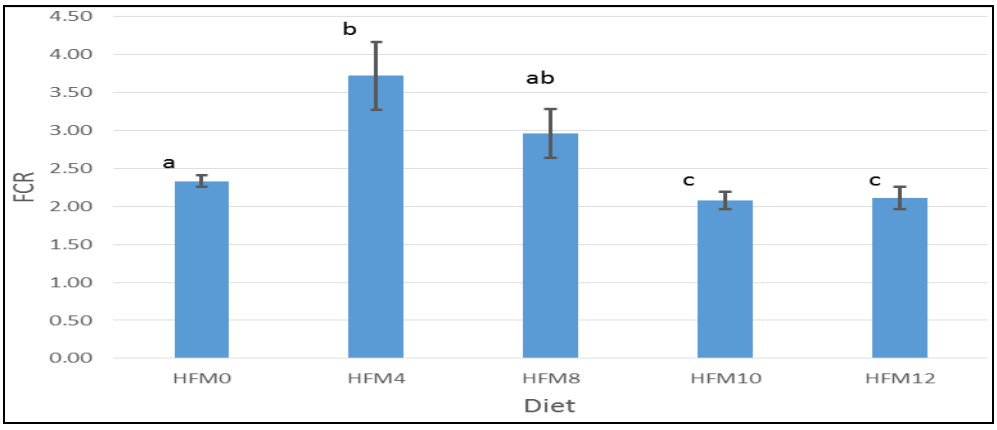

Fig 1: Comparison of mean food conversion ratio (FCR) of *O. mossambicus* fingerlings fed diets containing increasing inclusion levels of hydrolyzed feather meal (Mean±SEM). Bars with the same letters have no significant difference

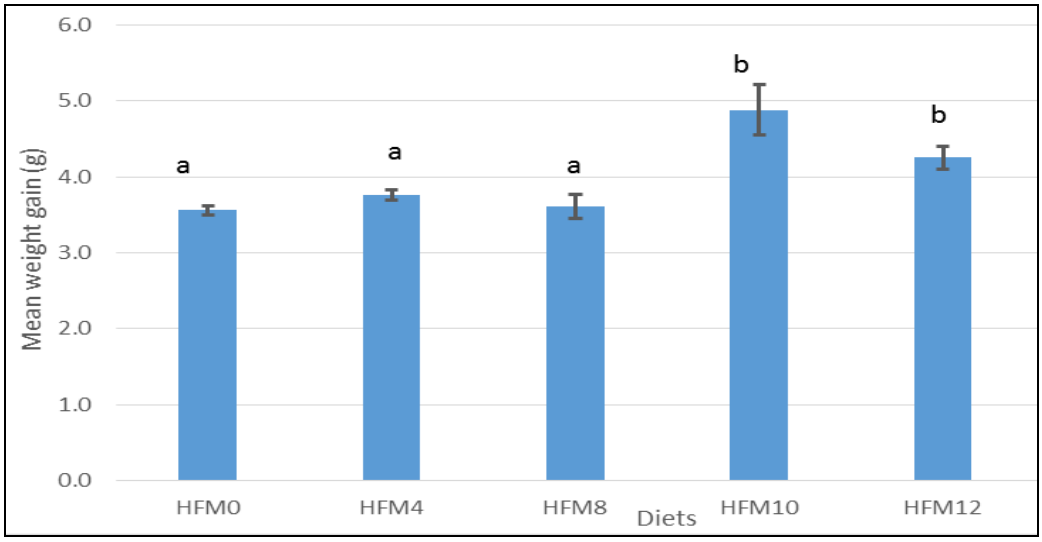

Fig 2: Comparison of mean weight gain of *O. mossambicus* fingerlings fed diets containing increasing inclusion levels of hydrolyzed feather meal (Mean±SEM). Bars with the same letters have no significant difference

The water temperature monitored during the experimental period ranged from 25.3 to 27.8 °C while dissolved oxygen (D.O) ranged from 6.5 to 8.6 mg/l, (Figure 3 and 4).

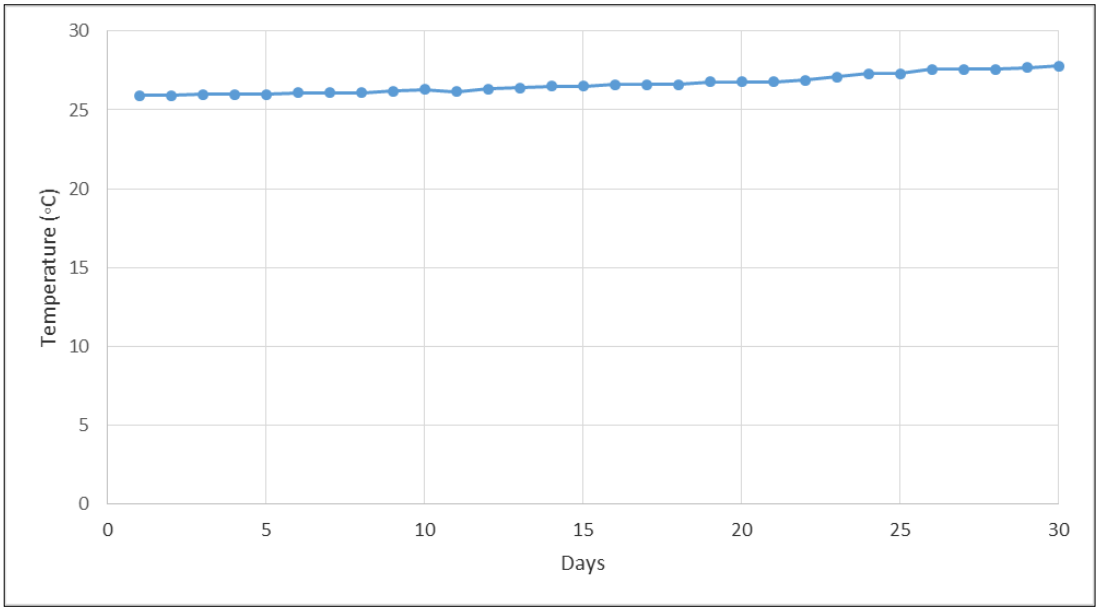

Fig 3: Temperature in the rearing system during the growth period.

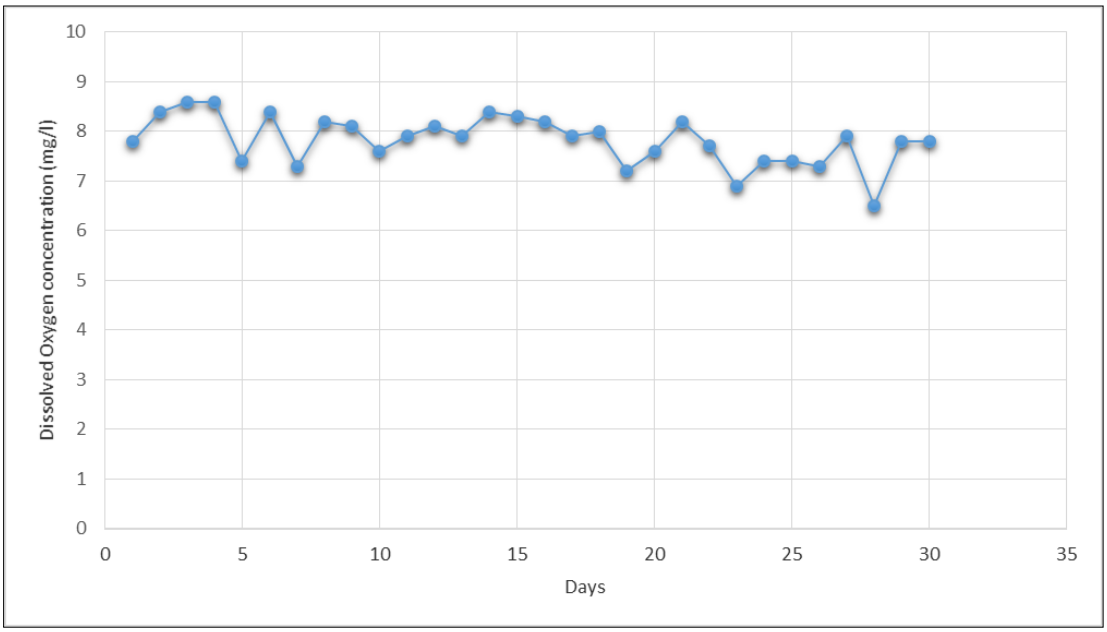

Fig 4: Dissolved oxygen concentration (mg/l) in the experimental system during the trial period.

3.2 Whole body composition of fish

Initial and final carcass compositions of tilapia mossambicus fed on the test diets are presented in table 5. All fish displayed a change in the whole body composition (compared with the initial composition). There were no marked variations between the final and initial protein content of the carcass even though there were significant differences in carcass composition amongst the dietary treatments. Final fat content was higher (P< 0.05) in all the diet treatments than the initial content and increased with increasing levels of the dietary HFM in the experimental diets. Diets HFM12 and HFM10 exhibited significantly higher fat content (P<0.05) than HFM0, HFM4 and HFM8. The final moisture content of the carcass was lower in all the treatments compared to the initial moisture content. There was a significant difference in the final moisture content amongst the treatments and it decreased with increasing inclusion levels of HFM (P<0.001).

Table 5: Proximate carcass composition of *O. mossambicus* fed increasing inclusion levels of HFM at the start and end of the experiment.

Parameter (%)	Initial body composition	Final body composition					
		HFM 0	HFM 4	HFM 8	HFM 10	HFM 12	P -Value
Moisture	72.72±0.88	71.1±0.79^{a}	69.5±0.15^{a}	69.7±0.18^{a}	68.5±0.06^{a}	66.4±0.16^{b}	P<0.001
protein	13.72±0.06	13.40±0.51^{a}	11.84±0.05^{b}	12.88±0.04^{ab}	12.23±0.11^{ab}	11.75±0.05^{b}	P=0.004
Fat	11.58±0.06	13.01±0.54^{a}	13.47±0.14^{a}	14.38±0.21^{ab}	15.15±0.08^{ab}	16.58±0.17^{b}	P<0.001
Ash	12.04±0.53	11.55±0.39^{a}	10.75±0.56^{a}	9.67±0.07^{a}	10.29±0.54^{a}	8.43±0.51^{b}	P=0.012

Values are Mean ± S.E of three replicates. Means having the same letter in the same row are not significantly different at P<0.05

Apparent digestibility coefficient (ADC) of protein and the degree of hydrolysis

The *in vivo* %ADC of CP showed that diet HFM12 and HFM10 had a significantly higher ADC (80 and 78.8% respectively) than the other diets. The lowest ADC was observed in diet HFM4 and HFM8, (Table 6). A similar trend is observed in the ADC of gross energy (GE) of the different diets although the highest ADC is recorded for diet HFM10.

Table 6: Apparent digestibility coefficient of crude protein, gross energy and the degree of hydrolysis of protein in the diets.

Digestibility (%)	HFM0	HFM4	HFM8	HFM10	HFM12	SEM
ADC of CP	73.9	70.9	73.4	78.8	80.0	0.87
ADC of GE	67.1	65.0	69.2	77.5	75.3	1.21
Degree of hydrolysis (%DH)	24.4	18.7	18.2	25.6	26.1	0.87

*ADC of CP: Apparent digestibility coefficient of crude protein
*GE: gross energy

In terms of the degree of hydrolysis, there were no significant differences (P>0.05) amongst diet HFM0, HFM10 and HFM 12 indicating values of 24.4, 25.6 and 26.1% respectively. HFM4 and HFM8 showed significantly lower DH of protein (18.7 and 18.2% respectively) than in the other diets. There was a significant correlation (R^2= 0.7187) between *in vivo* ADC of CP and *in vitro* DH of protein, Figure 5.

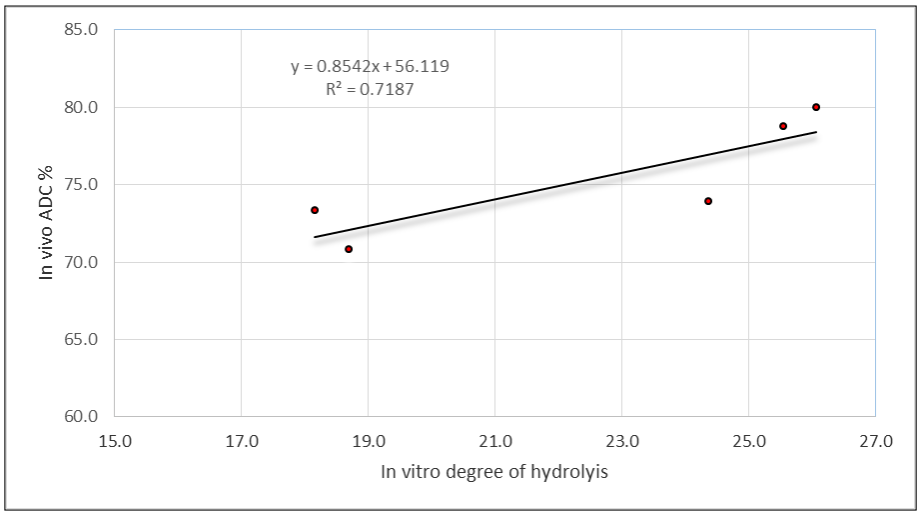

Fig 5: Relation of Degree of hydrolysis (*in vitro*) and apparent digestibility coefficient of crude protein (*in vivo*).

4. Discussion

Under the experimental conditions of the present study, survival of tilapia was high (above 75%) during the 30 days trial and was probably contributed by the overall quality and stability of the experimental conditions. Other studies have reported similar survival of tilapia while attempting to replace fish meal with HFM. Hasan *et al*., [10] evaluated HFM as a protein source in the diet of *Labeo rohita* and observed a survival of between 95-99%. Similarly, Suloma, *et al.*, [11], reported 97.5-98% survival of *O. niloticus* while a survival of 97-100% was observed in *Heterobranchus longifilis* fingerlings when fish meal (FM) was replaced with crab meal [12]. In the present study the lowest survival (77±1.8) was recorded in fish fed diet HFM4. This was probably not a result of dietary effect but rather a failure in management and handling.

Temperature and oxygen are critical parameters in fish culture systems and in this study, the temperature ranged between 25.3 to 27.8 ºC while dissolved oxygen (D.O) measured ranged between 6.5 to 8.6 mg/l. These values are within the recommended range for tilapia culture [13]. The proximate composition of the ingredients in this study differed with the estimated proportion as shown in Table 2. When formulating diets one can always expect some variations in exact chemical composition of ingredients from the approximated one. Additionally there are always some possible aberrance in the weighing and processing procedure of the experimental diets. The protein content of the diets were near isoproteinous although diet HFM4 had the highest percentage of 35.2%, while diet HFM0 had the lowest protein percentage of 32.9%.This however did not reflect on the variation in growth parameters. The same trend is repeated in the proportion of lipid in all the diets. This is probably due to the high lipid content in HFM of 18.1%. The proximate lipid content in HFM in this study is much higher than that reported by NRC, 2011 of 5.4%

There was difference in the gross energy in the diets. Diet HFM12 had the highest gross energy of 19.13±0.10 KJg^{-1} while diet HFM0 had the lowest gross energy of 13.7 KJ^{-1}. This observation could be due to the increasing lipid content as a result of the increasing level of HFM which is high in lipid (18.1%) in this study. HFM had gross energy of 23.7±0.13KJ^{-1} which is similar to that recorded by Bureau,*et al*., [14]. The high ash content in diet HFM0 and HFM4 and HFM8 is as a result of the high proportion of SSM, HFM is low in ash content and this is reflected in the proximate ash content of diet HFM10 and HFM12. HFM meal had protein content of 76.1% which is lower than the value of 80.26% reported by Bishop, *et al.*, [5]. This could be because of different processing methods of HFM.

The lowest mean weight gain (3.6±0.22g**)** was observed in diet HFM0 (control) while diet HFM10 and HFM 12 had a significantly higher MWG of 4.9±1.18g and 4.3±0.52g respectively. This shows that the fish responded positively to all the diets. The present study showed that inclusion of hydrolyzed feather meal in substitution of marine protein sources in the diet of *O. mossambicus* is feasible. The results indicated that inclusion of at least 12% HFM had positive effect on growth rate and weight gain in comparison of other tested diets, formulated with lower HFM inclusion. Fish fed the diet containing 10% and 12% HFM had a significantly higher FMW and SGR compared with the other diets (HFM0, HFM4, HFM8). A significantly lower FMW and SGR observed in the control diet (HFM0), HFM4 and HFM8) might be due to high proportion of SSM (60%) and progressively higher inclusion of wheat bran [15]. Feeds containing high ash content may have high protein content and favorable essential amino acid profile but still have poor digestibility. In this study diets, HFM0, HFM4 and HFM8 had a relatively high ash content of 20.02, 17.9 and 15.3% respectively. The growth performance recorded in diet HFM10 and HFM12 which replaced the animal protein by 30% and 63% respectively concurs with a similar study by Munguti, *et al.* [5] which demonstrated that the growth of *O. niloticus* was not affected by the replacement of up to 66% of the animal protein (9.9% of the total diet) by feather meal. Studies on replacement of fish meal and shrimp meal with HFM have been done on *Oreochromis niloticus* but few if any on *O. mossambicus*. Results of this study contradicts the findings of Munguti, *et al.* [2] who found significant decline in growth of *O. niloticus* fed diet containing 8.6% HFM. The highest weight gain (69.5%) was recorded for fish fed 4.5% HFM. This may have been attributed to different processing methods of the HFM and the different combinations of the ingredients in the treatments. Bureau, *et al*.,[6] reported 15% replacement of FM with HFM in the diet of rain bow trout and they found no significant differences in weight gain and feed efficiency in fish fed the diet containing HFM (15%) and those fed the control diet of 50% FM.

FCR is an important economic indicator of how efficiently the

fish utilizes the feed thereby reducing wastage. The FCR was generally high in this study due to the uneaten feeds due to the relatively bigger sizes of pellets fed to the fish. The lowest FCR (2.0±0.11and 2.11±0.14) was observed in the fish fed diet HFM10 and HFM12.This was significantly lower than those for the fish fed diet HFM0, HFM4 and HFM8 and therefore indicates the best utilized diet compared to the other diets. This could be because of the diets being relatively digestible as demonstrated by the significantly high degree of hydrolysis and ADC of CP in diet HFM10 and HFM12 (Table 6). This was followed by the FCR of 2.33±0.07, 2.96±0.32 and 3.72±0.44 for diets, HFM0, HFM8 and HFM4 respectively. When Poultry feather meal was used as a single animal protein at inclusion levels of 48%, Bag *et al*., [16] realized an FCR of 2.28 which had no significant difference with the other dietary treatments (earthworm meal and slaughter offal meal). This compares to the FCR recorded on fish fed the control diet in this study. This is perhaps due to the high inclusion HFM in the former hindering growth due to low levels of lysine and methionine amino acids in HFM.

At the end of the experiment the body moisture content was lowest in fish fed diet HFM10 and HFM12; 68.5±0.06 and 66.4±0.16% respectively, indicating better quality of flesh than the other diets which were significantly higher in the body moisture content. Body protein did not differ much from the initial composition in all the diet treatments. These values have similar trend as in the study by Bag *et al*., [16] on *O. mossambicus* using poultry feather meal where they recorded moisture and protein contents of 75.91% and 11.01% respectively at the beginning of the experiment and 75.28% and 11.03% respectively at the end of study.

Final body lipid increased with increasing level of dietary HFM and was highest in diet HFM12, further explaining the high weight gain in fish fed diet HFM10 and HFM12 and the lower moisture content compared to the other diets which had significantly lower lipid content in the carcass.

The ADC of protein increased with increasing inclusion levels of HFM. Diet HFM12 and HFM10, had higher ADC of protein than HFM0, HFM4 and HFM8. This indicates that the inclusion of HFM in this study improved digestibility of the diets. The significantly lower ADC of protein in diet HFM4 and HFM8 could be a result of the high fiber content resulting from the high proportion of wheat bran. The digestibility of shrimp shell meal can be poor due to high chitin content [17] and its inclusion is relatively high in in the first three diets.

The ADC of CP reported in this study are higher than the ones reported by Munguti, *et al*. [2], probably due to the different plant protein sources used in the diet formulations. HFM improved the digestibility of the diets and had no adverse effects on digestibility in this study. The same scenario is reported by Zhang *et al*.[4] in a study cotton seed meal and soy bean meal were partially replaced by HFM at inclusion levels of 12% in the diet of hybrid tilapia (*O. niloticus* × *O. aureus*) without any adverse effect on digestibility.

Degree of hydrolysis assays have been used before in aquafeeds to ascertain feed quality and to measure digestibility [18, 19, 9]. Following in the trend and consistent with growth parameters, diet HFM10 and HFM12 had the highest DH of 25.6±0.01 and 26.1±0.01% respectively while the rest had lower DH. It indicates, together with the highest measured ADC in this study, that the processing method of steam hydrolysis of the feathers did create reasonably good protein source. The high proportion of wheat bran in diet HFM4 and HFM could be the reason for the low DH as argued by Alarcón, *et al*, [20] where they realized that the DH decreased with increasing levels of plant proteins. High fiber content in diets might also affect the protein hydrolysis, both *in vitro* and *in vivo*. The significant correlation between DH and the ADC of CP (R^2= 0.7187) confirms that DH is a reliable indicator of the digestibility of protein in tilapia diet. González-Félix *et al*., [18] reported a non-significant correlation of R^2=0.6 in the diet of Nile tilapia while evaluating the impact of replacing fish meal with different plant protein sources.

5. Conclusion and recommendations

Results from this study have shown that feather meal is a feasible ingredient in formulation of aquafeeds for tilapia and it can be used to reduce the overreliance on fish meal and fresh water shrimp in Kenya. It is also clear from this study that feather meal can replace up to 63% (at inclusion levels of 12%) of shrimp shell meal in the diet of *O. mossambicus* when formulated together with plant protein such as rapeseed meal. DH assay by OPA method can be an accurate and quicker way of assessing the digestibility of ingredients and this should be done for all the ingredients to ascertain their quality. In this study the author recommends the ideal diet formulation for O. *mossambicus* diet to be HFM10 or HFM12 for best growth and feed efficiency.

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7. References

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