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Carcass fatty acid composition and sensory properties of Nile tilapia (Oreochromis niloticus) fed on oilseed meals with crude papain enzyme

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Abstract

In this study, proximate body composition, fillets fatty acid and sensory properties of Nile tilapia fed on oilseed meals with crude papain enzyme were determined. A control diet (D1) of 300g/kg crude protein (CP) and (2900Kcal/kg) was formulated using fishmeal (Rastrionaebola argentea) (FM) and test diets by replacing 10% CP of FM by soybean meal (SBM) (D2), canola meal (CM) (D3) and sunflower meal (SFM) (D4). One hundred and one days feeding trial was conducted in a 4x2 factorial design on 4 diets (D1, D2, D3 and D4) with (0.06%) and (0%) enzyme using 720 Nile tilapia fingerlings (7±3g). Fish were fed twice daily at 5% of their biomass at 10am and 4pm in two equal meals. At the end of feeding trial, fish were starved for 24 hrs and weighed. A sample of ten fish representing the average weight of each replicated group was used. The fillets fatty acid profiles were determined by MPA FT-NIR spectrometer. Organoleptic tests of both fresh and steamed fish were carried out by eight semi trained panellists using 5-point hedonic scale. There was increase ($p<0.05$) in carcass proximate composition in all the treatments. Fillets fatty acid levels were influenced by the crude papain enzyme ($p<0.05$). Palmitic acid (C16:0), linoleic acid (C18:2n-6) and oleic acid (C18:1n-9) were the most abundant fatty acids in the fillets. Fish fed on fishmeal based diet recorded higher percentage (22.56) of polyunsaturated fatty acids (PUFA). There was significant difference among the treatments ($p<0.05$) for general appearance. Fish fed on crude papain enzyme treated diets were less preferred. It is recommended that more research be done on the effect of papain enzyme on the carcass fatty acid and sensory properties of farmed fish.

Keywords: *Fatty acids; Nile tilapia; oilseed meals; papain enzyme; sensory properties*

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Introduction

Reduction of the feed costs is the major concern in aquaculture. Attempts to partially or wholly replace the fish meal component in fish feed with alternative feed ingredients have been made by researchers. However, the value and the feasibility of an alternative feed ingredient to fishmeal cannot simply be evaluated by its ability to maintain growth. Fish market value for human consumption depends in large part on the perceived quality (Sealey *et al.,* 2011). In view of this, the goal of fish nutrition as a scientific discipline is to produce feeds that support good growth rates, maintain fish health and quality. This will translate into a safe and healthy product

for the consumer (Singh *et al*., 2008). Fish provide an important source of essential fatty acids (EFA), amino acids, vitamins and minerals (FAO, 2016). The high nutritional value of fish makes it an important food source for humans (Murray and Burt, 2001).

However, farmed fish are less accepted by consumers on basis that they are of lower quality and poor flavor compared to wild fish (Rasmussen, 2001). The composition and sensory quality of fish meat is genetically and dietary controlled (Rasmussen, 2001; Zenebe 2010). Based on this, improvement of feed and nutrition in aquaculture provide an opportunity to enhance the quantity and nutritional quality of fish (Rasmussen, 2001). Utilization of nutrients and their interactions when alternative feed ingredients from plants are used to substitute fish meal have and are being studied (Sing *et al*., 2008). Oilseed meals represent suitable alternative plant ingredients for replacing fishmeal in fish diets. However, increasing interests is now focused on the fatty acid composition of fish when evaluating the suitability of oilseed meals to replace fishmeal in fish diets (Abou *et al.,* 2013). The fatty acid composition of fish tissue changes in response to the fatty acid composition of the diet (Leaver *et al.,* 2008). Due to relatively low nutrient digestibility in plant ingredients, addition of crude papain enzyme into the feed can improve the feed's protein hydrolysis. This will result in increased feed efficiency, improved growth and body deposition. This study therefore was carried out to determine carcass proximate, fatty acid composition and sensory attributes of Nile tilapia fed on oilseed meal diets with crude papain enzyme.

Materials and Methods

Study site and diet formulation

The experiment was conducted at Chuka University and Fisheries Department, Meru County, Kenya. Fatty acid analysis was done at Fletcher Scientific Solutions, Nairobi, Kenya. A control diet (D1) of 300 g/kg crude protein was formulated using fishmeal (*Rastrionaebola argentea*) (FM), soybean meal (SBM), canola meal (CM) and sunflower meal (SFM). The test diets were formulated by replacing 10% CP of FM by SBM (D2), CM (D3) and SFM (D4), respectively (Table 1). Feed was formulated as outlined in NRC (2011). Crude papain was collected from the locally grown *Carica papaya* (paw paw) plants and sun dried at 40°C for 14 hrs following the procedure by Adu *et al.,* (2009). The protease activity of crude papain extract was determined using Hammersten casein as substrate according to Macalood *et al.,* (2013) and supplemented at the rate of 0.06% (Kirimi *et al.,* 2019). The enzyme in powder form was dissolved in 50ml water and then reconstituted to 500mls of water. The solution was mixed thoroughly with the formulated marsh feed to form a paste and pelleted.

Experimental treatments

In a 4x2 factorial design, the study was based on 4 diets, FM (D1) SBM (D2) CM (D3) and SFM (D4) with crude papain enzyme levels of 0% and 0.06%. The experiment was conducted on liner pond in 24 net hapas (2m x 1m x 1m). Seven hundred and twenty sex- reversed Nile tilapia fingerlings from Sagana fish farm of average weight 7±3g were selected and acclimatized for two weeks during which time they were fed on commercial feed (Aller aqua®). After acclimatization the fingerlings were randomly picked and transferred into the twenty-four hapa nets at a rate of 30 fingerlings per unit net hapa. They were further divided randomly into eight groups with three replicates. Feeding with the experimental rations begun after the initial weight of the fish was taken. They were fed at 5% of body weight throughout the experimental period twice daily i.e. morning (10 am) and evening (4 pm) in two equal meals. The amount of supplementary feed provided was adjusted accordingly after weighing the fish at each sampling done fortnightly. Water temperatures in the hapas ranged from 22.06^{o}C to 29^{o}C with a mean of 25.53^{o}C. The pH ranged from 7.42 to 10.01 mean of 8.72. Dissolved oxygen ranged between 2.5 mg/l to 5.3 mg/l with a mean of 3.9 mg/l.

Table 1. Ingredient composition and calculated crude protein (g/kg) of the diets supplemented to Nile tilapia containing soybean meal, canola meal and sunflower meal as a replacement of fishmeal

Diet/Ingredient	D1	D2	D3	D4
Fish meal	165	90	90	90
Soybean meal	130	240	150	160
Canola meal	165	160	310	150
Sunflower cake	180	190	180	430
Maize grain	180	160	130	100
Wheat bran	180	160	140	70
Crude papain enzyme (%)	0.06	0.06	0.06	0.06
Total	1000	1000	1000	1000
Calculated crude protein	301	300	301	300
Calculated Digestible Energy(Kcal/kg)	2997.08	2965.08	2949.63	2878.11

Diet code: D1, fishmeal based diet; D2, soybean meal based diet; D3, canola meal based diet; D4, sunflower meal based diet

Sample collection for carcass analysis

Both at the beginning and end of feeding trial (final day of sampling), 10 fish samples were selected randomly from each group for carcass proximate and fatty acid analysis. Selected fish were killed by percussive stunning method which involves a forceful and accurate blow to the head with a blunt instrument (Daskalova *et al.,* 2016). The carcass was then eviscerated (gutted), descaled and rinsed in clean water. Fillets were removed from the muscles in preparation for fillets fatty acid analysis. The samples were packed in a cool box, preserved with clean ice blocks and transported to the deep freezer at -4^{0}C pending analysis (Maina *et al.,* 2003).

Fillet fatty acid and proximate analysis

Fish whole carcass and fillets were partly thawed and samples minced using meat mincer and homogenized in preparation for carcass proximate and fatty acid analysis (Maina *et al.,* 2003). Fatty acid analysis of fillets was performed by MPA FT-NIR spectrometer (Bruker, Germany) which is a non-destructive method of analysis. Near-infrared (NIR) spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range of 780–2500 nm (Osborne, 2006). Approximately 30-50 g of the sample was put into the sample cup, which was later put on the integrating sphere for measurement. Samples were analyzed for calibration and cross validation of the calibration performed (Kirimi *et al.,* 2020). The proximate analysis of the carcass was carried out in triplicates following the procedure AOAC (1995). Moisture contents were obtained after drying in an oven at 110 °C for 24 hours. The loss in weight was the moisture content and what was left is the dry matter (DM) of the sample. The crude protein was determined using the Kjeldahl method. Ash was determined by heating the samples in a muffle furnace set at 550 °C for 4 hours. Ether extraction was carried out through the soxhlet extraction method. Nitrogen free extracts (NFEs) were estimated by subtracting the total moisture, crude protein, ether extracts, ash and crude fibre from 100.

Sample collection and preparation for sensory analysis

After 101 days of the experimental trial, fish for the sensory test were randomly selected from the net cages for each treatment and transferred into fresh clean water where they were starved for one day. During the day of sensory test, five fish from each group were selected, gutted, descaled and washed in tap water.

Organoleptic/sensory evaluation

Organoleptic test was done following the procedure by Kirimi (2015). Fish were blind coded with three digit numbers and then double wrapped with aluminium foil. Two litres of water was put in a cooking pot and the gas heat used to boil the water to 100 ^{o}C. The wrapped fish in the aluminium foil were put in the boiling water in cooking pot covered with a lid. The temperature was measured by inserting a thermometer in the boiling water. They were left for 20 minutes to cook under steam and then heat put off. The

steamed samples were removed from the cooking pot and cooled to a room temperature. Eight-member panel (gender: 4 women, 4 men; age group 30-56 years) were selected. All volunteers were selected on the basis of their interest and availability. Prior to evaluation, a session was held to familiarize the panelists with the product. The panel was then asked to read through the questionnaires and understand the meaning of each attribute (texture, taste, aroma, appearance and juiceness) to avoid any misinterpretation (Kilcast and Subramaniam, 2000). Steamed and fresh fish were presented to the 8 semi-trained panelists in plates coded with three digit random numbers, along with distilled water to wash their mouth between the samples. Panelists evaluated whole fish first from each group coded with three digit numbers that did not indicate treatment. The panelists individually evaluated changes in skin, gills, texture and odour.

The assessment was based on 5-point hedonic scale (where 1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like, 5 = like very much). The descriptors for various sensory attributes were defined and the panelists were asked to rate their acceptance for general appearance, texture and colour while the attributes of steamed fish samples were, aroma, taste and juiceness. The samples were evaluated for aroma by sniffing when the aluminium foil was first opened partially. Panelists were advised to have at least 2 minutes break before tasting the next sample. The panelists were not allowed to discuss their findings with one another during the evaluation session.

Data analysis

Carcass proximate, fatty acid composition and sensory characteristics data were subjected to a two-way analysis of variance (ANOVA) using statistical package for social science version 20.0 at P = 0.05 confidence level, to determine whether there were significance differences and where the differences occurred, mean separation was done by least significance difference (LSD).

Results

Diets proximate composition

Proximate composition of the diets is shown in Table 2. Crude protein values for D1, D2, D3 and D4 were near isoproteinous i.e 30.57%, 30.76%, 30.34% and 31.35% respectively ($p>0.05$). Diet 3 recorded highest level of ether extracts (10.75%). Fishmeal based diet (D1) had highest ash content (6.16%) but low in crude fibre content (11.06%).

Table 2. Proximate composition of the diets (%), for Nile tilapia containing either soybean meal, canola meal or sunflower meal as a replacement of 10% (on CP basis) of fishmeal

Diet (D)	D1	D2	D3	D4	P-value
Proximate composition					
DM	90.9±0.07^{dbc}	91.31±0.16^{dbca}	91.00±0.09^{dbca}	91.56±0.19^{abc}	0.030
CP	30.57±0.43^{a}	30.76±0.53^{a}	30.34±0.31^{a}	31.35±0.33^{a}	0.077
EE	7.55±0.27^{cd}	7.67±0.18^{cd}	10.75±0.28^{a}	9.63±0.18^{b}	0.000
Ash	6.16±0.03^{abc}	5.60±0.24^{abc}	5.40±0.21^{dbc}	5.81±0.17^{abc}	0.081
CF	11.06±0.08^{d}	12.18±0.12^{c}	13.37±0.17^{b}	16.03±1.00^{a}	0.000
NFE	42.45±0.21^{ab}	42.79±0.65^{ab}	37.44±0.56^{cd}	36.09±0.51^{cd}	0.000
NDF	24.07±0.22^{cab}	24.41±0.31^{abc}	24.41±0.23^{bac}	23.08±0.34^{d}	0.029
ADF	8.37±0.25^{cd}	8.23±0.30^{dc}	11.83±0.20^{ba}	11.86±0.47^{ab}	0.000

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different ($p<0.05$)

Diet code: D1, fishmeal based diet; D2, soybean meal based diet; D3, canola meal based diet; D4, sunflower meal based diet;

Abbreviations: ADF, acid detergent fibre; CF, crude fibre; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; NFE, nitrogen free extracts

Diets fatty acid composition

The fatty acid composition of the diets is shown in Table 3. Soybean meal based diet (D2) had a high level of palmitic acid (16:0) (15.4mg/100g) compared to the other diets. However, sunflower meal based diet (D4) recorded the highest level of saturated fatty acid (23.6mg/100g). Diet 1 (FM based) recorded highest amount of polyunsaturated fatty acid (32.1mg/100g) followed by sunflower meal based diet (30.3mg/100g).

Table 3. Fatty acid composition of the diets (mg/100g) for Nile tilapia containing either soybean meal (D2), canola meal (D3) or sunflower meal (D4) as a replacement of 10% (on CP basis) of Fishmeal (D1)

Fatty Acids	D1	D2	D3	D4	P-value
Saturated					
C 14:0	4.14±0.01^{d}	4.47±0.01^{b}	4.36±0.01^{c}	5.11±0.01^{a}	0.000
C 16:0	15.5±0.01^{c}	15.4±0.01^{a}	14.7±0.01^{d}	15.2±0.00^{b}	0.000
C 18:0	2.98±0.01^{c}	2.12±0.01^{d}	3.22±0.01^{b}	3.26±0.01^{a}	0.000
ΣSFA	21.85±0.36	22.00±0.10	22.44±0.07	23.51±0.19	
Mono-unsaturated					
C 16:1 n-7	5.36±0.01^{ba}	5.17±0.01^{c}	4.81±0.01^{d}	5.37±0.01^{ab}	0.000
C 18:1 n-9	12.7±0.01^{d}	13.5±0.01^{b}	13.1±0.01^{c}	13.7±0.01^{a}	0.000
C 20:1 n-9	2.50±0.01^{d}	2.71±0.01^{b}	2.66±0.01^{c}	2.82±0.01^{a}	0.000
C 22:1 n-9	4.54±0.01^{c}	6.12±0.01^{b}	6.90±0.01^{a}	5.36±0.01^{d}	0.000
C 24:1 n-9	0.81±0.01^{b}	0.86±0.01^{a}	0.32±0.01^{d}	0.49±0.01^{c}	0.000
ΣMUFA	25.90±0.01	28.30±0.01	27.80±0.00	27.80±0.00	
Polyunsaturated					
C 18: 2n-6	15.6±0.01^{a}	14.7±0.01^{c}	15.2±0.01^{b}	12.2±0.01^{d}	0.000
C 18:3n-6	0.17±0.00^{ab}	0.11±0.00^{d}	0.13±0.00^{c}	0.15±0.00^{ba}	0.000
C 20:4n-6	0.62±0.01^{ab}	0.53±0.01^{cd}	0.52±0.01^{dc}	0.61±0.01^{ba}	0.000
C 20:3n-3	0.10±0.01^{a}	0.10±0.01^{a}	0.11±0.01^{a}	0.10±0.01^{a}	0.000
C 20:5n-3	6.89±0.01^{b}	6.33±0.01^{c}	7.14±0.01^{a}	5.93±0.01^{d}	0.000
C 22:6n-3	8.64±0.01^{d}	7.64±0.01^{a}	7.02±0.01^{c}	6.87±0.01^{d}	0.000
ΣPUFA	32.10±0.00	29.90±0.00	30.20±0.00	30.30±0.01	

Values are expressed as mean ± SE $^{a, b, c, d}$ .Values in the same row with different superscript letters show differences ($p<0.05$).

Diet code: D1, fishmeal based diet; D2, soybean meal based diet; D3, canola meal based diet; D4, sunflower meal based diet;

Abbreviations: SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Carcass proximate composition

Results of the whole fish body proximate composition at the start and end of the experiment is shown in Table 4. There was a change in whole body proximate composition at the end of experiment compared with that at the start of the experiment. Initial crude protein content was 60.19% while lipid was 17.23%. However, at the end of experiment, there was increase in crude protein content both in enzyme treated diets and non-treated diets. Diet 2 (SBM) recorded higher figures for CP (64.49%) closely followed by FM based diet (63.84%). FM based diets recorded lowest lipid content (16.83%) than oilseed meals.

Diet 1 (SFM) recorded highest concentration of the lipid content (20.62%). The ash content ranged from 13.60% SBM to 14.50% SFM. However, on crude papain treated diets, there was a slight increase in protein content (63.97%) and lipid content (19.58%) ***Fillets fatty acid composition***

Fatty acid composition of the fillets is shown in Table 5. Fillets fatty acid composition closely resembled dietary fatty acid composition though no definite pattern for all the fatty acids. Palmitic acid (16:0) constituted the largest proportion of

the saturated fatty acid in both enzyme treated and non-treated fish fillets. However, enzyme treated diets recorded highest figure (16.21 mg/100g). Linoleic acid (18:2n-6) was in highest concentration of the polyunsaturated fatty acid in all the treatments. However, monosaturated fatty acid; Oleic acid (18:1n-9) was highest in both treatments. The effect of enzyme treatment led to increase in saturated and monosaturated fatty acid and decrease in total polyunsaturated fatty acid in all the diets. Diet 3 (CM based diet) recorded highest proportion of the total polyunsaturated fatty acid (24.79 mg/100g).

Sensory analysis

Results of sensory analysis are as shown in Table 6. Enzyme treated diets scored low for all sensory attributes. However, there was no significant difference for taste and aroma (p>0.05) in fish on enzyme treated diets. There was no significant difference on texture and aroma of fish due to different protein sources (p>0.05), though FM based diet scored higher numerically closely followed by canola meal based diet. The taste of the meat appeared to be best for FM based diet (4.63) with CM and SFM recording similar figures (3.75). Juiceness and overall acceptability was high in FM based diet. **Discussion**

Fillets fatty acid composition

Fillets fatty acid composition closely resembled fatty acid composition in the diets though some slight variation in specific fatty acid (Table 3 and 5). This is in agreement with Mulligan and Trushenski, (2013) that fatty acid composition of cultured fish greatly depends on the dietary fatty acids. Fillets from fishmeal based diet recorded low values for total saturated fatty acids attributed to low levels of these fatty acids in the diets (Table 3). Palmitic acid (16:0) was the most abundant saturated fatty acid in fish fillets and this was a reflection of the fatty acid in the dietary treatments. Study conducted by Satue and Lopez (1996), palmitic acid was the predominant saturated fatty acid in Nile tilapia and this corroborates with the present study. However, myristic acid (14:0) was in lowest concentration of all the saturated fatty acid in the fillets. Myristic acid (14:0) and palmitic acid (16:0) are considered to be hypercholesterolemic fatty acids and thereby increase the synthesis of cholesterol, promoting the accumulation of low density lipoprotein, which is a risk factor for cardiovascular diseases (Moloney *et al.,* 2001). However, when consumed by humans, stearic acid (18:0) is transformed into oleic acid (18:1n-9) (monounsaturated), a fatty acid that does not carry any cardiovascular risks (Lima *et al.,* 2017). Oleic acid was the most abundant of the mono-unsaturated fatty acids in both the diets and fillets. Linoleic acids (18:2n-6) in the fillets were much lower than levels in the diets. This is because some dietary linoleic acid might have been converted to long chain polyunsaturated fatty acid by desaturase and elongase enzymes (Torstensen and Tocher, 2010). In this study, the levels of arachidonic acid (20:4n-6) in the fillets resembled those in the dietary treatments. However, study done by Visentainer *et al.,* (2005) where addition of flax seed oil rich in alpa linolenic acid (18:3n-3), was found to moderately increase concentration of arachidonic acid in tilapia fillets. The approach did little to increase long chain polyunsaturated fatty acids (LC-PUFAs). This is likely due to fact that tilapia has a limited hepatic capacity to elongate and desaturate eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) from dietary ALA precursor (Karapanagiotidis *et al*., 2007). Soybean meal, canola meal and sunflower meal based fillets had slightly similar levels of polyunsaturated fatty acid. However, they recorded higher levels for saturated fatty acids. Karapanagiotidis *et al.,* (2006) reported on the elevated level of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in the fillet of intensively farmed tilapia due to the increased fat deposition. In the present study, fish had low levels of decosahexanoic (22:6n-3) and eicosapentaenoic acid (20:5n-3). Although this might have been contributed by low levels in the diets, fish were frozen at -4^{o}c for a period of about 3 weeks before analysis was done which might have led to some levels of oxidation of polyunsaturated fatty acid (PUFA) hence low values observed. Highly unsaturated fatty acids are more susceptible to oxidation and may have undergone some degree of oxidation during storage (Maina *et al.,* 2003). Polyunsaturated fatty acid (PUFA) predisposes the meat to rancidity with linolenic acid (18:3) being twice as susceptible to oxidation as compared to linoleic acid (18:2) (Lima *et al.,* 2017).

Table 4. Carcass proximate composition (% DM basis) of Nile tilapia fed on oilseed meals with crude papain enzyme

		Diet (D)				**Enzyme (%) (E)**		**P- value**		
	Initial	FM (D1)	SBM (D2)	CM (D3)	SFM (D4)	0	0.06	D	E	DXE
DM	91.06±0.01	91.92±0.11^{cb}	92.08±0.12^{bc}	93.04±0.19^{a}	90.97±1.18^{d}	92.58±0.21	91.42±0.57	0.000	0.000	0.000
CP	60.19±0.19	63.84±0.63^{bc}	64.49±0.21^{a}	63.19±0.25^{dc}	63.48±0.20^{cbd}	63.53±0.22	63.97±0.33	0.000	0.017	0.000
EE	17.23±0.54	16.83±0.17^{d}	19.55±0.11^{c}	20.00±0.32^{b}	20.62±0.47^{a}	18.91±0.33	19.58±0.58	0.000	0.017	0.000
ASH	14.16±0.16	13.89±0.39^{cdb}	13.66±0.58^{dc}	13.96±0.37^{bc}	14.50±0.09^{a}	14.77±0.06	13.24±0.23	0.000	0.000	0.000
CF	0.88±0.13	1.00±0.14^{cb}	1.27±0.12^{ab}	1.17±0.17b^{ca}	0.73±0.04^{d}	1.16±0.10	0.93±0.10	0.001	0.001	0.001
NFE	4.06±0.40	4.44±0.37^{a}	1.04±0.41^{cd}	2.27±0.71^{b}	0.73±0.25^{dc}	1.64±0.52	2.60±0.52	0.000	0.020	0.000

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (p<0.05)
Diet code: D1, fishmeal based diet; D2, soybean meal based diet; D3, canola meal based diet; D4, sunflower meal based diet;
Abbreviations: CF, crude fibre; CP, crude protein; DM, dry matter; EE, ether extract; NFE, nitrogen free extracts.

Table 5. Fillets fatty acid composition (mg/100g) of Nile tilapia fed on oilseed meals with crude papain enzyme

	Diet (D)				**Enzyme (%) (E)**		**P- value**		
	FM (D1)	SBM (D2)	CM(D3)	SFM (D4)	0	0.06	D	E	DXE
Saturated									
C 14:0	2.83±0.27^{b}	2.62±0.26^{d}	2.79±0.38^{c}	2.91±0.37^{a}	3.50±0.06	2.07±0.03	0.000	0.000	0.000
C 16:0	15.02±0.80^{c}	14.60±0.21^{d}	15.69±0.42^{b}	15.72±0.28^{a}	14.30±0.21	16.21±0.21	0.000	0.000	0.000
C 18:0	3.61±0.24^{dcb}	3.62±0.22^{cdb}	3.65±0.34^{bcd}	3.84±0.45^{a}	2.98±0.37	4.37±0.88	0.000	0.000	0.000
ΣSFA	21.46±0.01	21.84±0.01	22.13±0.01	22.47±0.00	20.78±0.01	22.65±0.01			
Mono-unsaturated									
C 16:1 n-7	6.25±0.23^{d}	6.87±0.28^{cab}	7.10±0.18^{acb}	7.04±0.22^{bca}	6.35±0.12	7.29±0.12	0.000	0.000	0.000
C 18:1 n-9	22.49±0.74^{c}	22.07±0.83^{d}	22.99±0.63^{b}	23.22±0.54^{a}	21.16±0.21	24.23±0.61	0.000	0.000	0.000
C 20:1 n-9	3.02±0.30^{a}	2.19±0.43^{b}	2.14±0.47^{c}	2.09±0.43^{d}	1.45±0.16	3.27±0.07	0.000	0.000	0.000
C 22:1 n-9	1.92±0.29^{a}	1.39±0.47^{cb}	1.40±0.49^{bc}	1.32±0.46^{d}	0.55±0.12	2.46±0.03	0.000	0.000	0.000
C 24:1 n-9	0.41±0.04^{a}	0.38±0.05^{b}	0.35±0.07^{c}	0.32±0.04^{d}	0.48±0.01	0.25±0.01	0.000	0.000	0.000
ΣMUFA	34.09±0.00	32.90±0.06	33.98±0.00	33.99±0.00	29.99±0.00	37.5±0.67			
Polyunsaturated									
C 18: 2n-6	9.34±0.55^{b}	9.83±0.20^{a}	7.63±0.62^{d}	8.18±0.26^{c}	8.45±0.50	9.04±0.24	0.000	0.000	0.000
C 18: 3n-9	1.29±0.14^{cd}	1.54±0.08^{b}	4.45±1.48^{a}	1.26±0.06^{dc}	1.36±0.06	2.92±0.85	0.000	0.000	0.000
C 18: 3n-6	0.05±0.02^{d}	0.06±0.02^{d}	0.10±0.04^{ab}	0.10±0.04^{ba}	0.15±0.01	0.00±0.00	0.000	0.000	0.000
C 20: 4n-6	0.59±0.01^{a}	0.46±0.06^{d}	0.53±0.09^{b}	0.53±0.10^{c}	0.67±0.02	0.38±0.03	0.000	0.000	0.000

C 20: 3n-3	0.10±0.05^{b}	0.09±0.04^{c}	0.05±0.02^{d}	0.11±0.05^{a}	0.8±0.01	0.00±0.00	0.000	0.000	0.000
C 20:5n-3, EPA	4.09±0.11^{b}	3.72±0.18^{d}	4.27±0.32^{a}	3.78±0.27^{c}	4.35±0.13	3.59±0.13	0.000	0.000	0.000
C 22:6n-3, DHA	7.10±0.48^{d}	7.37±0.60^{c}	7.76±0.28^{b}	8.17±0.50^{a}	8.63±0.14	6.57±0.16	0.000	0.000	0.000
ΣPUFA	22.56±0.00	23.07±0.00	24.79±0.00	22.13±0.00	24.41±0.02	22.55±0.00			

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (p<0.05)
Diet code: D1, fishmeal based diet; D2, soybean meal based diet; D3, canola meal based diet; D4, sunflower meal based diet;
Abbreviations: SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

Table 6. Sensory evaluation scores of fresh and steamed Nile tilapia fed on oilseed meals with crude papain enzyme

	Diet (D)				Enzyme (%) (E)		P- value		
Attribute	FM (D1)	SBM (D2)	CM (D3)	SFM (D4)	0	0.06	D	E	DXE
Appearance	4.13±0.20^{bcda}	3.69±0.24^{db}	4.05±0.19^{abc}	3.94±0.17^{cba}	4.22±0.13	3.78±0.15	0.210	0.030	0.475
Texture	4.13±0.18^{a}	3.69±0.19^{a}	4.00±0.16^{a}	4.00±0.18^{a}	4.13±0.13	3.78±0.12	0.365	0.061	0.845
Aroma	3.94±0.25^{a}	3.50±0.242^{a}	3.63±0.20^{a}	3.56±0.273^{a}	4.03±0.17	3.28±0.14	0.477	0.001	0.023
Taste	4.63±0.16^{a}	3.94±0.21^{bcd}	3.75±0.19^{cbd}	3.75±0.28^{dc}	4.12±0.15	3.91±0.17	0.007	0.272	0.005
Juiceness	4.50±0.16^{a}	3.81±0.23^{bcd}	3.81±0.21^{cbd}	3.69±0.22^{dbc}	4.06±0.15	3.84±0.16	0.024	0.281	0.164
Overall acceptability	4.25±0.11^{a}	3.69±0.120^{dbc}	3.87±0.13^{bd}	3.81±0.19^{cd}	3.94±0.10	3.87±0.11	0.020	0.628	0.005

Values are expressed as mean ± SE.^{abcd}, Values in the same row between diets, having different superscript letters are significantly different (p<0.05)
Diet code: FM, fishmeal based diet; SBM, soybean meal based diet; CM, canola meal based diet; SFM, sunflower meal based diet.

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Although diet was the major cause of variation in fillet fatty acid composition in the present study; other factors could also have played a role. Fatty acid composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Zenebe, 2010). The present study was conducted in warm season i.e between the month of January and April. Lipid composition of farmed fish is more constant and less affected by seasonal variations than that of wild fish as its flesh fatty acid profile directly reflects the fatty acid composition of the diet (Ng *et al.,* 2007). However, the cold the water temperature, the more efficient fish are at converting saturated fatty acids into mono saturated and polyunsaturated fatty acids. This is possibly due to need to keep cell membranes fluid at lower temperatures and polyunsaturated fatty acids provide greater membrane fluidity (Desilva *et al.,* 1997).

In relation to the duration of feeding, Justi *et al.,* (2003) found that in Nile tilapia, the length of the feeding time (in a period of 30 days) is directly related to the incorporation of n-3 PUFA into fillet, mainly for α-linolenic acid. Tonial *et al.,* (2009) demonstrated that 45 days is the shortest time period required for the inclusion of linseed oil in tilapia feeds to raise the nutritional value (n-6 to n-3 of muscle tissue) of Nile tilapia. Based on these studies, the length of feeding time (101 days) in present work was sufficient to raise the nutritional value of n-6 to n-3.

Carcass proximate composition

Carcass proximate composition was little affected by dietary and enzyme treatments. However, there was increase in protein and fat in final carcass composition (Table 4). The carcass crude protein content in all the treatments was markedly high (60.19% and 64.49%) and formed the largest portion of the dry matter. The high protein content in Nile tilapia as in the present study makes it important living resource of dietary protein (Zuraini *et al.,* 2006). The increase in protein content above the initial can be attributed to the optimum protein and energy in the feed and proper feeding of fish. However, in case of underfeeding or restricted food supply to the fish, fat reserves are first mobilized. This reaches a critical low value before proteins begin to be utilized, ultimately causing a reduction in protein contents (Hassan and Javed, 1999). In this study, fish were fed twice in a day at 5% body weight to optimise feed utilisation. The protein content of muscle tissue increases slightly at first during periods of heavy feeding, then fat content shows a marked and rapid increase (Hassan and Javed, 1999).

The carcass dry matter content was high in all the treatments (Table 4). However, in case of restricted feeding, as protein is being utilized, water moves-in to take its place. This shift results in increased water content of the fish body hence decrease in dry matter content. Fat and water to a certain degree substitute each other such that with increasing fat content, the protein content is reduced with a simultaneous increase in dry matter (Daudpota *et al.,* 2014). In farmed fish, less effort is needed to get food and this enhance adipose deposition resulting in decreased water contents in the fish body (Rasmussen, 2001).

Lipids are the most important constituents which determine the quality of fish meat. In the present work, trends in the whole body lipid concentration slightly varied among the treatments. The marked difference in lipid content of fish may be due to effect of crude papain enzyme in the feed which might have enhanced fat deposition due to increased feed efficiency (Kirimi *et al.,* 2019). Though they have been described as lean fish, Tilapias store most of the dietary fat (Viola, 1988). This is because 40% of the body fat in tilapia is distributed around the viscera while muscle contains only 8% of the total fat (Hanley, 1991). Based on this, the fat content recorded in this study could be only 8% because viscera fat was discarded. The whole body lipid stores are influenced more by energy intake than by dietary lipid levels (Shearer, 1994). Excess energy in the diets may result in excessive deposition of carcass lipids. However, in the present study energy content in the diets (Table 1) was balanced across the treatments.

The ash content ranged from 13.24% to 14.50% in all the treatments. The results reveal significant difference (p<0.05) in body ash content between treatments. The ash content in this study indicates the presence of different minerals and

according to Murray and Burt (2001) constitutes 1-2% of the edible portion in fish hence a good source of minerals. The narrow range of final carcass proximate composition in the present study could have been attributed to narrow range of dietary nutrients treatments. However, in case of increasing protein levels in the diets, there is increase in whole body protein and decrease in lipid content due to the high carbohydrate and low protein content in the diet having low protein concentration (Daudpota *et al.,* 2014).

Sensory characteristics

There were variations observed in the organoleptic quality of Nile tilapia fed diets with and without enzymes. However, to the best of knowledge, no similar studies on the effect of crude papain enzyme on sensory traits of fish are available. In terms of general appearance, there was significant differences among the treatments (p<0.05). Fishmeal based diet scored higher numerically with sunflower based diet scoring lowest (Table 6). Fish on FM based diet were brighter in colour than those on oilseed meals. This can be attributed to the quality of FM based diet as appearance of coat colour reflects the quality of feed (Grigorakis *et al.,* 2003; Zenebe, 2010). With reference to the textural impression, all the treatments followed the same trend (p>0.05), with sunflower based diet recording firmer and more rigid fresh than others. This could probably be due to the slight variation in crude fibre and fat. Differences in texture of fish muscle tissue are related to the lipid, protein, fibre and water contents (Grigorakis *et al.,* 2003).

Fresh fish has a mild delicious taste and smell that is attributed by various volatile and non-volatile organic compounds. There was no significant difference (p>0.05) in aroma (odor) of enzyme treated fish whereas variation was observed as a result of different protein sources (p<0.05), for soybean meal, canola meal and sunflower meal. This agrees with study done by Regost *et al.,* (2003) who found significant difference in the odour of fish fed diets containing soybean oil and fish fed 100% fish oil. However, study done by Bjerkeng *et al.,* (1997) on organoleptic properties of juvenile Nile tilapia showed that use of soybean meal had no effect on flesh quality in terms of general appearance and organoleptic properties. In the present study, although all fish were fresh, FM based diet scored higher (4.63) for taste than the other treatments. In his study, Givens (2002) argued that feeding fish with vegetable based diet as in the present work produces a 'flat taste' to the cooked meat and over softens the texture that lead to oozing out. This could have contributed to the FM based diet being preferred more in terms of taste. Lipids noticeably influence the sensation of cooked fish in the mouth of the consumer. Fat-rich tissues usually taste very smooth and succulent (juicy), while on the contrary, when fat levels are low, the sensation of dryness or fibrousness (rough or coarse) describes the tissue better (Gustone, 2006). Consumer preference is biased towards meat that is tenderer. During the chewing process, fat is released, which stimulates salivation and increases the perception of both juiciness and tenderness. Hence, as a result of the lubricating effect of fat, meat with an increased content of fat is perceived as juicier and tenderer (Gustone, 2006). This could be the probable reason for FM based diet meat being juicier. In terms of the overall impression of the fish (acceptability) based on the above attributes, FM diet seemed to be more acceptable, closely followed by canola meal diet. SBM based diet was least preferred. This preference was expressed mainly in relation to the taste, juiciness and aroma.

Conclusion

There was slight increase in carcass proximate and fatty acid composition on oilseed meal substitution. However, crude papain enzyme resulted to increase in both saturated and monosaturated fatty acid and decrease in total polyunsaturated fatty acid, desirable in human health. In terms of sensory characteristics, crude papain enzyme supplemented fish were less preferred. Therefore, more research needs to be carried out on the effect of crude papain enzyme on the carcass fatty acid and sensory properties of farmed fish.

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