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ORIGINAL ARTICLE

Nutrients digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed on oilseed meals with crude papain enzyme

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Abstract

In this study, nutrients digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed on oilseed meals with crude papain enzyme were determined. A control diet (D1) of 30% crude protein (CP) was formulated using fishmeal (FM), soybean meal (SBM), canola meal (CM) and sunflower meal (SFM). The test diets were formulated with replacing 10% CP of FM by SBM (D2), CM (D3) and SFM (D4), respectively. Crude papain enzyme in powder form was incorporated at 0.06%. Digestibility trial was performed using chromium oxide as an inert marker. A feeding trial of 101 days was conducted using a 4 × 2 factorial design with 4 diets (D1, D2, D3 and D4) and 2 papain enzyme inclusion levels at 0.06% and 0% using 720 Nile tilapia fingerlings (7 ± 3 g). The fingerlings were randomly distributed into 8 groups of 3 replicates of 30 fingerlings per net hapa (2 × 1 × 1 m^3). Fish were fed twice daily at 5% of their biomass at 10 AM and 4 PM in two equal meals. Apparent digestibility coefficients increased ($p < 0.05$) on crude papain enzyme supplementation. There was increase in final body weight (47.32 ± 2.10 g) on 0.06% enzyme than without enzyme (0%) (46.17 ± 2.14 g) ($p > 0.05$). Irrespective of enzyme supplementation, fish fed FM-based diet were larger (56.89 ± 1.37 g) ($p < 0.05$) than those fed on oilseed meals; SBM (45.59 ± 0.91 g), CM (43.89 ± 2.12 g) and SFM (40.59 ± 1.60 g). Based on the study, 0.06% crude papain enzyme inclusion increased nutrients digestibility and growth performance of Nile tilapia. Therefore, crude papain enzyme is recommended as a feed additive in Nile tilapia diets to promote growth. However, more research is recommended to determine optimum inclusion levels of crude papain enzyme in Nile tilapia diets.

KEYWORDS
amino acid, body weight, dietary protein, proteolytic enzyme

1 | INTRODUCTION

In Kenya, aquaculture is the fastest growing subsector due to increased demand for fish and fish products. With the surging demand for locally farmed fish, innovative technologies have emerged to boost aquaculture production (Munguti et al., 2022). Increased aquaculture production should positively correlate with the production of quality feeds to meet the nutritional requirements of cultured fish (Agbo, 2008; Machena & Moehl, 2001). Dietary protein is the major and most expensive component of formulated aquatic feeds (Wilson, 2002). Fishmeal (FM) is the desirable animal protein ingredient in fish feeds due to high protein content, balanced amino acid profile, high digestibility, palatability and essential fatty acids (Hardy & Tacon, 2002; Kirimi, Musalia, Magana, et al., 2016). However, substitution of FM by plant protein ingredients is a necessity, being driven by both economic and sustainability issues (Shahabuddin et al., 2015). The use of plant protein sources in fish feeds has expanded considerably in recent years to meet the demand for feeds and sustain aquaculture production (Tacon & Metian, 2015). However, deficiency in essential amino acids like lysine, methionine and cysteine is a major limitation with plant protein sources (Ogunji et al., 2008; Kirimi et al., 2020).

Oilseed meals (soybean meal [SBM], canola meal [CM] and sunflower meal [SFM]) are alternative plant ingredients for replacing FM in fish diets as they are readily available in Kenya (Kirimi et al., 2022). SBM is suitable ingredient for replacing FM in fish diets because of high protein content, highly digestible, relatively well-balanced amino acid profile and reasonable price (Kikuchi, 1999; National Research Council, 1993; Storebakken et al., 2000; Kirimi et al., 2020). CM is second to SBM as the most commonly fed protein feedstuff in animal diets (Newkirk, 2009). The seed contains 21% crude protein (CP), whereas CM contains approximately 36% CP (Naczk et al., 1998; Uppstrom, 1995). Sunflower is cultivated extensively due to its adaptability to a wide range of climatic and soil conditions (Ravindran & Blair, 1992). Its seeds are inexpensive to process, and the cake remaining after oil extraction is used as a protein supplement in animal diets (Daghir et al., 1980; Maina et al., 2007). The CP content of sunflower cake ranges from 25% to 45% (air-dry basis) depending on the extent of dehulling and the efficiency of the oil extraction process (Maina et al., 2007).

However, concerns regarding low nutrient digestibility in plant protein ingredients has led to an increasing interest in feed enzymes. Not all compounds in fish feed are broken down by digestive enzymes; hence, some nutrients can be unavailable (Plumstead, 2013). Enzymes are protein in biological systems which catalyse the rate of a reaction but are not themselves altered. They are involved in all anabolic and catabolic pathways of digestion and metabolism (Kirimi et al., 2019; Khattak et al., 2006). Papain is a proteolytic enzyme that can break down peptide bonds of a protein molecule (Aravind et al., 2013; Kirimi et al., 2019). When used in fish feed, papain enzyme could increase digestion rate, catalyse protein hydrolysis into amino acids and subsequently increase protein absorption in the digestive tract of fish (Hamid et al., 2022; Rostika et al., 2018). This will result in increased feed efficiency, improved growth and body deposition (Kirimi et al., 2022). In Kenya, crude papain enzyme can be extracted from unripe fruits of *Carica papaya* (paw paw) plants that are readily available.

Nile tilapia is the most cultured species in Kenya, accounting for 80% of national aquaculture production but faces challenges of access to quality feeds (Munguti et al., 2022). Few studies have been performed with Nile tilapia to evaluate the effect of crude papain enzyme on growth performance. This study was therefore conducted to evaluate the effect of crude papain enzyme inclusion on oilseed meal-based diets on nutrient digestibility and growth performance of Nile tilapia.

2 | MATERIALS AND METHODS

2.1 | Ethics approval statement

The study protocol was reviewed and approved by Chuka University and granted permit (permit number: NACOSTI/P/17/40298/16344) by the National Commission for Science, Technology and Innovation (NACOSTI), Kenya.

2.2 | Preparation of diets, collection and enzymatic testing of crude papain enzyme

Feed ingredients were sourced from local feed dealers. A control diet (D1) of 30% CP was formulated using FM, SBM, CM and SFM. The test diets were formulated by replacing 10% CP of FM by SBM (D2), CM (D3) and SFM (D4), respectively (Table 1), using an Excel spreadsheet. Chromic oxide was incorporated at 1%. Crude papain was collected from locally grown *C. papaya* (paw paw) plants and sun dried at 40°C for 14 h (Adu et al., 2009). Protease activity of crude papain extract was determined using Hammersten casein as substrate (Macalood et al., 2013) and supplemented at the rate of 0.06% (Kirimi et al., 2019). The enzyme in powder form was dissolved in 50 ml water and then reconstituted to 500 ml of water. The solution was mixed thoroughly with the prepared marsh feed to form a paste, pelleted and dried.

2.3 | Proximate and amino acids analysis of ingredients and diets

The proximate analysis was carried out following the procedure AOAC (1995). Amino acid analysis was performed using an MPA FT-NIR spectrometer (Bruker, Germany) which is a non-destructive method of analysis. Approximately 30–50 g of sample was put into the sample cup and then into integrating sphere for measurement. Samples were analysed in triplicates for calibration and cross validation of the calibration performed (Osborne, 2006; Kirimi et al., 2020).

TABLE 1 Ingredients and proximate composition (%) of the diets supplemented to Nile tilapia containing soybean meal, canola meal and sunflower meal as a replacement of fish meal

Diet (D)	D1	D2	D3	D4
Ingredient				
Fish meal	16.5	9	9	9
Soybean meal	13	24	15	16
Canola meal	16.5	16	31	15
Sunflower cake	18	18	18	42
Maize grain	18	16	13	10
Wheat bran	17	16	13	7
Crude papain enzyme	0.06	0.06	0.06	0.06
Chromic oxide	1	1	1	1
Total	100.06	100.06	100.06	100.06
Analysed proximate composition (%)				
Dry matter	90.90 ± 0.07	91.31 ± 0.16	91.00 ± 0.09	91.56 ± 0.19
Crude protein	30.57 ± 0.43	30.76 ± 0.53	30.34 ± 0.31	31.35 ± 0.33
Crude fat	7.55 ± 0.27	7.67 ± 0.18	10.75 ± 0.28	9.63 ± 0.18
Ash	6.16 ± 0.03	5.60 ± 0.24	5.40 ± 0.21	5.81 ± 0.17
Crude fibre	11.06 ± 0.08	12.18 ± 0.12	13.37 ± 0.17	16.03 ± 1.00

Note: Values are mean ± SE of triplicate samples. Diet code: D1, fishmeal-based diet; D2, soybean meal-based diet; D3, canola meal-based diet; D4, sunflower meal-based diet.

2.4 | Growth performance trial

The experiment was conducted at the Fisheries Department, Meru County, Kenya. In a 4 × 2 factorial design, the study was based on four diets {FM (D1), SBM (D2), CM (D3) and SFM (D4)} with papain enzyme levels of 0% and 0.06%. The experiment was conducted in 24 net hapas dimension 2 m × 1 m × 1 m mounted in a liner pond (20 m × 15 m × 1.2 m). A total of 720 sex-reversed Nile tilapia fingerlings from Sagana fish farm of average weight 7 ± 3 g were selected and acclimatized for 2 weeks during which time fish were fed on commercial fish feed (Aller Aqua). Feeding with the experimental rations begun after the initial weight of the fish was taken. After acclimatization, the fingerlings were randomly picked and transferred into the 24 hapa nets at a rate of 30 fingerlings per unit net hapa. They were further divided randomly into eight groups with three replicates each.

2.5 | Feeding and data collection

After the adaptation period, the initial weight was measured, and the fish were offered the experimental diets. Feed was provided fish at a rate of 5% of body weight throughout the experimental period twice daily, that is morning (10 AM) and evening (4 PM) in two equal meals. The amount of feed provided was adjusted accordingly after weighing the fish at each sampling done fortnightly for the entire experimental period. Water parameters were monitored weekly (dissolved oxygen, pH and temperature) using multiparameter water quality meter, Hanna D.O. model H19147. Weight (g), total length (mm) of fish and feed consumed data were recorded.

Fish growth parameters were calculated as follows:

1. Daily weight gain (DWG %) = [(final body weight − initial body weight)/(initial body weight × days of experiment)] × 100.
2. Relative growth rate (RGR %) = $((W_f - W_i)/W_f) \times 100$, where W_f is the final average weight at the end of experiment; W_i is the initial average weight at the beginning of experiment (Otubusin et al., 2009).
3. Specific growth rate (SGR %/day) = [(L_n final body weight − L_n initial body weight) × 100]/experimental period, where L_n is the natural logarithm (Khalafalla & El-Hais, 2013).
4. Feed utilisation efficiency (FE) (%) = [weight gain (g)/feed intake (g)] × 100 (Guroy et al., 2005).
5. Survival rate SR (%) = (initial number of fish stocked − mortality)/initial number of fish stocked × 100 (Charo et al., 2006).

2.6 | Digestibility trial

The digestibility study started at the end of the growth performance trial using fish weighing 40 ± 2 g selected from each treatment and transferred into glass aquaria of dimensions 0.9 m × 0.6 m × 0.5 m in triplicate. The apparent digestibility coefficients (ADCs) of the test diets were measured by an indirect method using chromic oxide as an external marker at 1% level (Table 1). The experiment took 30 days of

faecal collection using Nile tilapia fish weighing 40 ± 2 g. Fish were acclimated to the experimental system for 7 days before the start of the experiment after which they were fed to satiation twice a day on each of the experimental diets. Faecal collection started 4 days after feeding test diets to allow evacuation of all previously ingested materials. The aquaria were cleaned to remove any uneaten feed and water completely exchanged in each glass aquarium every day. Faecal collection was done manually by siphoning and straining through a fine-meshed net (Baruah et al., 2007). Faecal matter collection from each aquarium was pooled and oven dried at 50°C for 5 h. The samples were analysed in triplicates for nutrients (dry matter, CP, ether extract and nitrogen free extracts) following the procedure by AOAC (1995). Chromic oxide in the diets and faeces was determined according to the method of Furukawa and Tsukahara (1966). The procedure involved the digestion of the sample by concentrated nitric acid and oxidising chromic oxide with 70% perchloric acid. Chromic oxide was calculated using the following formula:

$$\text{Chromicoxide}(\%) = [\{(\text{absorbance} - 0.0032)/0.2089\}/\text{sampleweight}] \times 100$$

ADCs for dry matter, CP, crude lipid and nitrogen free extracts in the diets were determined using the following formula:

Apparent nutrient digestibility (AND) (%) = 100 − {100 − (% chromium oxide in feed/% chromium in faeces) × (% of nutrient in faeces/% of nutrient in diet)} (De Silva, 1989; Bureau et al., 2002).

2.7 | Data analysis

Data on ANDs and growth performance parameters 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.

The basic linear model for 4 × 2 factorial design was as follows:

$$Y_{ijkl} = \mu + A_i + B_j + (AB)_{ij} + e_{ijkl}$$

where Y_{ijkl} is the observation on the ith EU; μ is the overall population mean; A_i is the effect due to diet; $i = 1–4$; B_j is the effect due to papain enzyme $j = 1–2$; $(AB)_{ij}$ is the effect of interaction of the diet and papain enzyme; and e_{ijkl} is the random error term.

3 | RESULTS

3.1 | Ingredients proximate composition

Results of the proximate nutrient composition of feed ingredients are as shown in Table 2. FM had highest CP content (62.6%) ($p < 0.05$). SFM recorded highest figures for crude fibre (36.4%) and acid detergent fibre (22.5%) ($p < 0.05$). CM had the highest lipid content (23.9%) and wheat bran lowest (4.3%) ($p < 0.05$). Maize meal recorded lowest figures for CP (10.7%) ($p < 0.05$).

3.2 | Amino acid composition of ingredients

Amino acid compositions of feed ingredients are shown in Table 3. Amino acid analysis revealed that fish meal (*Rastrineobola argentea*) recorded the highest level for essential amino acids ($p < 0.05$). Fish meal had the highest level of lysine (7.81 mg/100 g) followed by CM (4.01 mg/100 g), with maize meal recording the lowest values (1.42 mg/100 g) ($p < 0.05$). Methionine content was higher in FM (2.89 mg/100 g) compared to oilseed meals ($p < 0.05$).

3.3 | Amino acid composition of diets

Amino acid composition of diets (mg/100 g) is shown in Table 4. Diet 4 had the lowest content of essential amino acid methionine (0.86 mg/100 g), lysine (6.83 mg/100 g), phenylalanine (2.54 mg/100 g), histidine (1.50 mg/100 g) and valine (2.40 mg/100 g) ($p < 0.05$). Diet 1 recorded the highest values ($p < 0.05$) for essential amino acid lysine (8.12 mg/100 g), phenylalanine (3.42 mg/100 g), histidine (2.32 mg/100 g), valine (2.79 mg/100 g) and threonine (2.65 mg/100 g). Methionine content of diet 2 was the highest (1.05 mg/100 g) followed by diet 1 (0.93 mg/100 g), and there was significant difference ($p < 0.05$).

3.4 | Protease activity of crude papain enzyme

The protease activity of crude papain enzyme was 1.9 U/mg protein.

3.5 | Physico-chemical parameters

Water temperatures in the hapas ranged from 22.1 to 29°C with a mean of 25.5°C (Table 5). The pH ranged from 7.4 to 10 mean of 8.7. Dissolved oxygen ranged between 2.5 and 5.3 mg/L with a mean of 3.9 mg/L.

3.6 | Apparent digestibility coefficients of diets

ADC for dry matter was statistically similar ($p > 0.05$), although D1 recorded the highest value (76.1%) (Table 6). ADCs for protein were significantly different ($p < 0.05$) with D1 recording 94.5%, D2 (93.6%), D3 (93.5%) and D4 (93.3%). Enzyme-treated diets recorded significantly higher ADC's for all the nutrients ($p < 0.05$).

TABLE 2 Proximate composition of feed ingredients (%) used to formulate diets for Nile tilapia

Ingredient	Fish meal	Soybean meal	Canola meal	Sunflower meal	Maize meal	Wheat bran	*p*-Value
Proximate composition (%)							
Dry matter	92.33 ± 0.25^{cb}	92.37 ± 0.07^{bc}	91.07 ± 0.05^{e}	94.42 ± 0.21^{a}	88.42 ± 0.11^{f}	90.10 ± 0.03^{d}	0.000
Crude protein	62.60 ± 0.38^{a}	47.38 ± 0.32^{b}	34.39 ± 0.18^{c}	24.81 ± 0.03^{d}	10.65 ± 0.27^{f}	16.04 ± 0.43^{e}	0.000
Ether extract	7.49 ± 0.32^{d}	9.27 ± 0.30^{c}	23.88 ± 0.24^{a}	13.31 ± 0.10^{b}	4.73 ± 0.23^{ef}	4.30 ± 0.17^{fe}	0.000
Ash	15.22 ± 0.59^{a}	8.96 ± 0.26^{b}	5.50 ± 0.26^{dec}	5.08 ± 0.14^{ed}	1.41 ± 0.19^{f}	6.17 ± 0.24^{cd}	0.000
Crude fibre	1.04 ± 0.09^{f}	15.88 ± 0.32^{bc}	15.58 ± 0.20^{cb}	36.38 ± 0.20^{a}	3.79 ± 0.28^{e}	14.41 ± 0.22^{d}	0.000
Nitrogen free extract	5.92 ± 0.32^{f}	10.88 ± 0.26^{ed}	11.72 ± 0.32^{de}	14.83 ± 0.47^{c}	67.85 ± 0.44^{a}	49.18 ± 0.75^{b}	0.000
Neutral detergent fibre	34.24 ± 0.20^{d}	28.16 ± 0.38^{e}	21.07 ± 0.14^{f}	43.03 ± 0.30^{b}	40.79 ± 0.23^{c}	46.95 ± 0.18^{a}	0.000
Acid detergent fibre	15.22 ± 0.21^{b}	9.89 ± 0.22^{e}	11.99 ± 0.22^{dc}	22.45 ± 0.27^{a}	3.52 ± 0.32^{f}	12.28 ± 0.19^{cd}	0.000

Note: Values are expressed as mean ± SE of triplicate samples. Values in the same row having different superscript letters are significantly different ($p < 0.05$).

TABLE 3 Amino acid composition (mg/100 g protein) of feed ingredients used to formulate diets for Nile tilapia

	Fish meal	Soybean meal	Canola meal	Sunflower meal	Maize meal	Wheat bran	*p*-Value
Essential amino acids							
Lysine	7.81 ± 0.07^{a}	3.01 ± 0.01^{d}	4.01 ± 0.01^{b}	3.14 ± 0.01^{c}	1.42 ± 0.00^{f}	1.75 ± 0.01^{e}	0.000
Methionine	2.89 ± 0.01^{a}	0.61 ± 0.01^{de}	0.61 ± 0.01^{ed}	0.51 ± 0.01^{f}	2.16 ± 0.01^{b}	1.44 ± 0.01^{c}	0.000
Cysteine	0.95 ± 0.01^{e}	0.66 ± 0.00^{f}	1.16 ± 0.00^{c}	1.54 ± 0.02^{b}	1.04 ± 0.00^{d}	1.82 ± 0.02^{a}	0.000
Histidine	2.43 ± 0.01^{b}	1.26 ± 0.01^{f}	1.57 ± 0.00^{e}	5.44 ± 0.01^{a}	2.12 ± 0.01^{c}	1.81 ± 0.01^{d}	0.000
Arginine	5.87 ± 0.04^{a}	3.39 ± 0.01^{b}	3.05 ± 0.02^{c}	2.96 ± 0.00^{d}	2.42 ± 0.01^{f}	2.81 ± 0.01^{e}	0.000
Threonine	4.28 ± 0.01^{a}	1.96 ± 0.04^{f}	2.1 ± 0.06^{e}	3.87 ± 0.00^{b}	2.6 ± 0.01^{d}	3.16 ± 0.01^{c}	0.000
Valine	5.4 ± 0.01^{b}	2.24 ± 0.01^{f}	2.34 ± 0.01^{e}	6.27 ± 0.02^{a}	4.09 ± 0.01^{d}	4.93 ± 0.01^{c}	0.000
Isoleucine	4.55 ± 0.07^{a}	2.36 ± 0.01^{c}	2.55 ± 0.01^{e}	0.97 ± 0.02^{f}	3.26 ± 0.01^{d}	3.83 ± 0.01^{b}	0.000
Leucine	7.55 ± 0.04^{b}	3.69 ± 0.01^{f}	3.78 ± 0.01^{e}	10.06 ± 0.01^{a}	7.15 ± 0.01^{c}	6.85 ± 0.02^{d}	0.000
Phenylalanine	4.2 ± 0.01^{c}	2.71 ± 0.02^{f}	3.84 ± 0.01^{d}	5.83 ± 0.01^{a}	4.24 ± 0.01^{b}	3.79 ± 0.01^{e}	0.000
Tryptophan	1.15 ± 0.01	0.68 ± 0.04	0.62 ± 0.02	ND	ND	ND	

Note: Values are mean ± SE of triplicate samples. Values in the same row having different superscript letters are significantly different ($p < 0.05$).
Abbreviation: ND, not detected.

3.7 | Growth performance

The highest final average body weight (56.9 g) was recorded in D1 with D4 recording lowest (40.6 g) ($p > 0.05$) (Table 7). However, D2 (45.6 g) and D3 (43.9) were statistically similar ($p > 0.05$). Survival rates were 97.2%–99.3% and statistically the same ($p > 0.05$) for all diets. Fish fed FM-based diet (D1) had highest daily weight gain (0.49 g/d) followed by D2 (0.37 g/d), D3 (0.36 g/d) and D4 (0.33 g/d) ($p < 0.05$). The highest feed conversion efficiency was recorded in D1 (0.44) and D4 recorded the lowest (0.36). Crude papain enzyme-treated diets had the highest final body weight (47.3 g) with none treated diets recording 46.2 g. Moreover, daily weight gain, specific growth rate and relative growth rate were higher in enzyme-treated diets ($p < 0.05$). However, feed conversion efficiency was similar ($p > 0.05$).

4 | DISCUSSION

4.1 | Apparent digestibility coefficients

The results of the present study indicate that the dry matter, protein, lipid and nitrogen free extracts of the experimental diets were well digested by Nile tilapia. Upon crude papain enzyme supplementation, ADC increased. Papain enzyme is a protease enzyme capable of

TABLE 4 Amino acid composition (mg/100 g protein) of diets for Nile tilapia containing either soybean meal (D2), canola meal (D3) or sunflower meal (D4) as replacements of fish meal (D1)

Diet (D)	D1	D2	D3	D4	*p*-Value
Essential amino acids					
Isoleucine	2.3 ± 0.01^{b}	1.34 ± 0.01^{c}	1.23 ± 0.01^{d}	1.42 ± 0.00^{a}	0.000
Leucine	4.43 ± 0.01^{a}	3.12 ± 0.00^{d}	3.89 ± 0.01^{b}	3.57 ± 0.01^{c}	0.000
Arginine	5.52 ± 0.01^{a}	4.55 ± 0.01^{d}	4.61 ± 0.01^{c}	4.73 ± 0.01^{b}	0.000
Valine	2.79 ± 0.01^{a}	2.46 ± 0.01^{b}	2.42 ± 0.00^{cd}	2.40 ± 0.01^{dc}	0.000
Methionine	0.93 ± 0.01^{bc}	1.05 ± 0.01^{a}	0.92 ± 0.01^{cb}	0.86 ± 0.01^{d}	0.000
Lysine	8.12 ± 0.01^{a}	7.35 ± 0.01^{b}	7.19 ± 0.01^{c}	6.83 ± 0.01^{d}	0.000
Phenylalanine	3.42 ± 0.01^{a}	2.57 ± 0.01^{bc}	2.56 ± 0.00^{cbd}	2.54 ± 0.01^{dc}	0.000
Histidine	2.32 ± 0.01^{a}	1.65 ± 0.00^{c}	1.70 ± 0.01^{b}	1.50 ± 0.01^{d}	0.000
Threonine	2.65 ± 0.01^{a}	2.30 ± 0.01^{b}	2.15 ± 0.01^{d}	2.25 ± 0.01^{c}	0.000

Note: Values are expressed as mean ± SE of triplicate samples. Values in the same row 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.

TABLE 5 Range and average of physico-chemical parameters of water during 101 days feeding trial

Parameter	Range mean	
Temperature (°C)	22.1–29.0	25.5
Dissolved oxygen (mg/L)	2.5–5.3	3.9
pH	7.42–10.01	8.72

hydrolysing protein complex compound into simple elements (amino acids) (Kirimi et al., 2019). Addition of papain enzyme into the feed improves the feed protein hydrolysis (Hamid et al., 2022; Rostika et al., 2018). This results in the increased feed digestibility hence improved amino acid absorption into the body for growth. El Moussaoui et al. (2001) and Azarkan et al. (2003) argued that crude papain may cause better absorption of amino acids because cysteine proteinases constitute as much as 80% of the enzyme fraction in papaya latex. Thus, it is clear from the ADC values recorded in the present study that 0.06% crude papain enzyme supplemented in the diets played a considerable role in the digestion process (Kirimi et al., 2019). The results agree with work done by Singh et al. (2011) and Muchlisin et al. (2016), where fingerlings of *Cyprinus carpio* and Keureling fish (*Tor tambra*) were supplemented with papain showed higher protein digestibility values. Rachmawati et al. (2018) also reported increased protein digestibility when papain enzyme was supplemented to post larvae of freshwater crayfish (*Cherax quadricarinatus*) at 0.2%, 0.3% and 0.4% of pure papain.

The apparent protein digestibility values for all the diets were above 90% which is in-line with National Research Council (1993) that the digestion coefficients for protein rich feed stuffs are usually in the range of 75%–95%. Although SFM-based diet had relatively high crude fibre of 16%, the ADC was high. This is because in tilapia, as in other species, protein digestion is relatively high, even in feed containing high fibre (Anderson et al., 1991). However, high dietary crude fibre content, as recorded in the present work, may accelerate the rate of passage of digesta through the intestinal tract, thus reducing the digestibility of protein (Hossain et al., 2000; Chi et al., 2017). Low digestibility values of plant-based ingredients are because of the high fibre content and anti-nutritional factors (Chi et al., 2017; Yu et al., 2013). Based on this, D4 recorded slightly low ADC, which might have been due to high concentration of crude fibre. Despite similar CP levels in the diets, there was slight variation in the apparent nutrient coefficients. This could be attributed to varying proportions of different ingredients which have different digestibility figures. Higher ADC values are obtained in diets with higher protein contents due to decrease in the proportion of metabolic faecal nitrogen with the rise in protein content in the diet (Jauncey, 1982). The highest protein digestibility was found in D1, likely because FM formed the bulk of protein. FM protein is highly digestible, hence being the preferred animal protein ingredient in fish feed (Madrid et al., 2022). Although FM is rich in protein and amino acids, its digestibility tends to be variable due to the production process and high keratin content in cysteine amino acid with strong disulphide bridges which make it resistant to digestive enzymes (Madrid et al., 2022; Pfeuti et al., 2019). The ADC for crude lipid in all the diets was relatively high and this agrees with Aksnes and Opstvedt (1998) that when lipid is administered either alone or in a mixed diet, it originates digestibility values ranging from 85% to 95% for fish.

The difference in protein digestibility may also be due to the method of faecal collection and fish species (Koproco et al., 2004). In relation to the technique of faecal collection employed, Cho et al. (1982) argued that the method used to determine digestibility can affect the value of the coefficients obtained. In their study, Singh and Nose (1967) established that digestibility estimations obtained with faecal collection from the tanks were 10% greater compared with that obtained by stripping, indicating that some nitrogen compounds were lost in the water. This could have contributed to the relatively high coefficients figures obtained in the present work as the breakup of

TABLE 6 Apparent digestibility coefficients (%) of oilseed meal diets with crude papain enzyme fed to Nile tilapia

	Diet (D)				Enzyme (E) (%)		*p*-Value		
	D1	D2	D3	D4	0	0.06	D	E	D × E
DM	76.98 ± 1.82^{b}	75.63 ± 2.01^{d}	77.50 ± 1.44^{a}	76.28 ± 1.60^{c}	72.97 ± 0.45	80.23 ± 0.47	0.215	0.000	0.492
CP	94.45 ± 0.94^{a}	93.63 ± 1.07^{bcd}	93.47 ± 1.11^{cbd}	93.29 ± 0.85^{db}	91.53 ± 0.22	95.88 ± 0.19	0.015	0.000	0.309
EE	92.13 ± 0.42^{d}	94.34 ± 0.48^{ba}	94.80 ± 0.79^{ab}	93.47 ± 0.31^{c}	92.71 ± 0.31	94.66 ± 0.42	0.000	0.000	0.040
NFE	77.35 ± 0.44^{cb}	78.17 ± 0.95^{ab}	76.44 ± 1.74^{d}	77.79 ± 0.21^{bca}	75.71 ± 0.57	79.17 ± 0.38	0.002	0.000	0.000

Note: Values are expressed as mean ± SE of triplicate samples. 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: CP, crude protein; DM, dry matter; EE, ether extract; NFE, nitrogen free extracts.

TABLE 7 Growth performance parameters of Nile tilapia fed on oilseed meals with crude papain enzyme

	Diet (D)				Enzyme (E) (%)		*p*-Value		
	D1	D2	D3	D4	0	0.06	D	E	D × E
IBW	7.32 ± 0.85^{a}	7.42 ± 0.06^{a}	7.36 ± 0.08^{a}	7.54 ± 0.06^{a}	7.43 ± 0.05	7.39 ± 0.06	0.197	0.670	0.503
FBW	56.89 ± 1.37^{a}	45.59 ± 0.91^{bc}	43.89 ± 2.12^{cb}	40.59 ± 1.60^{cd}	46.17 ± 2.14	47.32 ± 2.10	0.000	0.512	0.987
DWG	0.49 ± 0.01^{a}	0.37 ± 0.01^{b}	0.36 ± 0.02^{c}	0.33 ± 0.01^{d}	0.38 ± 0.02	0.39 ± 0.02	0.000	0.514	0.997
SGR	0.88 ± 0.01^{a}	0.78 ± 0.01^{bc}	0.77 ± 0.02^{cbd}	0.72 ± 0.02^{dc}	0.78 ± 0.02	0.79 ± 0.02	0.000	0.516	0.972
RGR	86.90 ± 0.47^{a}	83.68 ± 0.35^{bc}	83.03 ± 0.88^{cbd}	81.26 ± 0.88^{dc}	83.42 ± 0.79	84.02 ± 0.74	0.001	0.433	0.960
SR	97.78 ± 1.41^{a}	98.67 ± 0.84^{a}	99.33 ± 0.67^{a}	97.22 ± 2.77^{a}	99.11 ± 0.62	97.39 ± 1.44	0.822	0.325	0.624
FCE	0.44 ± 0.01^{ac}	0.38 ± 0.01^{bcd}	0.39 ± 0.02^{cabd}	0.36 ± 0.02^{dbc}	0.39 ± 0.01	0.39 ± 0.01	0.049	0.923	0.947

Note: Values are expressed as mean ± SE of triplicate samples. 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: DWG, daily weight gain; FBW, final body weight; FCE, food conversion efficiency; IBW, initial body weight; RGR, relative growth rate; SGR, specific growth rate; SR, survival rate.

faeces by fish movement may have led to leaching of nutrients and, therefore, an over estimation of digestibility. However, to minimize this, upon removal of uneaten feed, faeces were continuously siphoned out from the glass aquaria. According to Bureau and Hua (2006) and Forster (1999), variability in apparent digestibilities may also be due to differences in chemical composition, origin and processing methods of various feed ingredients. To account for these issues, the ingredients used to formulate the four diets and their processing were the same.

4.2 | Growth performance

In this study, water quality parameters (temperature, pH and dissolved oxygen) were within recommended range for optimum biochemical reactions in digestion and metabolic utilization of feeds to enhance growth rate (Table 5). There was a slight increase in growth performance when 0.06% crude papain enzyme was incorporated in the diets. This can be attributed to the addition of crude papain enzyme in the diets which increased nutrient availability. Papain is a protease enzyme that hydrolyses proteins to short peptides in the diet, which is a key factor to increase protein digestibility and absorption. This helps to increase growth factors through fast metabolism (Wong et al., 1996). The protease enzyme from papain is effective in reducing the energy for activating metabolism process hence increase in growth rate (Rostika et al., 2018).

Results of the present study were in-line with those conducted by previous researchers. Nile tilapia yielded better results in terms of per cent weight gain and specific growth rate in diets supplemented with crude papain in the form of papaya leaf (Munguti et al., 2014; Hamid et al., 2022). Better growth performance of common carp was also reported when fed with 3% papain in feed and 10% papaya leaf mixed with feed, which was attributed to increased protein digestion due to papain (Tagare, 1992). Diet supplementation with 0.1% of papain resulted in better growth of post larvae of freshwater prawn (Patil & Singh, 2014). However, these studies lacked information on protease activity data for the papain enzyme to justify and standardize concentration to be used in Nile tilapia diets, hence likely under or over estimating it. In contrast, protease activity in the present study at the inclusion level of 0.06% was determined (1.9 U/mg). Despite low weight gain in addition to crude papain enzyme, there was no preference for diets with or without the enzyme. Thus inclusion level of 0.06% had no negative effect on palatability of the feed. The survival rate was high, ranging from 97% to 99% due to the fish positive reactions to the enzyme added feed. Therefore, crude papain extract had no negative effect on survival rate, and this corroborates the studies by Singh et al. (2011) and Hamid et al. (2022) where a similar trend was observed.

The markedly low growth performance in the diets despite crude papain enzyme supplementation could be attributed to crude fibre content in the diets (Table 1). Fibre reduces enzyme activities (Moron et al., 1989; Boisen & Eggum, 1991; Kirimi et al., 2019). Thus, the activity of papain enzyme could have been inhibited by dietary fibre in the diets, somehow explaining the low growth observed. The addition of protease enzyme at the right dose to the feed is helpful in accelerating fish growth (Rostika et al., 2018). However, considering the growth performance results in this study, it is not possible to affirm if papain enzyme dosage was insufficient or if the enzyme added in the diets was masked by the fibre content. Different ingredients were used to formulate the diets in order to balance and compensate for their deficiency in amino acids (Kirimi et al., 2020). However, this could have negatively impacted the activity of the enzyme; hence, the low growth observed. Cross-binding of proteins from different ingredients yields fewer degradable reaction products as a result of the diet formulation process (Tonheim et al., 2007; Kirimi et al., 2019).

Regardless of crude papain enzyme supplementation, D1 performed better compared to the oilseed meals (D2, D3 and D4)-based diets. This can be attributed to the high level of FM which formed the bulk of the 30% CP in D1. The quality of feed is a function of how well it meets nutrients requirement of fish (Desilva & Anderson, 1995). The results are in agreement with Hardy and Tacon (2002) and Kirimi, Musalia and Munguti (2016) that fish meal is the most desirable animal protein ingredient in aqua feeds because of its high protein content, balanced amino acid profile, high digestibility, palatability and $n-3$ polyenoic fatty acids. However, high costs and scarcity are the major limitations to its use (Kirimi, Musalia & Munguti, 2016).

There was a decline in growth performance of Nile tilapia on substitution of FM (*R. argentea*) with oilseed meals (D2, D3 and D4). However, among the oilseed meal-based diets, SBM (D2) performed better in terms of growth rate. The low growth performance observed in SBM-based diet (D2) compared to FM diet (D1) could be attributed to amino acid imbalances, especially methionine and isoleucine which were the most limiting. This study agrees with research by Jackson et al. (1982) who reported growth reduction when 50% or more FM was replaced with SBM, attributed to the methionine deficiency and the presence of trypsin inhibitors. Growth reduction in the hybrid tilapia when SBM substituted 24% of FM at 30% and 32% dietary protein level was recorded (Shiau et al., 1987). Liti et al. (2006) reported that SBM could fully substitute FM without a significant reduction in tilapia growth if the diets contained suboptimal (24%) levels of protein. Based on the present study, SBM cannot totally replace FM in Nile tilapia diets because 10% CP replacement of FM with SBM depressed growth. The contradiction among researchers regarding the use of SBM as a protein source for fish may be related to the quality and processing of SBM, fish species, size and culture systems (Ogello et al., 2014). It is unquestionable that substituting FM with oilseed meals reduced the growth performance of Nile tilapia. Substituting animal protein with plant protein at higher levels than the optimal dietary protein reduces the growth of tilapia, whereas growth is not affected below the optimal levels (Liti et al., 2006).

CM-based diet (D3) performed better than sunflower (D4). The decrease in growth of fish with increased CM level in the diet can be attributed to the suboptimal levels of the essential amino acid methionine and isoleucine. Moreover, the mustard smell of CM-based diet was still noticeable, and this might have adversely affected the acceptance of diets. Despite lower glucosinolate content in CM, the typical mustard smell is known to affect acceptance by fish (Adem et al., 2014). A decrease in growth performance has been reported at an inclusion

level of 31% (10% of the 30% dietary protein) in the present study. This is in contrast with Enami (2001) who indicated that protein from canola can replace up to 10% of protein from FM in the diets for tilapia. Jackson et al. (1982) reported a significant reduction in weight gain with rapeseed inclusion levels of 63% and higher.

From all oilseed meals tested to replace FM, sunflower-based diet (D4) was the one that performed more poorly. Decline in performance was likely due to their poor amino acid profile, namely in methionine and isoleucine. Moreover, the high crude fibre content in the diet might have led to the low growth performance. Increased dietary fibre can affect growth in tilapia because it reduces the total dry matter and lowers the digestibility of nutrients (Shiau & Kwok, 1989). The recommended fibre content in fish feed is 8%–12% (De Silva & Anderson, 1995; Lovell, 1998). Jackson et al. (1982) reported good growth in tilapia (*Sarotherodon mossambicus*) fed rations containing 35.2% SFM replacing 50% of the fish meal protein.

Generally, there was a progressive mean weight gain observed in all the dietary treatments due to their high nutritional composition, and a feature that promotes better growth and higher yields in fish (Madu et al., 2003). It is worth noting that vitamin and mineral premix was deliberately left out in the present experiment during diet formulation. This was to avoid introduction of exogenous amino acids present in most premixes in order to minimise sources of variation. However, the fertilized liner pond offered fish extra nutrition from natural food. This source of nutrients may have provided an extra supply of limiting essential amino acids, although it could not compensate for the deficient nutrients in oilseed meal-based diets (Kirimi, Musalia, Magana, et al., 2016; Kirimi et al., 2020; Munguti et al., 2014). The weight of fish at the beginning of the experiment was not significantly different, but mean final body weight within the same group was high. This large fluctuation in final weight gain in the same group, despite their identical dietary treatment, might have been due to inbreeding of brooder stock in the hatchery. Feed conversion efficiency varied among diets, with FM-based diet recording the highest values. This was likely a result of improved nutrient and energy utilization by fish fed this diet. However, the values obtained ranged between 0.36 (36%) and 0.44 (44%). According to Rostika et al. (2018), feed might be considered in good category when the feed efficiency value is above 50%. Thus, the feed efficiency recorded cannot be considered good.

5 | CONCLUSION

Crude papain enzyme has a potential to be used in Nile tilapia diets to promote growth. Oilseed meal-based diets were inferior compared to FM diet (D1), but upon crude papain enzyme supplementation, nutrients digestibility and growth performance increased. However, more research is needed to determine the optimal inclusion level of crude papain enzyme in Nile tilapia diets. Additional efforts should also be made to reduce the fibre content in the diets as it negatively affects enzyme activity.

AUTHOR CONTRIBUTIONS

James G. Kirimi. Conceptualization; Formal analysis; Funding acquisition; Methodology; Project administration; Writing-original draft; Writing-review & editing: Levi M. Musalia. Conceptualization; Funding acquisition; Supervision; Writing-original draft: Adiel Magana. Methodology, Supervision, Validation: Jonathan M. Munguti. Conceptualization, Methodology, Supervision, Validation

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CONFLICT OF INTEREST

We certify that there is no conflict of interests among the authors. Further authors declare no conflict of interest.

ETHICS STATEMENT

We certify that this is our original scientific research work, and it has not been submitted or published anywhere. The authors are responsible for all the content in the manuscript. We certify that the current study followed all the applicable guidelines for the care and use of fish.

DATA AVAILABILITY STATEMENT

We certify that the data used in this article were collected from the study and can only be availed through the request and permission of the third-party authors.

PEER REVIEW

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