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Fatty acids composition of Nile tilapia *(Oreochromis niloticus)* fingerlings fed diets containing different levels of water spinach *(Ipomoea aquatica)*

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ABSTRACT

Fish is a major source of n-3 LC-PUFA for humans. Fatty acids especially n-3 and n-6 polyunsaturated fatty acids (PUFAs) play important role in human health. This study was conducted to evaluate the effects of different inclusion levels of *Ipomoea aquatica* on fatty acids composition of *Oreochromis niloticus* fingerlings. Five diets containing 0%, 5%, 10%, 15% and 20% *Ipomoea aquatica* were formulated. The results indicated that 18 types of fatty acids with different saturation levels were detected. Total saturates, n-3 PUFAs, n-6 PUFAs in all the tissues were not significantly affected by the different levels of *I. aquatica*. Fish fed 10% diet recorded the highest level of muscle docosahexaenoic acid (DHA). The tissue composition of docosahexaenoic acid (DHA) was significantly higher than eicosapentaenoic acid (EPA). There was an increase in PUFAs with increased levels of *I. aquatica*. There was no significant difference (P *>* 0.05) in fatty acids in all the tissues. The study suggests that 20% dietary inclusion of *I. aquatica* resulted into high DHA in all tissues thus *I. aquatica* can be used to increase fatty acid.

1. Introduction

Fish is one of the most important protein sources for the population worldwide hence the needs to increase fish production to meet the increasing demand for protein. Due to rapid expansion of aquaculture, fish feed is considered as an essential component, which constitutes over 50–70% of total operating cost in aquaculture [[1](#page-6-0)]. Although fish farming contributes to the global production of n-3 LC-PUFA to meet human dietary requirements, marine ingredients, fish meal and oil are the only raw materials in aquafeeds that can supply n-3 LC-PUFA to farmed fish. The use of expensive and limited fish meal and fish oil to maintain n-3 LC-PUFA levels in farmed fish is not a sustainable approach [[2](#page-6-0)]. Obtaining alternative sources of n-3 LC-PUFA from other non-conventional sources such as aquatic macrophytes is promising in fish farming because they are in mass supply [\[3\]](#page-6-0).

Freshwater fishes contain saturated fatty acids (SFA), monosaturated fatty acids (MUFAs) and long-chain polyunsaturated fatty acids (PUFAs) that are important in human health. They help in prevention of diseases [[4](#page-6-0)], neural and immune development [\[5\]](#page-6-0), inflammatory, cardiovascular and neurological diseases [\[6\]](#page-6-0) and some types of cancers including prostate cancer [\[7\]](#page-6-0). PUFAs are grouped into two mainly omega-3 and omega-6 depending on the position of double bond from the methyl end group of the fatty acids [[8](#page-6-0)]. The main n-3 PUFAs are ɑ-linolenic acid (ALA), docosahexaenoic acids (DHA), eicosapentaenoic acid (EPA) and docosapentaenoic (DPA) which are important in human health, n-6 PUFAs include linoleic acids (LA) and arachidonic acid (ARA) [\[8\]](#page-6-0). These PUFAs are not synthesized in human body and hence must be supplemented in the diet [[9\]](#page-6-0).

Dietary lipids acts as a source of fatty acids, phospholipids, steroids and fats soluble vitamins required for proper functioning of physiological processes [[10\]](#page-6-0). However, excess dietary lipids decreases feed consumption and utilization of other nutrients resulting in reduced growth rates [[10\]](#page-6-0) and increased fat deposition [\[11](#page-6-0)] which should be considered during feed formulation. Tilapia need higher amount of n-6 FA compared to n-3 FA for maximum growth [\[12\]](#page-6-0) with a dietary requirement of approximately 1% of n-6 FA in their diets [[13\]](#page-6-0).

Water spinach (*Ipomoea aquatica*) is an aquatic macrophyte belonging to the family Convolvulaceae. Its leaves contain significant amount of crude protein (28–30%) which is one of the major fish feed component, besides considerable amount of carbohydrates and crude

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lipids thus a promising ingredient of fish feed. Many studies have been carried out to evaluate the effects of non-convectional ingredients used in diets as substitutes of fish meal on FA composition. However, research information on utilization of such alternative fish feed source is scanty. Therefore, this study aimed at assessing the effect of different levels of *I. aquatica* on fatty acid composition in *O. niloticus*.

2. Methodology

2.1. Experimental diets

Fresh *I. aquatica* were harvested from irrigation canals in Ahero, 23 km south-east of Kisumu City and 323 km west of Nairobi, Kenya. These were sorted, cleaned, dried, milled into fine powder and their proximate composition was determined in the laboratory. Nutritional analysis was also determined. Five isonitrogenous diets of 30% crude protein (CP) with different levels of *I. aquatica* ranging from 0% (control), 5%, 10%, 15% and 20% were formulated. Linear program software was used to formulate feeds with *I. aquatica* together with other dietary ingredients. The proximate and fatty acid composition of experimental diets is presented in Table 1.

2.2. Experimental set up

A six months culture trial was conducted using *O. niloticus* monosex. A total of 450 fingerlings weighing 2.0 ± 1 g were obtained from the Kenya Marine and Fisheries Research Institute (KMFRI) Sagana, in Kirinyaga County, Kenya. Prior to trial, fish were acclimatized for three days in circular tanks (1 m^{3}) at KMFRI, Kegati Aquaculture Centre. At the beginning of the experiment, fish were starved for 24 h, weighed and randomly distributed using 5×1 design at a stocking density of 30 fish per tank. Water was maintained at 45 cm throughout the experimental period, temperature, dissolve oxygen and pH, were monitored daily using YSI Multi probe model. NH_4 and NO_2 were analyzed spectrophotometrically on a weekly basis using standard methods [\[14](#page-6-0)]. During the experimental period, fish were fed twice daily at 10 a.m. and 4 p.m. at 5% body weight.

Table 1

SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFApolyunsaturated fatty acids.

2.3. Sample collection and analysis

2.3.1. Samples collection

A total of 9 fish per treatment were sampled using a scoop net (3 fish per tank for each treatment) and were anaesthetized using 70% ethanol. Muscle, intestine, gills, head, liver and whole body were extracted, packed in sampling bags and frozen at − 74 ◦C for further analysis. Each sample per tank was stored in well labeled ziplock. Analyses were done at the Food Biochemistry laboratory, Jomo Kenyatta University of agriculture and Technology (JKUAT).

2.3.2. Proximate composition

Proximate compositions for whole body fish sample were determined according to AOAC methods specification 950.46 [[15\]](#page-6-0). Moisture contents were obtained after drying in an oven at 110 ◦C for 24 h and ash content determined after incineration at 600 ◦C for 16 h. Crude protein was determined using Kjeldahl analysis.

2.3.3. Analysis of lipids and fatty acids composition

Lipids extraction was done according to the procedure by Bligh and Dyer [\[16\]](#page-6-0). Lipids in the muscles, intestines, gills, head, liver and whole body were extracted by homogenization of finely ground 0.5 g of samples in chloroform–methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant and cold isotonic saline, 0.9% sodium chloride. This was mixed vigorously and allowed to stand for 20 min.

The mixture was then centrifuged at 3000 rpm for 10 min and the aqueous layer was then separated from organic layer using a micropipette. The bottom layer, chloroform, was then transferred to 100 ml reflux flask, quick fit, and evaporated to dryness under vacuum evaporator.

Fatty acid methyl esters (FAME) were then prepared from vegetable oils and extracted total lipid by acid-catalyzed *trans*-esterification by adding 5 ml of 1% H_2SO_4 (v/v) in methanol at 70 °C, for 3 h. FAME were then extracted into 750 ml of distilled water and 10 ml of hexane, dehydrated using anhydrous sodium sulphate, Na₂SO₄ and concentrated to 0.5 ml under vacuum evaporator. The concentrated FAME were then transferred to GC vials for later GC analysis.

2.3.4. Gas chromatography analysis

FAME were separated and quantified by gas-liquid with on-column injection, equipped with a fused silica capillary column (SUPELCO Column Omegawaxtm530, 30 m \times 0.5 mm x 0.5 µm) with nitrogen as carrier gas and temperature programming from 170 ◦C to 220 ◦C for 18 min^{-1} and final time of 47 min totaling to a run time of 75 min. Injection and detection temperatures were 240 ◦C and 260 ◦C respectively. The programmer rate for both GC and decoder were set at 5min⁻¹ with an attenuation of 3. All the GC analyses were done under same conditions. Individual methyl esters in the sample were identified by comparison with known FAME standards obtained from Kobian chemicals.

2.4. Statistical analysis

The data was tested for homogeneity of variance using Lavene test. The data was then analyzed using One way analysis of variance (ANOVA) to compare the levels of n-3 PUFAs. Tukey's HSD multiple comparisons test was done to evaluate specific differences in levels of selected n-3 PUFAs among the treatments in cases where there were significant differences. Values with P *<* 0.05 were considered significant. All analyses were done using Statistical Package for Social Sciences (SPSS) version 20 for windows.

3. Results

3.1. Water quality

Water quality parameters monitored during the experimental period are presented in Table 2 below. Temperature ranged between 22.52 ◦C and 23.26 ◦C among the treatment groups. Mean pH values ranged between 7.42 and 7.68 whereas dissolved oxygen ranged between 2.68 and 3.06 mg/l. NH₄ and NO₂ values ranged between 0.25 to 0.75 and 0.04–0.68 mg/l respectively. Water quality parameters monitored did not vary significantly (p *>* 0.05) among treatments.

3.2. Proximate composition of experimental fish

The effects of different inclusion levels of *I. aquatica* on proximate analysis, macroelements and vitamins of *O. niloticus* at the end of experimental period are presented in [Table 3](#page-3-0). Proximate composition ranged from 56.3 to 62.2 and 6.9–13.6 mg/100 for crude protein and carbohydrates respectively. Crude protein decreased with increase in the level of *I. aquatica. O. niloticus* fed with 5% diet recorded the highest crude protein content (56.3 mg/100). Macroelements ranged from 11.2 to 27.8, 365.2 to 1104.3, 1.0 to 3.4 and 1.4–3.03 mg/100 for Mg, Ca, Zn and Fe respectively. Vitamins ranged from 2.5 to 4.5 and 67.9–82.6 mg/ 100 for Vitamin E and Retinol respectively. Proximate composition showed no significant difference in all the diets.

3.3. Fatty acids composition in tissues (dry matter) of cultured O. niloticus

3.3.1. Muscles

[Table 4](#page-3-0) shows the fatty acid composition in muscle of experimental fish. SFA, MUFA, n-3 and n-6 ranged from 18.78% to 29.37%, 12.24%– 18.14%, 7.43%–8.85% and 9.9%–10.74% respectively. The dominant SFA was palmitic acid recorded in 20% diet Total SFA in fish fed with 20% diet was higher compared to other diets. Oleic acid was the dominant MUFA recorded in all dietary treatments. The most dominant n-3 recorded in the muscles was DHA ranging from 2.5 to 3.7% ([Table 4\)](#page-3-0). Increasing the composition of *I. aquatica* also increased the amount of omega-3 in the muscles from 7.72% in 0% diet to 8.85% in 20% diet. Total PUFA from n-6 series recorded for fish fed with 20% diet was higher than other treatments and increased with increased composition level. Muscles recorded the highest amount of both DHA and EPA compared to other tissues.

3.3.2. Intestines

The fatty acid composition in intestines of experimental fish is showed in [Table 5](#page-4-0). SFA, MUFA, n-3 and n-6 ranged from 24.69% to 32.09%, 22.23%–30.18%, 11.14%–17.51% and 4.91%–8.42% respectively. The most dominant SFA recorded was stearic acid, with fish fed 5% diet recording the highest amount. The levels of stearic acid decreased with increase in levels of *I. aquatica*. Oleic acid was the most dominant MUFA and the highest amount was recorded in fish fed 5%

diet. Linolenic acid and DHA were the most dominant n-6 and n-3 respectively. Inclusion of *I. aquatica* increased the amount of Omega-3 in the intestine from 5.68% in 0% diet to 8.42% in 20% diet.

3.3.3. Gills

Fatty acids composition in the gill is shown in [Table 6.](#page-4-0) SFA, MUFA, n-3 and n-6 ranged from 24.71% to 30.45%, 14.29%–23.46%, 5.99%– 8.68% and 11.19%–14.06% respectively. The most dominant SFA was stearic acid recorded from fish fed with 5% diet. Total SFA from fish fed with 5% diet recorded the highest amount compared to other treatments. Oleic acid was the most dominant MUFA recorded from fish fed with 10% diet. Linoleic acid was the most common n-6 from fish fed with 0% while DHA from fish fed with 20% diet was the most dominant. Increasing the composition of *I. aquatica* also increased the amount of Omega-3 in the gills from 7.77% in 0% diet to 8.68% in 20% diet.

3.3.4. Head

[Table 7](#page-5-0) shows the fatty acids composition on the head. SFA, MUFA, n-3 and n-6 ranged from 25.64% to 29.91%, 19.34%–28.44%, 4.30%– 7.37% and 12.16%–18.92% respectively. The most dominant SFA was palmitic acid recorded from fish fed with 10% diet while Oleic acid being the most common MUFA recorded from fish fed with 10% diet. Arachidonic acid was the most dominant n-6 from fish fed with 5% while linoleic was the most dominant n-3 from fish fed with 10% diet. The n-3 PUFAs level increased with the increasing level of *I. aquatica*.

3.3.5. Liver

Fatty acids compositions in the liver are shown below ([Table 8\)](#page-5-0). SFA, MUFA, n-3 and n-6 ranged from 23.82% to 32.88%, 10.76%–20.62%, 4.80%–9.40% and 7.67%–13.18% respectively. The most dominant SFA was stearic acid recorded from fish fed with 5% diet while Oleic acid and Linoleic acid were the most abundant MUFA and n-6 respectively, from fish fed with 5% diet. Relatively high linolenic acid was recorded from fish fed with 0% diet.

3.3.6. Whole body

[Table 9](#page-6-0) shows fatty acids composition of the whole body. SFA, MUFA, n-3 and n-6 ranged from 23.91% to 32.14%, 15.60%–20.90%, 11.45%–19.78% and 4.87% to 9.13%respectively. Stearic and palmitic acids were the most dominant SFA while relatively high Oleic acid (MUFA) was recorded from fish fed with 5% diet. Linoleic acid was the most dominant n-6 PUFA with significantly high composition observed in fish fed with 5% dietary composition of *I. aquatica*. DHA was the prominent n-3 PUFA.

4. Discussion

Studies have reported the effect of diet on fatty acids composition of fish tissues [\[17](#page-6-0)] Omolo et al., 2017 and [\[18](#page-6-0)]. There is almost direct influence of dietary fatty acid on the tissue fatty acid profile of fish, a conclusion that has been demonstrated through previous studies. In studied cases, palmitic acid, C16:0, is the dominant saturated fatty acid

Temperature (Temp) = °C, Dissolved Oxygen (DO) = mg/l, Salinity (Sal) = g/l, Ammonium (NH₄) = mg/l, Nitrite (N.O₂) = mg/l. Values are presented as mean \pm standard error (SE).

Proximate composition of experimental fish.

 $Mg = Magnesium$, Ca= Calcium, Zn = Zinc and Fe= Iron. Values are presented as mean \pm standard error (SE).

Table 4

Fatty acid (% total FA) composition of muscle of *O. niloticus* fed diets containing different dietary inclusion of *I. aquatica*.

Values are expressed as mean ± SE. SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid, PUFApolyunsaturated fatty acid.

(SFA) in fish [[19,20\]](#page-6-0), since palmitate is the primary product of fatty acid synthase, a regulary enzyme in the *de novo* synthesis of fatty acids. Elongation and desaturation of palmitate either in the mitochondria or on the surface of endoplasmic reticulum generate longer saturated and unsaturated fatty acids in organism. Both stearic acid and palmitic acids were dominant in all the tissues in the current study, a finding that agrees with studies of Omolo et al. (2017) , Suloma et al. [[21\]](#page-6-0) and Akpinar et al., [[22\]](#page-6-0). Excess carbohydrates are sources of acetyl CoA, precursor of palmitic acid which is the first fatty acid to be produced during fatty acid synthesis. Stearic acid, C18:0, is elongation product of palmitate thus its composition is likely to be determined by levels of palmitic acid. Palmitic acid is also an anabolic precursor to biosythesis of longer fatty acids.

MUFAs constituted the largest proportion of total unsaturated fatty acids in all the tissuesWith Oleic acid, C18:1, n9 being the most dominant MUFA, a finding which collaborates with Satue and Lopez [\[23](#page-6-0)], Alemu [\[24](#page-6-0)], Mwanja et al. [\[25\]](#page-6-0), Olsen et al. [\[26](#page-7-0)], Jabeen and Chaudry [[9](#page-6-0)] and Luo et al., [\[27\]](#page-7-0). In animals, oleic acids are formed from oxidative desaturation of stearic acids; therefore, there is a correlation between cellular strearic acid and oleic acid compositions. The observed composition of oleic acid in this study could be attributed to the reported elevated stearic levels in this study. This finding corroborate earlier studies where oleic acid was observed as the dominant MUFA (Jamal et al., 2020). In addition, high amounts of oleic acid in all the tissues could be linked to the abundance of these fatty acids in diets. Moreover, studies have reported the effect of diet, temperature among other factors, on fatty acids composition of fish tissues [[17](#page-6-0),[18\]](#page-6-0), findings that corroborate the current study. In the present study, *O. niloticus* had higher n-3 PUFA in liver tissues. However, reverse results were recorded for n-6 PUFA in the liver. This resulted to an increase in n3/n6 ratio in experimental fish as reported by De Silva et al. [\[28](#page-7-0)] and Mnari et al., [[29\]](#page-7-0). This could be attributed to inherent mechanism of fish physiology

Values are expressed as mean ± SE. SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid, PUFA-Polyunsaturated fatty acid.

Table 6

Fatty acid (%total FA) composition of gills of *O. niloticus* fed diets containing differentdietary inclusion of *I. aquatica*.

Values are expressed as mean ± SE. SFA-saturated fatty acid, MUFA-monounsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid, PUFApolyunsaturated fatty acid.

to maintain internal homeostasis.

The decreasing levels of n-6 values in intestines, head, liver and whole body with increasing levels of *I. aquatica* is in line with the studies presented by Kaushik [[30\]](#page-7-0) on *O. niloticus* fed diets containing elevated levels of palm oil. Christian et al. [[31\]](#page-7-0) also reported similar results on *O. niloticus* fed high levels of vegetable oil. The n-6 series reported in all the tissues is lower compare to that of the diets. This is because n-6 is an intermediate pathways of desaturation and elongation of fatty acids [[32\]](#page-7-0), thus utilized in the biosynthesis of long chain n-6 fatty acids. The n3/n6 ratio of experimental fish ranged from 1 to 4 in all the tissues. However, Cowey [[33\]](#page-7-0) and Valfre et al. [[34\]](#page-7-0) reported a ratio of 1–6 while Ackman et al. [[35\]](#page-7-0) reported ratio between 1 and 2. In the present study, n3/n6 ratio decreases with a decrease in temperature as reported by Ref. [[36\]](#page-7-0). This is due to inability to digest dietary lipids at high temperatures. The n3/n6 ratio of experimental fish were much higher than n3/n6 ratio of experimental diets. A similar result to Christian et al., [[31\]](#page-7-0). This is because fish is able to adjust their own n3/n6 ratio for their own physiological adaptation [\[27](#page-7-0)]. There was a higher n3/n6 ration in

Values are expressed as mean ± SE. SFA-saturated fatty acid, MUFA-monounsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid, PUFApolyunsaturated fatty acid.

Table 8

Fatty acid (%total FA) composition of liver of *O. niloticus* fed diets containing different dietary inclusion of *I. aquatica*.

Values are expressed as mean ± SE. SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid, PUFApolyunsaturated fatty acid.

the muscles compare to diets, similar results were obtained by Christian et al., [\[31](#page-7-0)]. This could indicate that a threshold level in the muscles was obtained, probably adjusted to a narrowly define physiological level [[21](#page-6-0)[,37](#page-7-0)].

[[32\]](#page-7-0).

5. Conclusion

In all the selected tissues, the amount of DHA was more than EPA. Similar results were also reported by Refs. [\[27](#page-7-0),[38\]](#page-7-0). This is mainly because EPA is highly oxidized compared to DHA [\[39](#page-7-0)] due to complex catabolism of fatty acids (Bell et al., 2001). In addition, EPA is an intermediate in the biosynthetic pathway of DHA to maintain LC-PUFAs

This study confirmed a direct influence of dietary composition of *I. aquatica* on fatty acid composition of different tissues of *O. niloticus*. Dietary inclusion of 20% of *I. aquatica* in the feed resulted into elevated levels of tissue n-3 fatty acids especially DHA, suggesting a possible utilization of *I aquatica* in dietary formulation of fish feeds. However,

Values are expressed as mean ± SE. SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid, PUFApolyunsaturated fatty acid.

more studies on the nutritional properties of *I. aquatica* is necessary to understand-its nutritional and anti-nutritional traits for possible use in fish feeds formulation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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