AquaTechnica 6(3): 194-208(2024)
ISSN 2737-6095
DOI https://doi.org/10.33936/at.v6i3.7146
https://doi.org/10.5281/zenodo.14510951

Poultry fat as a main fat source in a mixture to replace fish oil in diets for *Oreochromis niloticus*

Grasa de ave como fuente principal de lípidos en una mezcla para reemplazar el aceite de pescado en dietas de *Oreochromis niloticus*

Eulalio Arambul-Muñoz, Luis Alonso Galindo-Valdez, Maria Teresa Viana
Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California (UABC), Km 107 carretera Tijuana-Ensenada, 22860 Ensenada BC, México.
Correspondencia: *Eulalio Arambul-Muñoza* ***E-mail:*** *eulalio.arambul@uabc.edu.mx*

Artículo original | *Original article*

Keywords
fish free feeds
aquaculture
rendered foods
poultry meal

ABSTRACT | The present work aimed to test poultry fat as the main fat source to replace fish oil in tilapia *Oreochromis niloticus* diets. For this purpose, a blend of poultry fat (71 %), kernel fat (PKF) (25 %) a rich source of lauric acid (12:0), and microalgae oil (4 %) rich in DHA and EPA was used to replace the fish oil. The isoproteic and isolipidic diets (37.5 % crude protein and 8 % crude lipids) contained four levels of the fat mixture in replacement of fish oil, 0, 1.65, 3.32 and 5 % in the total diet and were used to feed tilapia juveniles (6.04 ± 0.13 g) four times a day in triplicate groups. Six weeks later, all fish were individually weighted, and a strong correlation was found using a polynomial regression analysis showing a lower feed conversion ratio using a higher poultry fat mixture (r^2=0.933), whereas a higher growth was registered (r^2=0.762). The fatty acid analysis in the hepatopancreas showed no sign of lauric acid contained in the PKF and also indicated a significant reduction in oleic and linoleic acids, which were the main energy sources, whereas a substantial accumulation of palmitic acid 16:0 was observed. Further, the hepatosomatic index was significantly positive at higher amounts of poultry fat mixture (r^2=1), revealing the capacity to maintain a higher weight in the hepatopancreas, despite there being no clear differences among the histology analyses obtained from the different dietary treatments. This study concluded that poultry fat can substitute fish oil from tilapia diets with a clear positive relationship with the overall performance of tilapia *O. niloticus*.

Palabras clave
alimentos libres de pescado
acuicultura
alimentos processados
harina de ave

RESUMEN | El presente trabajo tuvo como objetivo probar la grasa de ave como la principal fuente de grasa para reemplazar el aceite de pescado en dietas para tilapia *Oreochromis niloticus*. Con este propósito, se utilizó una mezcla de grasa de ave (71 %), grasa de palmiste (25 %), una fuente rica en ácido láurico (12:0), y aceite de microalgas (4 %), rico en DHA y EPA, para reemplazar el aceite de pescado. Las dietas isoproteicas e isolipídicas (37,5 % de proteína cruda y 8 % de lípidos crudos) contenían cuatro niveles de la mezcla de grasas para reemplazar el aceite de pescado: 0, 1,65, 3,32 y 5 % de la dieta total, y se usaron para alimentar juveniles de tilapia (6,04 ± 0,13 g) cuatro veces al día en grupos por triplicado. Seis semanas después, todos los peces fueron pesados individualmente, y se halló una fuerte correlación mediante un análisis de regresión polinómica, mostrando una menor tasa de conversión alimenticia con mayores niveles de la mezcla de grasa de ave (r^2=0,933), mientras que se registró un mayor crecimiento (r^2=0,762). El análisis de ácidos grasos en el hepatopáncreas no mostró presencia de ácido láurico contenido en la grasa de palmiste, pero sí una reducción significativa en los ácidos oleico y linoleico, principales fuentes de energía. Por otro lado, se observó una acumulación significativa de ácido palmítico (16:0). Además, el índice hepatosomático fue significativamente positivo con mayores cantidades de la mezcla de grasa de ave (r^2=1), lo que revela la capacidad de mantener un mayor peso en el hepatopáncreas, a pesar de no haberse observado diferencias claras en la histología obtenida de los diferentes tratamientos dietéticos. Este estudio concluyó que la grasa de ave puede sustituir al aceite de pescado en dietas para tilapia, con una relación claramente positiva con el desempeño general de *O. niloticus*

INTRODUCTION

Aquaculture feed production is a crucial component of fish farming, significantly influencing the growth, survival, and quality of fish products (Tacon 2015). Traditionally, fish diets have relied heavily on fishmeal and fish oil. However, the need to reduce or eliminate these ingredients has emerged as a key objective over the past two decades. This shift is primarily driven by the scarcity of these resources, which has led to substantial price increases, particularly for fish oil (Tacon 2015, Yossa *et al*. 2022).

Recibido: 28-11-2024 | **Aprobado:** 10-12-2024 | **Versión final:** 14-12-2024

Research has extensively explored alternative feed ingredients that do not harm fish intestinal health, such as those that prevent enteritis, a condition especially prevalent when high concentrations of vegetable meals are used, particularly in carnivorous and omnivorous fish (Yuangsoi *et al*. 2014, Sankian *et al*. 2019, Nakharuthai *et al*. 2020, Khieokhajonkhet *et al*. 2024).

The melting point of oils and fats varies due to their differing compositions of fatty acids. Fats, which contain lower levels of polyunsaturated acids, remain solid at room temperature, while oils are liquid. This property arises from the relative proportions of saturated to unsaturated fatty acids in these substances. Despite their differences, all oils and fats share a common set of fatty acids in varying ratios, allowing the formulation of blends to achieve desired profiles tailored to specific species, even mimicking fish oil.

In the last decade, significant research has focused on using blends of animal and vegetable oils as sources of omega-3 (n3) fatty acids in aquaculture. According to Turchini *et al.* (2009), various fats and oils are available for this purpose. However, essential fatty acids such as Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), and Arachidonic acid (ARA) which are vital for growth and health, are predominantly sourced from fish oil. Nowadays, commercial products rich in these essential oils, particularly DHA and EPA (and to a lesser extent ARA), are widely available (Oliver *et al*. 2020, Napier and Betancor 2023).

A study by Ng *et al*. (2018) found that incorporating a mixture of soybean oil, palm oil, and fish oil into the diet of Nile tilapia resulted in reduced growth performance and lower levels of n3 fatty acids compared to diets that exclusively used fish oil. Maintaining an appropriate ratio of n3 to n6 fatty acids in fish feed is essential, as excessive n6 fatty acids can adversely affect fish health. While vegetable oils offer a viable and cost-effective alternative to fish oil, their high n6 content can negatively impact health and growth if used in excess (Tacon 2015).

Another promising alternative for aquaculture feed is high-quality terrestrial animal fats, sourced from the inedible parts of domestic animals such as poultry, pork, and cattle. According to Bureau and Meeker (2010), approximately 12 million metric tons of animal fats were produced globally in 2010, a figure that likely remains stable today due to the growing preference for meat cuts that contain fat.

While terrestrial animal fats do not provide essential fatty acids, the addition of microalgae oil can help address this deficiency (Santiagosa *et al*. 2020). By utilizing a variety of oil and fat sources, we can create blends that achieve a desirable fatty acid profile. Notably, medium-chain fatty acids can enter mitochondria through diffusion, bypassing the need for carrier enzymes like carnitine palmitoyltransferase I (CPTI), which is responsible for transporting long-chain fatty acids. This characteristic allows medium-chain fatty acids to be utilized more efficiently in energy production.

Among these sources, Palm Kernel Fat (PKF), derived from the seeds of the African palm fruit and commonly used in the pharmaceutical industry, is rich in lauric acid, a 12-carbon fatty acid. This makes it an excellent candidate for fish feed formulations (Kader *et al.* 2018), which have demonstrated beneficial effects on fish health and growth (Adedeji *et al.* 2016, Emehinaiye *et al.* 2018).

The present study aims to introduce a mixture comprising three primary fat sources: poultry fat, palm kernel fat, and DHA from algae concentrate. This blend was used as a partial to total replacement for fish oil in isoprotein and isolipid diets based on low fishmeal and defatted poultry meal. The effectiveness of this mixture on zootechnical performance is evaluated.

MATERIALS AND METHODS

Fish and experimental design and fish handling

A total of 2 000 juvenile tilapia (*Oreochromis niloticus*) were sourced from Grupo AQUAMOL from Chapala Jalisco, Mexico, and transported by plane to the Laboratory of Nutrition and Physiology of Aquatic Organisms at the Institute of Oceanological Research of the Autonomous University of Baja California (IIO-UABC, Mexico).

The fish were acclimatized in a 3 000 L tank for two weeks and were fed commercial feed until apparent satiety three times a day.

The experiment was conducted in a recirculating aquaculture system (RAS) comprising 12 tanks of 500-L each, equipped with a pump, protein skimmer, UV filter, and biological filter (PolyGeyser®; PneumaticDrop Bead Filter model PG7 International Filter Solutions, TX, USA). The fish, with an initial weight of 6.04 ± 0.13 g, were randomly distributed at a density of 20 fish per tank, corresponding to triplicate groups per dietary treatment. They were fed experimental diets four times daily (08:00, 11:00, 14:00, and 17:00) for six weeks, starting with a feeding rate of 6 % of the total biomass per tank, readjusted every two weeks to a final rate of 13 %. To adjust the feed rate, partial biometry was performed by weighing at least 10 fish per tank.

Water parameters, including temperature (27.08 ± 0.10 °C), salinity (0 ± 0.5 ppt), and dissolved oxygen (6.04 ± 0.30 mg/L), were monitored daily using a YSI-55 (YSI Inc, Yellow Springs, OH, USA). Nitrogen compounds such as ammonium (0.25 ± 0.02 mg/L) and nitrite (0.35 ± 0.04 mg/L) were measured weekly using API test kits (Mars Fishcare Inc., Chalfont, PA, USA).

Experimental diets

Defatted poultry by-product meal served as the primary protein source, supplemented with defatted fish meal and soybean meal (containing 65 % crude protein and 1% crude fat). Four isoprotein and isolipid diets were formulated, each containing 37.5 % crude protein and 8 % lipids (see Table 1). The diets included varying levels of a fat mixture in replacement of fish oil (Poultry fat 71 % + Kernel Fat 25 % + DHA-Nature 4 %) to replace fish oil, categorized as Control (0 % fat mixture, but 5% fish oil), low-fat mix (Low-Mix 1,65 %), medium-fat mix (Med-Mix 3.32 %), and high-fat mix (High-Mix 5 %).

The experimental diets were prepared at IIO-UABC following internal protocols. First, macronutrients were pulverized to 0.5 mm (Inmimex M-300, Mexico) and then sieved (Kemutek-Gardner K300, USA). The components were mixed in a vertical mixer-cutter (Robot Coupe R-60, USA) until a homogeneous blend was achieved. Micronutrients were added to the bulk mixture, and the fat sources were pre-mixed to ensure diet consistency. The fat mixture was incorporated into the diets, which were then thoroughly mixed using a Robot-Coupe (model R10, USA) and cold extruded to 5 mm using a meat grinder (Tor-Rey, model M32-5, Mexico) before being dried at 60 °C in a forced-air oven for 24 h. After drying, the diets were refrigerated at 4 °C throughout the experiment.

After the six-week feeding trial, the starved fish for 18 h, were counted and weighed individually to assess overall performance using the following metrics:

Weight Gain (%) = (Wt – Wo) x 100 / Wo
Specific Growth Rate (SGR, %) = 100 x ((ln Wt – ln Wo) / days of feeding)
Feed Conversion Ratio (FCR) = total feed consumed / wet weight gained
Hepatosomatic Index (HSI, %) = (hepatopancreas weight / body weight) x 100
Viscerosomatic Index = (viscera weight / Wt) x 100
Thermal Growth Coefficient (TGC) = [(Wt^(1/3) – Wo^(1/3)) / (T°C x days)] x 1000
Survival Rate (Sv, %) = (Nt x 100) / No
Where Wt and Wo are the final and initial fish weights, respectively, and Nt and No correspond to the final and initial number of fish.

At the end of the trial, fish were handled carefully to minimize stress. Three fish per tank were euthanized through hypothermia, following ethical protocols established by the UABC, and were subsequently stored at -80 °C for analysis.

Chemical analysis

All experimental diets and muscle samples were analyzed for proximate composition in triplicate samples according to AOAC (2015) methods, muscle tissues were individually analyzed (three per experimental unit, nine per dietary treatment). In summary, the dry weight and ash content of the diets were determined by drying the samples at 60 °C for 24 h. The samples were then ashed in a muffle furnace at 550 °C for 6 h. Next, crude protein was analyzed using the micro-Kjeldahl method (UDK 129, Velp, Italy), and protein content was calculated by converting nitrogen (%N) using a factor of 6.25. Finally, lipid content was quantified using the Soxhlet method with petroleum ether as the solvent, by AOAC standards.

Fatty acids from the experimental diets and muscle tissues were analyzed following a methodology adapted from Folch *et al*. (1957), using dichloromethane instead of chloroform. The crude oil obtained from the diets was separated for further methylation following the method described by Castro-Gómez *et al*. (2014). Muscle tissue samples were individually analyzed, three per experimental unit and nine per dietary treatment. Fatty acid methyl esters (FAME) were analyzed by gas chromatography equipped with a flame ionization detector (Agilent GC 6880, Agilent Technologies, Santa Clara, CA, USA), with hydrogen as the carrier gas. The conditions for the GC column (60 m × 0.25 mm with 0.25 µm film thickness; Agilent 122-2362 dB-23) were: initial oven temperature of 50 °C for 1 min, then ramping from 50 °C to 140 °C at 30 °C per minute, maintaining 140 °C for 5 min, transitioning from 140 °C to 240 °C at 4 °C per min, and finally holding at 240 °C for 20 more min. The injector and detector temperatures were maintained at 230 °C and 260 °C, respectively. FAMEs were identified and quantified by comparing retention times with those of a standard mixture (a 37-component mixture of FAMEs, PUFA 1, and PUFA 3, Supelco/Sigma-Aldrich, St. Louis, MO, USA), using C19 as an internal marker to calculate concentration.

Apparent digestibility was determined using acid-insoluble ash as an internal marker. The percentage of acid-insoluble ash in the experimental diets and feces collected during the bioassay was measured. Feces were collected two weeks after the start of the experimental procedure. The methodology described by Tejada *et al*. (1992) with some modifications (Montaño-Vargas *et al*. 2002) was employed. In summary, the collected feces were weighed, introduced in porcelain crucibles and placed in a furnace at 550 °C for 6 h. After calcination, the samples were weighed and transferred to 50 mL beakers; 25 mL of 2 N HCl was added, and the beakers were covered with a watch glass and placed on an electric hot plate at 100 °C until boiling. The samples were allowed to remain at boiling for 5 min before cooling to room temperature. After cooling, the contents were filtered through 47 mm diameter GF-F glass fiber filters (Whatman®), which had been pre-weighed to a constant dry weight. A flask connected to a vacuum pump was used to filter the ashes, and hot distilled water was applied to ensure all ash residues were dissolved. The filters were then placed in aluminum foil and ashed again in a muffle furnace at 550 °C for 6 h. Once the samples were calcined, they were weighed, and ash content was calculated according to Tejada *et al*. (1992).

$$\% IA = \frac{(AW) - (CW)}{DMW} X\ 100$$

Where

IA: Acid insoluble ash

AW: Ash weight (g)

CW: Crucible weight (g)
DMW: Dry matter weight

Liver histology analysis

Liver tissue samples used for histological analysis were fixed in Davidson solution for 24 h, then transferred to 70 % ethanol. The fixed samples were dehydrated through a series of increasing ethanol concentrations, cleared in dimethyl benzene (xylene) solution, and embedded in Paraplast® according to routine histological procedures. Sections 5 µm thick were prepared using a microtome (Leica HistoCore AutoCut) and mounted on slides coated with poly-L-lysine solution. The sections were stained with hematoxylin-eosin and examined using a computerized

image analyzer (Leica DM1000 LED microscope, Leica MC170HD camera). For morphometric analysis, the average area of cells and lipid vacuoles was measured using Leica LAS Interactive Measurements software.

Serum chemistry analysis

Hematocrit (HCT) was measured using a blood sample in a heparinized capillary tube filled two-thirds full (LEEX Equipment, Mexico). The tubes were sealed and centrifuged for 10 min at 7 000 rpm in a micro-hematocrit centrifuge (Premiere® XC-3012, Mexico). The packed cell volume was measured using a hematocrit reader and reported as a percentage (Zaragoza *et al*. 2008). Hemoglobin (HGB) in erythrocytes was determined using a HemoCue Hb 201 analyzer according to the manufacturer's instructions (HemoCue® AB, Angelholm, Sweden). The method described by Natt and Herrick (1952) was used for counting total red blood cells (RBC) and white blood cells (WBC) plus thrombocytes. The diluted sample was placed in a Neubauer hemocytometer (Marienfeld-Superior, Lauda-Königshofen, Germany), and the cells were counted using an optical microscope (Karl Zeiss, Primo Star, Mexico). The following indices were calculated using standard formulas based on HCT, RBC, and HGB: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Total protein, albumin, globulin, glucose, and triglycerides were determined from blood serum using colorimetric assay kits (MexLab Group, Jalisco, Mexico) following the manufacturer's instructions. Globulin was calculated by subtracting the albumin value from the total protein.

Statistical analysis

The homoscedasticity and normality of the data were verified using the Levene test and the Shapiro-Wilk test, respectively. Zootechnical performance variables were analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey test when a significant difference was detected among treatments (IBM SPSS Statistics V26.0.0, Copyright IBM Corporation 1989, 2011, USA). Differences were considered significant at the 95 % level. A polynomial (quadratic) regression was performed among treatments for most overall performance parameters. A significance value of $P < 0.05$ was applied for all statistical tests (IBM SPSS Statistics V26.0.0, Copyright IBM Corporation 1989, 2011, USA).

RESULTS

Tables 1 and 2 present the formulation, proximate composition, and fatty acid profiles of the experimental diets and fat sources (fish oil and fat mixture). The protein content (37.3 ± 0.15), lipid content (8 ± 0.05), and ash content (7 ± 0) were consistent across all formulated diets; however, the Med-Mix diet had the lowest protein percentage compared to the other diets. After six weeks of the experimental procedure, the response variables were analyzed to assess the zootechnical performance of tilapia fed with the experimental diets (Table 3). No significant differences were observed among repetitions per each dietary treatment ($P<0.05$). The polynomial regression analysis (Fig. 1) revealed strong positive correlations between the poultry fat mixture inclusion levels and various performance metrics: FBW ($r^2=0.795$), WG ($r^2=0.762$), FCR ($r^2=0.933$), Sv ($r^2=0.933$), VSI ($r^2=0.967$), and HI ($r^2=1$). Accordingly, the Control treatment demonstrated lower indices, including FBW (82.07 ± 0.74), WG (76.04 ± 0.74), and SGR (3.89 ± 0.02), compared to the other treatments. Conversely, the Control treatment had higher FCR (0.61 ± 0.01) and Sv (%) (98.33 ± 1.66) values. Additionally, the High-Mix treatment exhibited higher HI and VSI.

Table 4 displays the proximate composition of tilapia muscle, showing no significant differences across analyzed variables ($P>0.05$), without any indication that dietary composition affected the muscle composition.

Table 5 reports that apparent dry matter digestibility was consistent across treatments, with the lowest percentages observed in the Med-Mix (72.16 ± 0.34) and High-Mix (72.56 ± 2.85) treatments, again with no significant differences among treatments ($P>0.05$). The polynomial regression analysis (Fig. 2) indicated a strong positive correlation between the fat mixture inclusion level and apparent dry matter digestibility ($r^2=0.737$).

Fatty acid profiles of the muscle indicated efficient assimilation of certain fatty acids, such as Lauric (12:0) and Myristic (14:0). Notably, Lauric acid was not detected in the fish muscle, and the concentration of Myristic acid

decreased proportionally with increasing the fat mixture blend levels (Table 6). While Linoleic acid was present in lower percentages than in the diets, its deposition in fish muscle was inefficient as inclusion levels increased. The concentrations of EPA and DHA were also lower than those observed in the various diet treatments, while Oleic acid (18:1n9) accumulation was higher than in the dietary levels.

Table 1. Ingredients and proximate composition (% on a dry matter basis, DM) of four isoproteic and isolipidic diets formulated to contain different fat mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %) levels replacing Fish oil fed to fish *Oreochromis niloticus* for 6 weeks. Low-Mix (low inclusion), Med-Mix (medium inclusion), High-Mix (high inclusion).

Ingredients, % of DM	Dietary treatments			
	Control	Low-Mix	Med-Mix	High-Mix
Fish meal [a]	5	5	5	5
Poultry by-product meal [b]	25	25	25	25
Soybean 65% ADM [c]	10	10	10	10
Wheat meal [d]	19	19	19	19
Gelatin[e]	7	7	7	7
Fish oil [f]	**5**	**3.35**	**1.68**	**0**
Fat mixture [g]	**0**	**1.65**	**3.32**	**5**
Corn starch [h]	24	24	24	24
Methionine [e]	1	1	1	1
Rovimix [j]	2.5	2.5	2.5	2.5
Stay C [k]	0.10	0.10	0.10	0.10
Phospholipids [l]	1.29	1.29	1.29	1.29
Sodium benzoate [m]	0.10	0.10	0.10	0.10
BHT [m]	0.01	0.01	0.01	0.01
Proximate composition (% dry matters basis)				
Crude Protein	37.6	37.4	37.3	37.6
Crude Lipid	8.1	8.1	8	8
Ash	7	7	7	7
NFE*	50.8	50.8	50.8	50.8

*NFE (g kg^{-1}) = 100 % − (crude protein+crude lipid+moisture+ash)
[a]Procesadora Mar de Ensenada S. de R.L. de C.V.
[b]Scoular de México S. de R.L. de C.V
[c]Soycomilk ADM
[d]Molinera del Valle S.A. de C.V., México
[e]Progel Mexicana SA de CV, Léon, Guanajuato, México
[f]Proteinas Marinas y Agropecuarias S.A de C.V.
[g]Chicken Fat (Proan Alimentos, S. de R.L. de C.V.) + DHA (Purac México, S. de R.L. de C.V.+ Palm oil (Comercializadora Abanor, S.A. de C.V)
[h]Maizena™, Unilever Food Solutions, México
[i]Future Foods, México

Table 2. Fatty acid composition (%) of formulated diets containing different inclusion of Fat Mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %) in replacement of Fish oil.

Fatty acid	Fat Sources		Dietary treatments			
	Fish oil	Fat mixture	Control	Low-Mix	Med-Mix	High-Mix
8:0	ND	1.2				
10:0	ND	1.4				
12:0	ND	18.3	0.69	6.24	9.6	12.15
14:0	10.4	7.3	4.80	6.25	7.92	8.54
16:0	27.9	18.7	21.96	24.03	25.03	25.38
18:0	6.0	6.3	4.67	5.02	5.31	6.22
∑SFA	**44.3**	**53.2**	**32.12**	**41.54**	**47.86**	**52.29**
16:1	10.8	5.6	9.34	7.72	6.75	5.01
18:1n7	3.3	ND				
18:1n9	14.2	32.5	21.35	25.95	28.53	29.87
∑MUFA	**15.3**	**38.1**	**30.69**	**33.67**	**35.28**	**34.88**
18:2n6	3.5	2.6	13.67	11.23	9.45	7.54
20:4n6	1.2	ND	ND	ND	ND	ND
∑PUFAn6	**4.7**	**2.6**	**13.67**	**11.23**	**9.45**	**7.54**
18:3n3	1.3	0.6	3.67	1.15	0.95	0.23
20:5n3	5.6	1.2	6.85	2.66	0.56	0.4
22:6n3	4.8	1.6	10.14	7.23	5.9	4.66
∑PUFAn3	**11.7**	**3.4**	**20.66**	**11.04**	**7.41**	**5.29**
∑PUFAs	**16.4**	**6.0**	**34.33**	**22.27**	**16.86**	**12.83**
Others			2.86	2.52	0	0

Low-Mix (low inclusion), Med-Mix (medium inclusion), High-Mix (high inclusion). ∑SFA, ∑MUFA, ∑PUFA, are the sum of saturated, monounsaturated, polyunsaturated respectively.

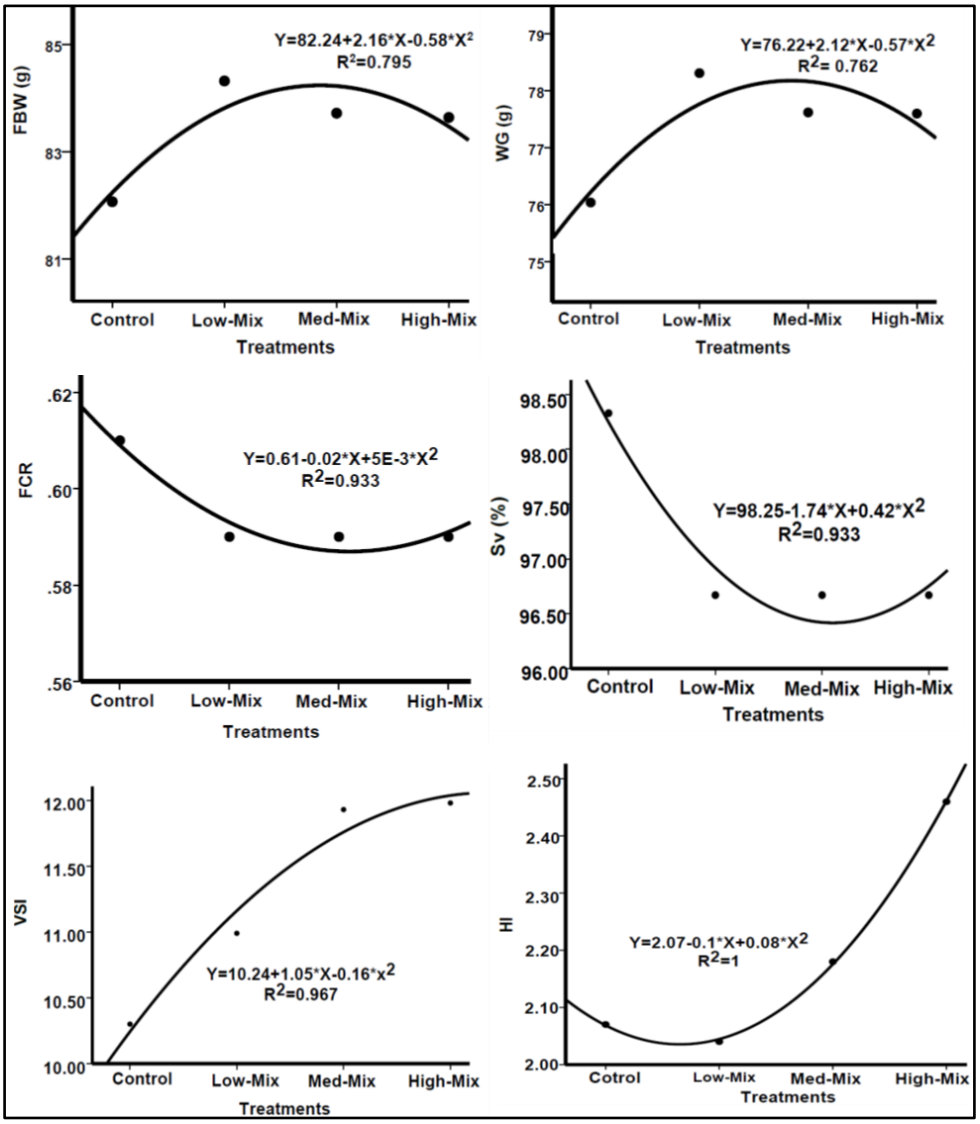

Figure 1. Polynomial (quadratic) regression analysis between levels of fat mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %) in the overall performance (FBW, WG, FCR, Sv, VSI, HI). The markers indicate the values shown in Table 3. The lines indicate the best-fit polynomial regression equations and the r^2 values indicate the power of the model. Treatments: 0 (Control), 1.65 (Low-Mix), 3.32 (Med-Mix), and 5 % (High-Mix) in replacement of Fish oil.

Table 3. Overall performance of tilapia *Orechromis niloticus*, fed with four diets containing four different Fat Mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %) levels; 0, 1.65, 3.32, and 5 % in replacement of Fish oil for 6 weeks.

Index	Treatment				
	Control	Low-Mix	Med-Mix	High-Mix	*P value*
IBW (g)	6.03 ± 0.06	6 ± 0.10	6.09 ± 0.09	6.04 ± 0.07	0.917
FBW(g)	82.07 ± 0.74	84.32 ± 0.70	83.72 ± 0.83	83.64 ± 2.28	0.392
WG (g)	76.04 ± 0.74	78.31 ± 0.79	77.62 ± 0.79	77.60 ± 2.22	0.506
SGR %)	3.89 ± 0.02	3.94 ± 0.03	3.91 ± 0.02	3.92 ± 0.03	0.687
FCR	0.61 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.147
HI (%)	2.07 ± 0.15	2.04 ± 0.18	2.18 ± 0.12	2.46 ± 0.03	0.085
VSI	10.30 ± 0.33	10.99 ± 0.10	11.93 ± 0.29	11.98 ± 0.77	0.083
TGC	1.36 ± 0.01	1.38 ± 0.01	1.37 ± 0.01	1.38 ± 0.01	0.636
Sv (%)	98.33 ± 1.66	96.67 ± 1.66	96.67 ± 1.66	96.67 ± 1.66	0.815

Low-Mix (low inclusion), Med-Mix (medium inclusion), High-Mix (high inclusion).

Mean ± standard error values in each row with different superscripts were significantly different (P<0.05, Tukey's test).
IBW= Initial body weight, FBW= final body weight, WG= weight gain, RWG= relative weight gain, SGR= specific growth rate, FCR= conversion ratio, HI= hepatosomatic index, VSI= viscerosomatic index, TGC= thermal growth rate and Sv= survival.

Table 4. Proximal composition of muscle (dry matter) of tilapia *Oreochromis niloticus* fed diets containing four different Fat Mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %); 0, 1.65, 3.32, and 5 % in replacement of Fish oil, for 6 weeks.

Muscle (%)	Dietary Treatment				
	Control	Low-Mix	Med-Mix	High- Mix	P value
Dry matter	24.88 ± 0.14	25.02 ± 0.15	25.48 ± 0.85	25.11 ± 0.24	0.061
Crude protein*	85.62 ± 0.78	85.25 ± 0.48	86.12± 0.44	85.19 ± 0.32	0.056
Crude lipid*	5.14 ± 0.66	5.66 ± 0.54	5.84 ± 0.34	6.01 ± 0.71	0.175
Ash*	3.11± 0.13	3.18 ± 0.08	3.35 ± 0.16	3.38 ± 0.11	0.15

* Values given as percentage of total dry matter
Low-Mix (low inclusion), Med-Mix (medium inclusion), High-Mix (high inclusion). Mean ± standard error. Values different superscripts were significantly different (P<0.05, Tukey's test).

Table 5. Apparent digestibility coefficient calculated using insoluble ash as internal market in diets containing four different Fat Mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %); 0, 1.65, 3.32, and 5 % in replacement of Fish oil, for 6 weeks.

Dietary treatment	Apparenet dry matter digestibility (%)
Control	74.49 ± 0.75
Low-Mix	74.42 ± 1.43
Med-Mix	72.16 ± 0.34
High-Mix	72.56 ± 2.85
P value	0.757

Low-Mix (low inclusion), Med-Mix (medium inclusion) High-Mix (high inclusion). Mean ± standard error. Values with different superscripts were significantly different (P<0.05, Tukey's test).

Table 6. Fatty acid (% total FA) composition of muscle of tilapia *Oreochromis niloticus* fed diets containing four different Fat Mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %); 0, 1.65, 3.32, and 5 % in replacement of Fish oil, for 6 weeks.

Fatty acid	Dietary treatments				
	Control	Low-Mix	Med-Mix	High-Mix	*P value*
14:0	4.16 ± 0.01^{a}	3.53 ± 0.04^{b}	3.50 ± 0.03^{b}	3.18 ± 0.04^{c}	0.001
16:0	26.19 ± .55^{c}	28.20 ± 0.19^{b}	30.93 ± 0.07^{a}	31.84 ± 0.30^{a}	0.001
18:0	10.33 ± .07^{a}	9.66 ± 0.05^{b}	9.52 ± 0.04^{b}	9.26 ± 0.11^{c}	0.001
20:0	2.11 ± 0.06^{b}	2.45 ± 0.02^{a}	2.16 ± 0.01^{b}	1.81 ± 0.02^{c}	0.001
22:0	2.12 ± 0.04^{a}	1.97 ± 0.03^{ab}	1.81 ± 0.02^{b}	1.64 ± 0.03^{c}	0.001
Σ SFA	**44.93**	**45.83**	**47.93**	**47.74**	
14:1	0.62 ± 0.04^{b}	0.71 ± 0.03^{ab}	0.81 ± 0.03^{a}	0.83 ± 0.02^{a}	0.015
16:1	6.11 ± 0.03^{a}	5.89 ± 0.12^{a}	5.85 ± 0.04^{a}	5.48 ± 0.04^{b}	0.002
18:1n9	34.15 ± .06^{a}	33.72 ± 0.07^{b}	32.64 ± 0.15^{c}	32.58 ± .04^{c}	0.001
20:1n9	1.67 ± 0.11	1.45 ± 0.01	1.4 ± 0.06	1.38 ± 0.02	0.053
Σ MUFA	**42.57**	**41.79**	**40.71**	**40.28**	
18:2n6	4.15 ± 0.06^{a}	3.71 ± 0.05^{b}	3.61 ± 0.04^{b}	3.36 ± 0.04^{c}	0.001
20:4n6	1.56 ± 0.04^{a}	1.42 ± 0.02^{ab}	1.36 ± 0.04^{b}	1.25 ± 0.06^{b}	0.008
∑PUFAn6	**5.72**	**5.13**	**4.97**	**4.63**	
18:3n3	0.91 ± 0.04	0.90 ± 0.01	0.89 ± 0.02	1.01 ± 0.02	0.081
20:5n3	0.73 ± 0.03	0.66 ± 0.03	0.62 ± 0.03	0.67 ± 0.03	0.252
22:6n3	1.73 ± 0.03^{b}	1.97 ± 0.02^{a}	1.72 ± 0.03^{b}	1.92 ± 0.03^{a}	0.002
∑PUFA n3	**3.39**	**3.54**	**3.25**	**3.63**	
Others	3.38 ± 0.06^{b}	3.71 ± 0.03^{a}	3.14 ± 0.05^{c}	3.72 ± 0.02^{a}	0.001

Low-Mix (low inclusion), Med-Mix (medium inclusion), High-Mix (high inclusion). Values represent Mean ± standard error. ∑SFA, ∑MUFA, ∑PUFA n6, ∑PUFA n3, ∑PUFA are the sum of saturated, monounsaturated, polyunsaturated, polyunsaturated n3 and polyunsaturated n6 respectively.
^{abc} Different letters mean statistical differences among experimental diets, according to Tukey´s test (P<0.05).

Table 7. Serum chemistry analysis of tilapia *Oreochromis niloticus*, fed with four diets containing different Fat Mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %) levels; 0, 1.65, 3.32, and 5 % in replacement of Fish oil, for 6 weeks. Three samples per experimental unit were analyzed.

Parameters	Treatment				P value
	Control	Low- Mix	Med-Mix	High-Mix	
TP (g dL^{-1})	2.98 ± 0.12	2.62 ± 0.09	3.01 ± 0.14	2.73 ± 0.07	0.083
ALB (g dL^{-1})	1.21 ± 0.04	1.14 ± 0.02	1.16 ± 0.03	1.17 ± 0.11	0.741
GLO (g dL^{-1})	1.77 ± 0.11	1.47 ± 0.10	1.85 ± 0.13	1.56 ± 0.07	0.136
GLU (mg dL^{-1})	65.45 ± 3.02^{b}	57.22 ± 3.51^{a}	56.29 ± 1.70^{a}	55.57 ± 2.07^{a}	0.016
CHO (mg dL^{-1})	12.37 ± 3.07^{a}	8.91 ± 3.42^{c}	10.80 ± 3.42^{b}	10.65 ± 4.30^{b}	0.001
TG (mg dL^{-1})	169.70 ± 17	153.98 ± 9.16	212.67 ± 17.40	179.84 ± 13.16	0.119

Low-Mix (low inclusion), Med-Mix (medium inclusion), High-Mix (high inclusion).
Mean ± standard error values in each row with different superscripts were significantly different (P<0.05, Tukey's test).
TP =Total Protein, ALB = Albumin, GLO =Globulin, GLU = Glucose, CHO, Cholesterol, TG = Triglycerides.

Chemical analysis of serum (Table 7) revealed significant differences in glucose and cholesterol levels (P<0,05). The Control treatment had the lowest glucose concentrations and the highest cholesterol levels. Polynomial regression analysis (Fig. 2) demonstrated a strong positive correlation between fat mixture inclusion and glucose levels (r^2=0.960).

Histological examinations using Hematoxylin-Eosin staining (3 samples per treatment) revealed no significant changes in liver tissue or sinusoids tubular spaces across treatments (Fig. 3), nor in the associated hepatopancreatic tissue. Hepatocytes were observed between blood capillaries known as sinusoids, forming hepatic cellular cords, with erythrocytes present in the sinusoids' lumen.

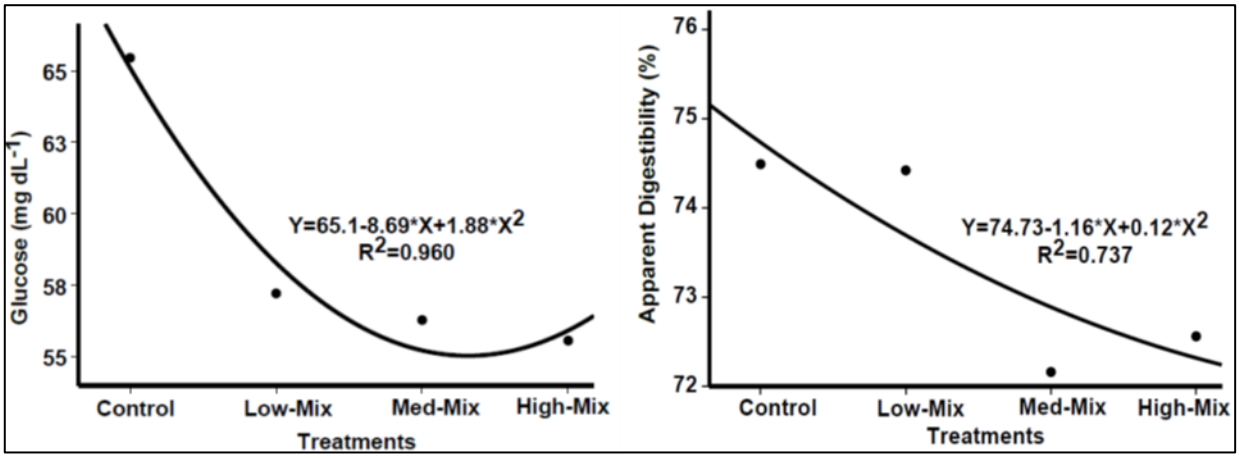

Figure 2. Polynomial (quadratic) regression analysis between levels of fat mixture (Poultry fat 71 %; Kernel fat 25 %; DHA concentrate 4 %) in the apparent dry matter digestibility and glucose. The markers indicate the values shown in Table 5 and 7 (respectively). The lines indicate the best-fit polynomial regression equations and the r^2 values indicate the power of the model. Treatments: 0 (Control), 1.65 (Low-Mix), 3.32 (Med-Mix), and 5 % (High-Mix) in replacement of Fish oil.

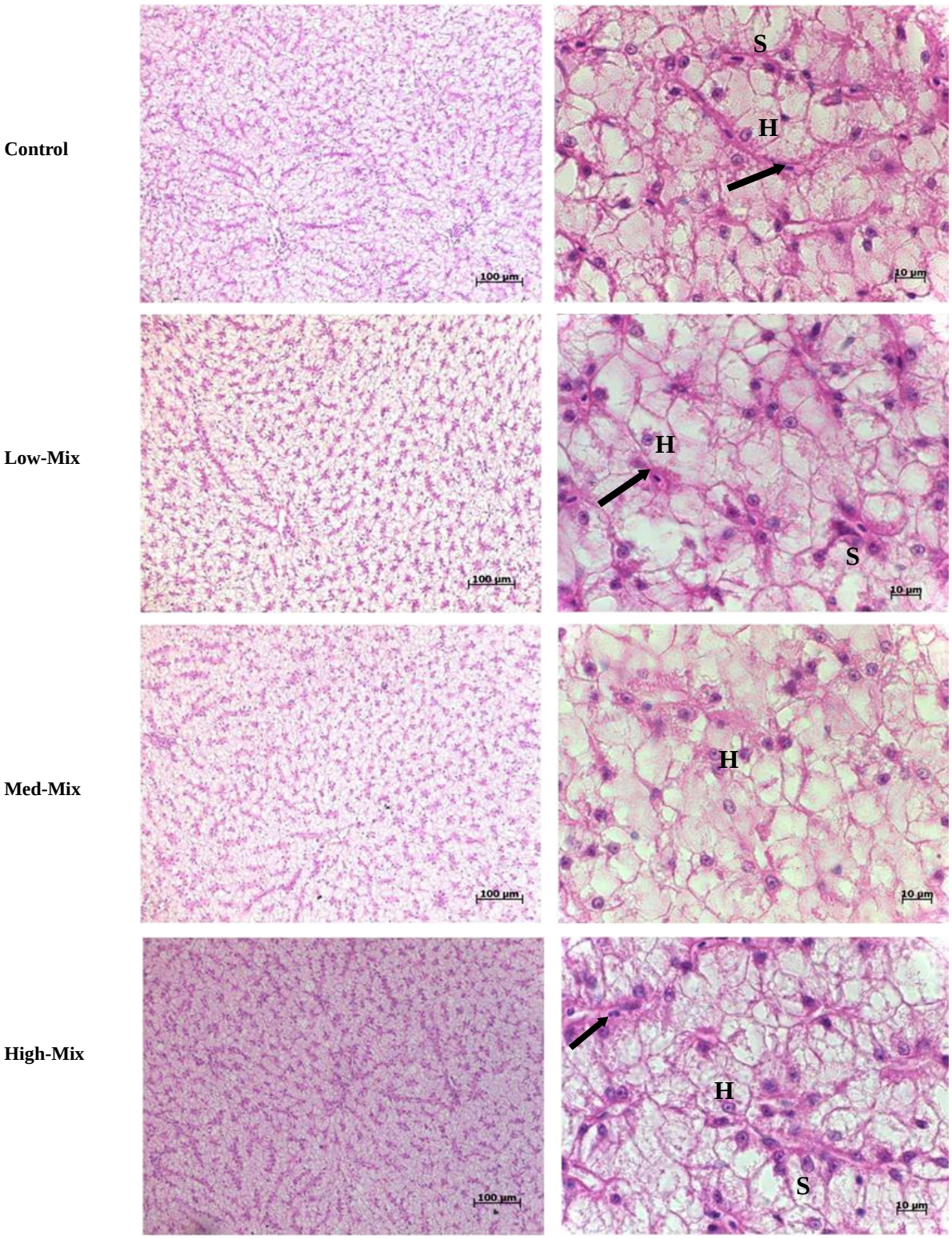

Figure 3. Morphology of hepatocytes in juvenile tilapia (*Oreochromis niloticus*) fed with different experimental diets. H= Hepatocytes, S= Sinusoids, arrows indicate erythrocytes. Hematoxylin-eosin staining.

DISCUSSION

The present study demonstrates that incorporating 71 % poultry fat significantly benefited the overall performance of tilapia (*Oreochromis niloticus*), as shown with the polynomial regressions, in terms of survival rates, weight gain, final weight, specific growth rate, hepatosomatic index, and viscerosomatic index. Additionally, the inclusion of 5 % palm kernel fat (PKF) in the fat mixture (comprising 25 % of the diet) may help spare energy by

facilitating the diffusion of lauric acid into the mitochondria. The polynomial regression analysis revealed a strong correlation across the various indices (Fig. 1 and 2). This finding suggests a potential reduction in reliance on fish oil (FO), which has become unsustainable due to fluctuating market prices, while still offering nutritionally competitive feeds (Ochang *et al.* 2007; Abdel-Tawwab *et al*. 2010; Adjanke *et al*. 2021).

The Control group, rich in FO, exhibited the lowest performance metrics, while the experimental groups demonstrated superior results. These findings are consistent with previous research indicating substantial benefits of poultry fat for tilapia's zootechnical indicators, sometimes exceeding traditional performance standards (Apraku *et al*. 2017, FAO 2018, Tacon 2020). The growing Nile tilapia farming industry has increased demand for nutrient-rich feeds, leading to higher prices for raw materials. This trend has prompted the exploration of alternative, cost-effective ingredients to replace conventional nutritional components (Ragaza *et al*. 2021; Gule and Geremew 2022). However, many alternative products, particularly plant-based ones, contain high levels of anti-nutrients that can significantly reduce their nutritional value (Eeckhout and De Paepe 1994, Medale and Kaushik 2009, Bu *et al*. 2018), especially linoleic acid (18:2n-6). The feed conversion ratio (FCR) is a critical indicator of feed quality for farmed fish, with an FCR of < 2 generally considered satisfactory for tilapia species (Craig *et al*. 2017). In this study, the overall FCR was < 1, with the Control treatment showing the highest FCR (0.61 ± 0.01) compared to the treatments with the fat mixture, which were similar (0.59 ± 0.01). Although no significant differences were found, this suggests that increasing the fat mixture, particularly with high poultry fat levels, did not lead to a proportional rise in FCR, indicating comparable digestive capacities across all treatments (Ayisi *et al*. 2021).

Fish growth was consistent across all treatments, indicating that the inclusion level of the fat mixture did not compromise growth. The overall performance indices (final body weight, weight gain, specific growth rate, hepatosomatic index, and viscerosomatic index) were higher in the experimental groups, likely due to the readily utilized poultry fat and the lauric acid (12:0) in PKF meeting the energy requirements of the cultured organisms, comparable to FO. This may result from optimal β-oxidation of fatty acids in mitochondrial processes (Babalola *et al*., 2011; Ayisi *et al.* 2021). Survival rates did not show statistically significant differences (P>0.05), with all experimental groups presenting a survival rate of 96.67 ± 1.66 %, slightly below the Control group (98.33 ± 1.66 %). The mortalities observed across all treatments were attributed to stress during periodic biometrics rather than to the diets themselves (Ayisi *et al*. 2021).

No adverse effects were observed on hematological indicators, including total protein (TP), albumin (ALB), globulin (GLO), and triglycerides (TG), with values remaining within the optimal range for the species (Svoboda *et al*. 2001, de Lucas *et al*. 2003, Cnaani *et al*. 2004). Furthermore, no significant differences (P>0.05) were noted among treatments. Previous studies (Zaragoza *et al*. 2008) indicated that TP concentrations can be influenced by stressors, such as acute heat stress, suggesting that the experimental diets did not adversely affect the health status of the organisms.

In this study, significant differences (P<0.05) were observed in the concentrations of glucose (GLU) and cholesterol (CHO). Plasma cholesterol serves as an important indicator of fish health, and documented reductions in this parameter have been noted (Yoneyama *et al*. 2009). Despite these statistical differences, both parameters remained within optimal ranges for the species, indicating no adverse effects on blood chemistry (Sukasem and Ruangsri 2007, de Azevedo *et al*. 2013, Wattanakul *et al*. 2021). Notably, as the concentration of poultry fat mixtures increased, glucose levels decreased, likely due to the organisms' demand for sugars for physiological processes rather than health concerns. This suggests that the observed differences may not significantly impact the organisms' responses to the treatments.

Some vegetable oils have been reported to cause excessive lipid accumulation in the livers of tilapia, leading to hepatic vacuolization and steatosis (Peng *et al*. 2014, Ayisi *et al*. 2017). However, in our investigation, liver histology showed no apparent damage; liver cells maintained their normal shape, distribution, and abundance (Wattanakul *et al*. 2021). This contrasts with the findings by Apraku *et al.* (2019), who reported increased hepatic vacuole amounts with different lipid sources compared to a control group. In our study, the liver structure remained consistent across all treatments, suggesting that the tested percentages of poultry fat mixture with PKF, which contains DHA and EPA, did not alter the hepatic structure of *Oreochromis niloticus*.

Crude protein levels in muscle tissue remained stable across diets. Lipid and ash content were consistent across all treatments, with experimental diets performing similarly to the control. Additionally, Nile tilapia appeared to preferentially uptake fatty acids (FA), favoring n-6 series derivatives (linoleic acid, 18:2n-6) over n-3 series derivatives (linolenic acid, 18:3n-3) (Jiao *et al*. 2020; Luo *et al*. 2023).

These results highlight poultry fat and PKF as potential alternatives to replace fish oil in the diets of tilapia (*Oreochromis niloticus*), as they are both sustainable and cost-effective. The polynomial regression analysis indicated a high positive significance of most parameters with an increase in growth and a decrease in feed conversion ratio. Therefore, the fat mixture used here may support better growth performance, adequate survival, and feed efficiency compared to fish oil. No adverse effects were detected at hepatic and hematological levels, underscoring the viability of these sources for aquaculture feeds.

These findings contribute to the search for alternative lipid sources that enhance nutritional efficacy, economic viability, and environmental sustainability while promoting the growth of Nile tilapia aquaculture. Future research should focus on the long-term impacts of these fat mixtures on productive performance, and commercial-scale production, as well as molecular analyses of specific gene expressions related to lipid metabolic pathways.

CONCLUSION

The study demonstrates that poultry fat as the primary fat source, together with palm kernel fat (PKF) and DHA from microalgae, are sustainable and cost-effective alternatives to fish oil in tilapia diets, resulting in a better performance in terms of survival, growth, and the feed conversion ratio without compromising the fish hepatic or hematological health.

ACKNOWLEDGEMENTS

We thank Alfredo Molina from AQUAMOL, for the kindly donation of fish. Thanks to ADM for the donation of DHA Nature™. This project was financed by CONAHCYT (Project CF-2023-G-192).

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

ETHICAL STATEMENT

The feeding trial and sampling procedures were approved by the Ethical Committee of the Instituto de Investigaciones Oceanológicas from the UABC.

REFERENCES

Abdel-Tawwab M., Ahmad M.H, Khattab Y.A.E., Shalaby A.M.E. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 298:267–274. https://doi.org/10.1016/j.aquaculture.2009.10.027

Adedeji O.B., Adeyemo G.A., Adeparusi E.O., Adegbola T.A., Fagade O.E. (2016). Evaluation of Palm Kernel Oil (*Elaeis guineensis*) as a replacement for Fish Oil in the Diet of Nile Tilapia, *Oreochromis niloticus* Fingerlings. *Pakistan Journal of Nutrition*, 15(9):834-840.

Adjanke A., Tona K., Toko I.I., Gbeassor M. (2021). Effect of palm kernel meal *(Elaeis guineensis*, Jacq, 1763) in the diet on digestive transit and some serum parameters in Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758). *International Journal of Biological and Chemical Sciences*, 15(5):1725-1733. https://doi.org/10.4314/ijbcs.v15i5.2

AOAC (2015). Official Methods of Analysis. Association of Analytical Chemists, Arlington, VA, USA.

Apraku A., Huang X., Yusuf A., Cornel A., Ayisi C.L., Asiedu B. (2019). Impact of dietary oil replacement on muscle and liver enzymes activity, histomorphology and growth-related genes on Nile tilapia. *Comparative Biochemistry and Physiology Part C: Toxicology Pharmacology*, 223:15-25. https://doi.org/10.1016/j.cbpc.2019.05.002

Apraku A., Liu L., Ayisi C.L. (2017). Trends and status of dietary coconut oil in aquaculture feeds. *Reviews in Fisheries Science Aquaculture*, 25(2):126-132. https://doi.org/10.1080/23308249.2016.1245275

Apraku A., Liu L., Leng X., Rupia E.J., Ayisi C.L. (2017). Evaluation of blended virgin coconut oil and fish oil on growth performanc and resistance to *Streptococcus iniae* challenge of nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Basic and Applied Sciences*, 4(3):175-84. https://doi.org/10.1016/j.ejbas.2017.06.002

Ayisi C.L., Alhassan E.H., Sarfo F. (2021). Substitution of fish oil with palm kernel oil in diets of *Oreochromis niloticus* fry: effects on growth, feed utilization and economic estimates. *Indonesian Aquaculture Journal*, 16(2):99-107. https://doi.org/10.15578/iaj.16.2.2021.99-107

Azevedo R.V.D., Tonini W.C.T., Braga L.G.T. (2013). Palm oil and palm kernel cake in diets for juvenile Nile tilapia. *Pesquisa Agropecuária Brasileira*, 48:1028-1034. https://doi.org/10.1590/S0100-204X2013000800031

Babalola T.O., Apata D.F., Omotosho J.S., Adebayo M.A. (2011). Differential effects of dietary lipids on growth performance, digestibility, fatty acid composition and histology of African catfish (*Heterobranchus longifilis*) fingerlings. *Food and Nutrition Sciences*, 2:11-21. https://doi.org/10.4236/fns.2011.21002

Bu X.Y., Wang Y.Y., Chen F.Y., Tang B.B., Luo C.Z., Wang Y.,Yang Y.H. (2018). An evaluation of replacing fishmeal with rapeseed meal in the diet of *Pseudobagrus ussuriensis*: growth, feed utilization, nonspecific immunity, and growth-related gene expression. *Journal of the World Aquaculture Society*, 49(6):1068-1080. https://doi.org/10.1111/jwas.12470

Bureau, D.P., & Meeker, D.L. (2010). Terrestrial animal fats. Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, FL, 245-266. https://doi.org/10.1201/9781439808634-c8

Castro-Gómez P., Fontecha J., Rodrígues-Alcalá L.M. (2014). A high-performance direct transmethylation method for total fatty acids assessment in biological and foodstuff samples. *Talanta* 12:518–523. https://doi.org/10.1016/j.talanta.2014.05.051

Cnaan A., Tinman S., Avidar Y., Ron M., Hulata G. (2004). Comparative study of biochemical parameters in response to stress in *Oreochromis aureus*, *O. mossambicus* and two strains of *O. niloticus*. *Aquaculture research*, 35(15):1434-1440. https://doi.org/10.1111/j.1365-2109.2004.01167.x

Craig S.R., Helfrich L.A., Kuhn D., Schwarz M.H. (2017). *Understanding fish nutrition, feeds, and feeding*. Virginia Cooperative Extension, Virginia State University, USA.

de Lucas Rodrigues Bittencourt N., Maria Molinari L., de Oliveira Scoaris D., Bocchi Pedroso R., Vataru Nakamura C., Ueda Nakamura T., Dias Filho P. (2003). Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system. *Hemoglobin (g/dl)*, 10(3.09): 6-58.

Eeckhout W., De Paepe M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology*, 47(1-2):19-29. https://doi.org/10.1016/0377-8401(94)90156-2

Emehinaiye P.E., Ezeri G.N., Abolagba O.J. 2018. Evaluation of the use of palm kernel oil as an alternative to fish oil in the diet of Nile tilapia (*Oreochromis niloticus*) juveniles. *Aquaculture Research*, 49(11):3715-3722.

FAO (2020). The State of World Fisheries and Aquaculture 2020. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/3/ca9229en/ca9229en.pdf

FAO (2018). The State of World Fisheries and Aquaculture 2018. Meeting the sustainable development goals. Rome: Food and Agriculture Organization of the United Nations.

Folch J., Lees M., Sloane-Stanley G.H., (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 276:497–507. https://doi.org/10.1016/S0021-9258(18)64849-5

Gule T.T., Geremew A. (2022). Dietary strategies for better utilization of aquafeeds in Tilapia farming. *Aquaculture Nutrition*, 2022. https://doi.org/10.1155/2022/9463307

Jiao J.G., Liu Y., Zhang H., Li L.Y., Qiao F., Chen L.Q., Du Z.Y. (2020). Metabolism of linoleic and linolenic acids in hepatocytes of two freshwater fish with different n-3 or n-6 fatty acid requirements. *Aquaculture*, 515:734595. https://doi.org/10.1016/j.aquaculture.2019.734595

Kader M.A., Bulbul M., Abol-Munafi A.B., Sheriff S.B., Keong N.W., Ali M.E., Koshio S. (2018). Effect of replacing fishmeal with palm kernel meal supplemented with crude attractants on growth performance of *Macrobrachium rosenbergii*. *Aquaculture, Aquarium, Conservation Legislation*, 11(1):158-166.

Khieokhajonkhet A., Ngoenthong W., Inyawilert W., Aeksiri N., Kaneko G., Ratanasut K., Phromkunthong W. (2024). Assessment of chaya meal (*Cnidoscolus chayamansa*) as an alternative feed ingredient for Nile tilapia (*Oreochromis niloticus*): Growth performance, hematology, histology, and growth-and appetite-related gene expression. *Aquaculture,* 593: 741288. https://doi.org/10.1016/j.aquaculture.2024.741288

Luo Y., Jiao J.G., Jin A.H., Hussain D., Chen L.Q., Qiao F., Du Z.Y. (2023). Metabolism of linoleic and linolenic acids in muscle cells of two freshwater fish with n-3 or n-6 fatty acid requirements. *Aquaculture*, 563:738994. https://doi.org/10.1016/j.aquaculture.2022.738994

Medale F., Kaushik S. (2009). Protein sources in feed for farmed fish. *Cahiers Agricultures*, 18(2):103-111.

Nakharuthai C., Rodrigues P.M., Schrama D., Kumkhong S., Boonanuntanasarn S. (2020). Effects of different dietary vegetable lipid sources on health status in Nile tilapia (*Oreochromis niloticus):* Haematological indices, immune response parameters and plasma proteome. *Animals*, 10:1377. https://doi.org/10.3390/ani10081377

Napier J.A., Betancor M.B. (2023). Engineering plant-based feedstocks for sustainable aquaculture. *Current Opinion in Plant Biology*, 71:102323. https://doi.org/10.1016/j.pbi.2022.102323

Natt M.P., Herrick C.A. (1952). A new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poultry Science*, 31(4):735-738. https://doi.org/10.3382/ps.0310735

Ng W.K., Law A.T., Wong S.H., Lim C.C., Lim S.J. (2018). Potential use of a vegetable and animal oil blend in the diet of tilapia, *Oreochromis niloticus*. *Aquaculture Nutrition*, 24(3):1063-1070.

Ochang S.N., Fagbenro O.A., Adebayo O.T. (2007). Growth performance, body composition, haematology and product quality of the African catfish (*Clarias gariepinus*) fed diets with palm oil. *Pakistan Journal of Nutrition*, 6:452-459. https://doi.org/10.3923/pjn.2007.452.459

Oliver L., Dietrich T., Marañón I., Villarán M.C., Barrio R.J. (2020). Producing omega-3 polyunsaturated fatty acids: a review of sustainable sources and future trends for the EPA and DHA market. *Resources*, 9(12):148. https://doi.org/10.3390/resources9120148

Peng M.O., Xu W., Mai K., Zhou H., Zhang Y., Liufu Z., Ai Q. (2014). Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil. *Aquaculture*, 433:442-449. https://doi.org/10.1016/j.aquaculture.2014.07.005

Ragaza J.A., Hossain M.S., Koshio S., Ishikawa M., Yokoyama S., Kotzamanis Y., Kumar V. (2021). Brown seaweed (*Sargassum fulvellum*) inclusion in diets with fishmeal partially replaced with soy protein concentrate for Japanese flounder (*Paralichthys olivaceus*) juveniles. *Aquaculture Nutrition*, 27(4):1052-1064. https://doi.org/10.1111/anu.13246

Sankian Z., Khosravi S., Kim Y.O., Lee S.M. (2019). Total replacement of dietary fish oil with alternative lipid sources in a practical diet for mandarin fish, *Siniperca scherzeri*, juveniles. *Fisheries and Aquatic Sciences* 22:1-9. https://doi.org/10.1186/s41240-019-0123-6

Santigosa E., Constant D., Prudence D., Wahli T., Verlhac-Trichet V. (2020). A novel marine algal oil containing both EPA and DHA is an effective source of omega-3 fatty acids for rainbow trout (*Oncorhynchus mykiss*). *Journal of the World Aquaculture Society* 51(3):649-665. https://doi.org/10.1111/jwas.12699

Sukasem N., Ruangsri J. (2007). Effects of palm kernel cake (PKC) on growth performance, blood components and liver histopathology of sex reversed red tilapia (*Oreochromis niloticus*). J. Sci. Technol., 29:1283–1299.

Svoboda M., Kouřil J., Hamáčková J., Kalab P., Savina L., Svobodova Z., Vykusova B. (2001). Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre-and postspawning period. *Acta Veterinaria Brno*, 70(3):259-268. https://doi.org/10.2754/avb200170030259

Tacon A. G. (2020). Trends in global aquaculture and aquafeed production: 2000–2017. Reviews in Fisheries Science Aquaculture, 28(1):43-56. https://doi.org/10.1080/23308249.2019.1649634

Tacon A. G., Metian M. (2015). Feed matters: satisfying the feed demand of aquaculture. *Reviews in Fisheries Science Aquaculture*, 23(1):1-10. https://doi.org/10.1080/23308249.2014.987209

Tejada de Hernández I. (1992). Control de calidad y análisis de alimentos para animales (No. SF95. T44 1992.).

Turchini G.M., Ng W.K., Tocher D.R. (2009). Fish oil replacement and alternative lipid sources in aquaculture feeds. *Reviews in Aquaculture*, 1:10-57. https://doi.org/10.1111/j.1753-5131.2008.01001.x

Wattanakul W., Thongprajukaew K., Hahor, W. Suanyuk, N. (2021). Optimal Replacement of Soybean Meal with Fermented Palm Kernel Meal as Protein Source in a Fish Meal-Soybean Meal-Based Diet of Sex Reversed Red Tilapia (*Oreochromis niloticus*× *O. mossambicus*). *Animals* 11(8):2287. https://doi.org/10.3390/ani11082287

Yoneyama Y., Yonemori Y., Murata M., Ohnuki H., Hibi K., Hayashi T., Endo H. (2009). Wireless biosensor system for real-time cholesterol monitoring in fish "Nile tilapia". *Talanta*, 80(2):909-915. https://doi.org/10.1016/j.talanta.2009.08.014

Yossa, R., Ahmad Fatan, N., Fairchild, J., Schrama, J. W. (2022). Apparent digestibility coefficients of local palm kernel cakes, rice bran, maize bran and sago flour in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Journal of Applied Aquaculture*, 34(2):502-526. https://doi.org/10.1080/10454438.2020.1869635

Yuangsoi B., Klahan R., Charoenwattanasak S. (2014). Partial replacement of protein in soybean meal by moringa seed cake (*Moringa oleifera*) in bocourti's catfish (*Pangasius bocourti*). *Songklanakarin Jounal of Science of Technology*, 36(2):125-135.

Zaragoza O.D.R., Rodríguez M.H., Bückle Ramirez L.F. (2008). Thermal stress effect on tilapia *Oreochromis mossambicus* (Pisces: Cichlidae) blood parameters. *Marine and Freshwater Behaviour and Physiology*, 41(2):79-89. https://doi.org/10.1080/10236240801896223