Volume 6 • Issue 1 • 2024

Research Article

Journal of Biotechnology and Immunology

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Fish Oil Replacement with Virgin Coconut and Corn Oil: Impact on Growth Performance, lipogenic and Digestive Enzyme Activity, and mRNA Expression of Genes Involved in lipid Metabolism of Nile Tilapia

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Key words: *mRNA, Lipogenic, Dietary supplementation, Enzymes, Physiology*

Citation: Escientific Publishers. (2024). Fish Oil Replacement with Virgin Coconut and Corn Oil: Impact on Growth Performance, lipogenic and Digestive Enzyme Activity, and mRNA Expression of Genes Involved in lipid Metabolism of Nile Tilapia. *Journal of Biotechnology and Immunology* 6(1).

Introduction

Dietary lipids have a reflective effect on the expression of genes which leads to changes in cell differentiation, growth and metabolism apart from playing a role in membrane lipid composition and as energy source (Martinez-Rubio et al. 2013; Goldberg et al. 2009).

and optimizing diet formulation. Although several studies have been conducted, the molecular mechanism responsible for changes in lipid deposition due to replacement of fish oil with virgin coconut oil (VO) and corn oil (CO) has not been studied extensively.

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in *O. niloticus*.

Tilapia (Oreochromis niloticus) is considered a suitable species for aquaculture worldwide owing to its ability to grow rapidly, high disease and stress resistance (Deng et al. 2010). Tilapia although have been noted to be capable of utilizing n-6 FAs, it still requires a certain number of n-3 FAs to enhance growth performance for maximum yield. This has resulted in several studies to establish its nutritional requirement especially on their FA (n6 FA) requirements

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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible].00
Rapeseed meal	30.00	30.00	30.00	30.00	30.00	30.00
Wheat meal	22.95	22.95	22.95	22.95	22.95	22.95
Fish oil	6.00	3.00	3.00	-	-	-
Virgin coconut oil	-	3.00	-	6.00		3.00
Corn oil	-		3.00		6.00	3.00
Vitamin & mineral premix	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50

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Inositol	0.05	0.05	0.05	0.05	0.05	0.05
$Ca(H_2PO_4)$	1.50	1.50	1.50	1.50	1.50	1.50
Soybean phos-	2.00	2.00	2.00	2.00	2.00	2.00

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14:0	0.61 ± 0.00	4.35 ± 0.07	0.43 ± 0.02	9.04 ± 0.09	0.27 ± 0.01	4.03 ± 0.01
16:0	10.40 ± 0.02	11.02 ± 0.06	10.25 ± 0.19	12.30 ± 0.29	9.99 ± 0.02	10.68 ± 0.06
18:0	3.02 ± 0.05	3.10 ± 0.04	2.41 ± 0.04	2.97 ± 0.07	1.68 ± 0.00	2.16 ± 0.02
20:0	0.29 ± 0.01	0.24 ± 0.01	0.31 ± 0.00	0.00 ± 0.00	0.32 ± 0.00	0.25 ± 0.00
22:0	0.23 ± 0.00	0.20 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

∑ SFA's	14.54 ± 0.05	28.49 ± 0.06	13.44 ± 0.04	45.35 ± 0.06	12.34 ± 0.04	26.59 ± 0.04
16:1 (n-7)	1.29 ±	0.91 ±	0.94 ±	0.80 ±	0.58 ±	0.60 ±

∑MC-PUFAs	52.32 ± 0.43	42.84 ± 0.06	52.01 ± 0.78	29.73 ± 0.26	53.63 ± 0.08	43.70 ± 0.08
n-3: n-6	0.12 ± 0.01	0.10 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.04 ± 0.00	0.05 ± 0.00

Table 2: *Fatty acid (FA) profiles (% total FA) of experimental diets.*

Total saturated fatty acids (SFAs) includes all FAs without double bonds

Total monosaturated fatty acids (MUFAs) constitute all FAs with one double bonds

Total polyunsaturated fatty acids (PUFAs) are sum of all FAs with

two or more double bonds

Total long-chain (LC) polyunsaturated fatty acids constitutes the sum of PUFAs with chain length of 20 or more carbon atoms and

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starved and fifteen (15) from each tank was randomly sampled on every two weeks (fifteenth day).

At the end of the feeding trial on the 56th day, all surviving fish were counted and weighed after starvation for 24h and sampled for various indices to be checked.

Sample collection and growth performance

Five fish per tank at the end of the trial were randomly sampled, euthanized with an overdose of tricaine methane sulfonate (MS-222 at 200mg/L in culture water), weighed individually, pooled and stored at -80°C for subsequent determination of proximate composition. Digestive tract and liver samples were taken, pooled and stored at -80°C for subsequent lipogenic enzyme activity and

bio-engineering). In brief, 0.5–1.0 ml of serum was pipetted into 1.5ml eppendorf tubes and inserted into the biochemical analyzer. The analyzer was calibrated according to the manufacture instruction and results read on attached computer.

Proximate composition and fatty acid analysis

Fish composition was determined using standard methods (AOAC, 1995). Total lipid was measured following the method of Folch, Lees & Stanley (1957). FA methyl esters (FAME) was produced from total lipid aliquots and methylated with boron trifluoride (BF_3) in

methanol. The FA composition of total lipid in the diets were determined using gas chromatography (GC-7890A, USA) according to the method of Huang, Todorčević, Ruyter & Torstensen (2010). FA content was determined using the normalization method, while

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was expressed per mg of hepatic soluble protein specific activity or per gram of liver tissue wet weight.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Measurement of RNA expression of target genes (PPAR-α, CD36, FABP4, G6PD, 6PGD) were performed using Real-time PCR. Trizol reagent (Invitrogen, China) was used to extract total RNA from liver samples that had been sampled and stored at -80°C. Through absorbance measurement, the concentration of RNA was determined. In brief, the RNA samples were digested by RNase-free DNase I (Takara) incubation for 15 minutes at 37°C. Next, 2ug of RNA was transcribed into cDNA using M-MLV reversed transcriptase. All cDNA samples were stored at -20°C until analysis. Samples were then

ferentiation); 6GPD: 6-Phosphogluconate dehydrogenase; G6PD: Glucose 6-phosphate dehydrogenase; β-Actin: Beta actin

Statistical analysis

No significant interactions were obtained when multivariate and univariate GLM were employed in the data analysis and so only one-way analysis of variance (ANOVA) was used to compare treatments, where significant, Tukey's multiple test was used to compare all pairs of columns. Significant levels were set at 0.05 probability ($P<0.05$). Homogeneity of variance and data normality were

tested using Bartlett's test and Shapiro-Wilk normality test respectively. All analysis was in triplicates and was also performed using

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was significantly different from VO (3.13 ± 0.13).

Protein content in muscle was significantly higher in group VO (72.43 ± 4.90) than VCO (85.64 ± 2.63) although, both were not different from the other treatments. However, whole body protein content in FO, FVO and CO were significantly higher than recorded in groups VO and VCO but not different from FCO.

Dietary alternatives had no effect on survival and feed conversion ratio. Weight gain (WG) and specific growth rate (SGR) were low-

cantly higher value. ARA in vegetable diets was significantly higher than those blends with FO and it's exclusive. While n-3 was higher in those fed FO (9.19 ± 0.17), n-6 was higher in CO group (51.15 ± 0.39) whereas VO group recorded the least amongst all. EPA and DHA was also significantly least in diets with vegetable lipid inclusive diets.

Moisture	FO	FVO	FCO	VO	CO	VCO
Muscle	75.99 ± 0.61	76.18 ± 0.65	76.65 ± 0.44	77.90 ± 0.54	75.09 ± 1.50	75.63 ± 0.65

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20:2(n-6)	1.60 ± 0.12[ab]	1.23 ± 0.15[bc]	1.51 ± 0.17[abc]	1.00 ± 0.01[c]	1.95 ± 0.06[a]	1.48 ± 0.04[abc]
20:3(n-6)	0.84 ± 0.03[abcd]	0.79 ± 0.01[bd]	0.81 ± 0.00[abcd]	0.56 ± 0.15[cd]	1.09 ± 0.04[ac]	1.09 ± 0.00[ab]
20:4(n-6) ARA	1.50 ± 0.08[b]	1.19 ± 0.05[c]	1.18 ± 0.03[c]	1.77 ± 0.04[ab]	1.65 ± 0.09[ab]	1.79 ± 0.02[a]
∑ n-6	42.16 ± 3.90[bc]	41.60 ± 0.42[bc]	48.74 ± 1.32[ab]	30.99 ± 0.36[d]	51.15 ± 0.39[a]	40.47 ± 0.61[c]
18:3(n-3)	2.82 ± 0.28[a]	2.68 ± 0.04[a]	2.37 ± 0.02[a]	1.31 ± 0.16[b]	1.32 ± 0.06[b]	1.32 ± 0.01[b]
20:3(n-3)	0.56 ± 0.03[a]	0.51 ± 0.01[a]	0.42 ± 0.00[b]	0.13 ± 0.03[d]	0.25 ± 0.01[c]	0.28 ± 0.01[c]
20:5(n-3) EPA	0.32 ± 0.02[a]	0.19 ± 0.03[b]	0.17 ± 0.02[bc]	0.18 ± 0.00[b]	0.10 ± 0.01[c]	0.12 ± 0.00[bc]
22:6(n-3) DHA	5.48 ± 0.29[a]	3.32 ± 0.07[b]	2.79 ± 0.07[bc]	2.56 ± 0.09[cd]	1.93 ± 0.12[d]	1.98 ± 0.05[d]
∑ n-3	9.19 ± 0.17[a]	6.69 ± 0.28[a]	5.74 ± 0.13[a]	4.16 ± 0.30[b]	3.59 ± 0.10[b]	3.70 ± 0.11[b]

∑PUFAs	51.35 ± 3.73^{ab}	48.28 ± 0.70^{ab}	54.47 ± 1.39^{a}	35.15 ± 0.40^{c}	54.75 ± 0.44^{a}	44.17 ± 0.72^{b}
∑LCPUFAs	8.70 ± 0.32^{a}	5.99 ± 0.39^{b}	5.36 ± 0.02^{b}	5.18 ± 0.22^{b}	5.02 ± 0.27^{b}	5.26 ± 0.11^{b}
∑ MCPUFA	41.05 ± 3.79^{ab}	41.07 ± 0.20^{ab}	47.60 ± 1.25^{a}	28.97 ± 0.32^{c}	47.78 ± 0.39^{a}	37.44 ± 0.58^{b}

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16:1(n-7)	1.70 ± 0.16^{b}	2.32 ± 0.07^{ab}	1.77 ± 0.12^{b}	3.09 ± 0.34^{a}	2.25 ± 0.06^{ab}	2.84 ± 0.26^{a}
18:1(n-9)	27.50 ± 2.57^{ab}	31.42 ± 0.89^{a}	27.89 ± 1.37^{ab}	22.69 ± 1.71^{b}	32.67 ± 1.51^{a}	32.30 ± 0.69^{a}
20:1(n-9)	1.11 ± 0.16	1.19 ± 0.09	0.88 ± 0.12	0.98 ± 0.06	1.37 ± 0.11	1.06 ± 0.11
22:1(n-9)	0.48 ± 0.09^{a}	0.09 ± 0.06^{bc}	0.22 ± 0.03^{abc}	0.10 ± 0.06^{b}	0.04 ± 0.04^{c}	0.06 ± 0.06^{c}
24:1(n-9)	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
∑ MUFAs	30.78 ± 2.79^{ab}	35.04 ± 1.08^{ab}	30.76 ± 1.35^{ab}	26.86 ± 2.06^{b}	36.33 ± 1.63^{a}	36.26 ± 1.01^{a}
18:2(n-6)	32.31 ± 6.99^{a}	21.59 ± 1.42^{ab}	35.16 ± 1.92^{a}	16.62 ± 0.99^{b}	26.96 ± 1.86^{ab}	20.44 ± 2.38^{ab}
18:3(n-6)	0.84 ± 0.08^{ab}	0.58 ± 0.02^{ab}	0.46 ± 0.23^{a}	0.45 ± 0.01^{a}	0.10 ± 0.06^{b}	0.62 ± 0.04^{ab}

20:2(n-6)	2.19 ± 0.38[a]	1.48 ± 0.09[ab]	1.56 ± 0.09[ab]	0.98 ± 0.04[b]	1.77 ± 0.04[ab]	1.14 ± 0.05[b]
20:3(n-6)	0.74 ± 0.37	0.76 ± 0.01	0.85 ± 0.09	0.97 ± 0.07	1.10 ± 0.07	0.78 ± 0.03
20:4(n-6) ARA	2.86 ± 0.31[ab]	2.50 ± 0.16[b]	2.88 ± 0.37[ab]	4.52 ± 0.79[a]	3.65 ± 0.09[ab]	2.75 ± 0.01[ab]

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ALT (U/L)	24.26 ± 5.14	29.93 ± 5.26	24.89 ± 1.36	22.47 ± 2.22	34.92 ± 5.78	26.13 ± 4.03	0.3936
AST (U/L)	32.16 ± 5.02	38.03 ± 5.54	31.67 ± 2.01	29.40 ± 2.21	42.63 ± 6.08	32.38 ± 4.74	0.3722

Note* TP: Total protein; TG: Triglyceride; TC: Total cholesterol; LDL: Low-density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; ALP: Alkaline phosphatase; ALT: Alanine amino transferase; AST: Aspartate amino transferase;

Table 8: *Hematological and antioxidant profile of fish fed different experimental diets.*

Digestive enzyme activity

Table 9 shows no significant differences were obtained between groups and sections when amylase activities were measured. the AI sections when lipase activity was measured. While only FVO (3.428 ± 0.0010) and CO (3.424 ± 0.0007) were significantly different in the MI, no differences in activity was obtained in the PI.

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Trypsin (IU/L)	4.027 ± 0.0065[b]	4.051 ± 0.0072[b]	4.029 ± 0.0105[b]	4.143 ± 0.0402[a]	4.055 ± 0.0080[b]	4.054 ± 0.0123[b]
Pepsin (U/L)	1.159 ± 0.0066	1.204 ± 0.0113	1.193 ± 0.0233	1.201 ± 0.0300	1.209 ± 0.0106	1.231 ± 0.0220
Protease (IU/L)	3.424 ± 0.0010	3.425 ± 0.0005	3.425 ± 0.0008	3.425 ± 0.0006	3.426 ± 0.0007	3.427 ± 0.0010
Lipase (mU/L)	5.048 ± 0.1900	4.937 ± 0.0392	4.819 ± 0.0410	4.843 ± 0.0753	5.054 ± 0.1085	5.123 ± 0.0935

Parameters/ Groups	FO	FVO	FCO	VO	CO	VCO
PPAR-α (U/L)	1.165 ± 0.0002	1.165 ± 0.0005	1.167 ± 0.0007	1.167 ± 0.0001	1.165 ± 0.0004	1.166 ± 0.0006

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ological functioning of O. niloticus will be maintained when FO is replaced with vegetable oils at a 50% level. The study results are in agreement with previous study reports when vegetable oils replaced FO at different levels in Juvenile Nile and Mozambique tilapia and common carp (Ochang, Fagbeno & Adebayo, 2007; Demir, Tüker, Acar & Kesbiç, 2014; Sun, Ye, Chen, Wang & Chen, 2011). Also, the performance of FCO can be as a result of an enhanced metabolic activities due to the preference for 18: n-9 content in vegetable oils which is a substrate for energy production (Peng, Li, Lin & Chen, 2016). Further, synthesis of FA is essentially obtained from 18: 3n-3 (α-linoleic acid, ALA) which is in abundance in FCO.

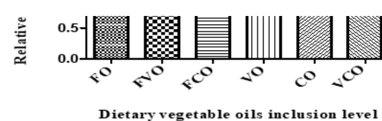

Figure 1: *Relative mRNA zxpression of (A) 6PGD, (B) G6PD (C) CD36, (D) FABP4 and (E) PPAR-α of fish fed different experimental diets.*

limited in vegetable diets which agrees with Tengjaroenkul, Smith, Caceci, and Smith (2000) indicating the limiting effects of low fats that are broken down and leads to triacylglycerol and digestion.

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which disagrees with other studies when FO was substituted with palm and canola oils in O. niloticus and yellowtail kingfish (seriola lalandi) respectively (Ayisi et al., 2017). VO had a limiting effect on lipase activity in AI indicating digestion of VO and its utilization as an efficient energy source although it was nominally lower. However, in the MI, VO seemed to stimulate higher lipase activities between groups. Although, nutrient digestion usually takes place in the AI and MI to some extent, it also plays a major role (Magalhães et al., 2015). This could explain the high activity of lipase in the VO group. The possible drag of secreted mucous to this part in the VO group could also be a reason for this activity. In general, lipase activity was

was not contaminated and shows the liver was in good functional status as opposed to Lin & Shiau (2007). Also, metabolic transport and associated activities were not influenced as the results for HDL-C and LDL-C shows. As such, cholesterol transport can be said to be efficient and thereby present a quality and healthy fish (Fei, Xiaoqin, Baian, & Xiangjun, 2015).

To our knowledge, studies on hepatic enzymes activities (PPAR-α, FABP4, CD36, G6PD and 6GPD) have not been done and this result basically presents the first-hand information on these activities. FABP4 and PPAR-α enzymatic activities in the liver was not

influenced by dietary alternatives. These are key enzymes in the transport of FAs (Oku et al., 2006). As such, this result could be explained that, dietary alternatives do not influence or impact the

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LC-PUFAs in dietary vegetable alternatives affect the regulation of FABP4 gene expressions when fed to fish (Li et al., 2016).

The lowest mRNA expression levels of 6GPD was obtained in fish fed VCO, which indicates blends of dietary vegetable oils might affect lipid synthesis by reducing NADPH supply which is essential for lipid synthesis. However, with the exception of VCO effect, a positive relationship between G6PD and 6GPD was obtained. This suggest a synergistic effect on pentose phosphate pathways (Zheng et al., 2013).

The uptake of FAs in the VCO group was lower as shown in the mRNA expression levels of CD36. This correlates with the results obtained in the expression of 6PGD mRNA levels where lower sup-

ence and Technology Commission (14320502000).

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