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Influence of Dietary Palm Oil on Growth Response, Carcass Composition, Haematology and Organoleptic Properties of Juvenile Nile Tilapia, Oreochromis niloticus

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Abstract: An 8-week feeding experiment was conducted to determine the effect of replacing fish (codliver) oil with palm oil at 0, 33.33, 66.67, 100 % for diets 1, 2, 3, 4 respectively, of Nile Tilapia, *Oreochromis niloticus*. Each diet was formulated to contain 28% crude protein and fed to triplicate groups of 10 juvenile *Oreochromis niloticus* (mean wt., 9.09g). Fish mortality decreased with increasing palm oil substitution level. There was a significant difference (P< 0.05) in the survival of fish fed Diet 4 compared with other treatments. The result showed that carcass quality of experimental fish was not compromised as a result of the use of palm oil in diets. Blood parameters of fish fed experimental diets showed a common trend, namely the higher the palm oil substitution level, the higher the blood parameters. Organoleptic properties of experimental fish showed that palm oil replacement for fish oil had no negative effect on their sensory evaluation and eating quality. The present study provides evidence that palm oil can effectively replace cod liver oil in diets for Nile Tilapia, *O. niloticus*.

Key words: Oreochromis niloticus, palm oil, haematology, sensory evaluation.

Introduction

Aquaculture has traditionally relied on products from industrial fisheries, namely fishmeal and oil, to service the aquafeed industry. But, of the global fish oil production in 1996 of 1.4 million mt, 576,000 mt were used for salmon and trout production alone (Bell *et al.*, 2002). It has been estimated that aquafeeds currently use about 60% of the global supply of fish oil (Barlow *et al.*, 2000). The predicted use of fish oil for aquaculture is estimated to rise to about 90% of the total available by 2010 (Barlow *et al.*, 2000).

Fish oil is produced from small marine pelagic fish and represents a finite fishery resource (Ng et al., 2003). Because of several factors including over fishing resulting in dwindling catch and environmental changes which necessitate tight regulations, future demand for wild-caught fish will exceed supply (Sargent et al., 1999). Hence the need to evaluate potential substitutes for fish oil, an important ingredient in the formulation of aquafeeds. Palm oil, currently the second most abundant vegetable oil in the world, presents a viable alternative to fish oil in aquafeeds. Studies on the use of crude palm oil, and refined, bleached and deodorized palm oil as dietary lipid for catfish have shown good results (Legendre et al., 1995; Ng et al., 2000; Ng et al., 2003). In this study, the nutritive value of crude palm oil substitution for cod liver oil in juvenile Nile Tilapia (Oreochromis niloticus) diets were evaluated. The influence of different dietary palm oil levels on the growth performance, haematology and organoleptic properties of juvenile Nile tilapia were investigated.

Materials and Methods

Fish meal (Atlantic menhaden, Brevoortia tyrannus), mineral/vitamin premix and binder used in this experiment/study were obtained from a feed/feedstuff store in Akure. Bovine Blood meal, cassava starch and palm oil were obtained from Akure main market. Cod liver oil was obtained from a pharmacy shop at Akure. Triplicate samples of fish meal and blood meal were analyzed for proximate composition (moisture, crude protein, crude lipid, crude fibre, ash) according to AOAC (1990) methods. Crude protein was determined using a Kjeltec Auto 1003 Analyzer after digestion with concentrated H2SO4 in a digester. Crude lipid was estimated by extracting in chloroform: methanol (2:1) using a Soxtec extraction HT6 unit. Crude fibre was determined using a Fibretec System 1020 Hot Extractor and ash content was determined by igniting at 550°C in a muffle furnace for 12 hours. Gross energy content was determined using a ballistic bomb calorimeter (model OC-5182, Gallenkamp & Co. Ltd., Loughborough, England).

Four isoproteic diets (28% crude protein) were formulated (Table 1). The control diet (diet 1) contained 6% cod liver oil, which was replaced by palm oil in diets 2, 3, and 4 at 33.3%, 66.7% and 100%, respectively. Each of the diets had 2% fish oil as a residual of the fishmeal component of the diet. The feedstuffs were thoroughly mixed in a Hobbart A-200 (Troy, Ohio, USA) pelleting and mixing machine to obtain a homogenous mass. Diets were passed through a mincer with die of 0.8mm and milled, blended, moistened, pelleted and

sun-dried at ambient temperature for three days and stored in air-tight plastic at ambient temperature (26°C). Proximate composition of the diets and fishes were determined in triplicates according to AOAC (1990) methods. Groups of 10 O. niloticus fingerlings (9.09 g) which had been acclimated for 14 days were randomly stocked in 12 circular plastic tanks (21 litre) containing 15 litres of water each. Each of the diets was fed to the fishes in triplicate containers at 4% body weight twice daily (9.00-10.00 and 16.00-17.00 h) for 56 days. Dissolved oxygen concentration and temperature were measured three times every week using the combined Jenway meter (Jenway model 9071, UK), while pH was also monitored thrice weekly using an electronic pH meter (Mettler Toledo 320 model, UK). The tanks were cleaned regularly and fresh water was used to change the water in the tanks daily. Aquarium Air-pumps (Resun AC-9802, Guangdong Risheng Group Co. Ltd., China) were used to aerate the water in

the experimental tanks throughout the study.

The weight of each group of fish was taken fortnightly using a Triple Beam Balance (700 series, Ohaus Florham park, N.J. 07932, USA), and the feed adjusted accordingly.

At the beginning and at the end of the feeding trial, six tilapia, randomly selected from the initial pool and each treatment group were homogenized, packed in airtight polythene bags and stored in a deep freezer (-20°C), prior to analysis. After two weeks from start of the feeding trial, faeces were collected from each tank early each morning, pooled for each treatment and dried; and the proximate composition determined using the methods of AOAC (1990). The dried faecal samples were then ashed, and the ashes were digested by Acid Insoluble Ash (AIA) method (Halver et al., 1993), Mean weight gain (MWG), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) were estimated from the bi-weekly weight data. For the carcass analysis, two fish were pooled together for each replicate, homogenized in a blender and the proximate composition determined.

The fish, from which blood for haematology was collected, were anaesthetized with 150 mg/l solution of tricaine methane sulphonate (MS-222, Sigma Chemical co. St. Louis, MO, USA). (Wagner et al., 1997). Blood samples were taken with 2ml heparinized syringes and 21swg needles from the caudal vein of a set of three O. niloticus fingerlings from each treatment and put separately in 2ml heparinized tubes and taken to the laboratory for determination of haematocrit (Hct), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), white blood cells (WBC), and red blood cells (RBC) using the methods of Svobodova et al. (1991). The haematological indices of mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated using the

total red blood cell count (RBC), haemoglobin concentration (Hb), and hematocrit (Hct) according to the following formulae (Dacie and Lewis, 2001):

MCHC (g l^{-1}) = [Hb (g dl^{-1})x 10]/Hct x 100 MCH (pg) = [Hb (g dl^{-1})x 10]/ RBC (10⁶ μl^{-1})

 $MCV (fl) = Hct/RBC (10^6 \mu l^{-1})$

Assessment of organoleptic properties carried out to determine the effect of palm oil substitution on fish quality was performed on the day the trial ended. The fish fillets were assessed by a panel of trained panelists of 4 individuals selected for their interest, availability and sensorial capacities of memorizing stimuli or discriminating intensities (Regost *et al.*, 2003).

Products were assigned three-digit numbers from a table of random numbers. The coding was necessary because 'people generally associate "1" or "A" with "best" and it is recommended that the three —digit random numbers be used' (Larmond, 1977). The samples were evaluated using a 9-point hedonic scale, with 1 being the lowest (poorest) and 9 the best. Both fresh and cooked samples were assessed. Fresh samples attributes assessed were general appearance and colour; while the attributes of cooked samples assessed were texture, aroma, taste and juiciness.

All data collected from the experiment were subjected to one way analysis of variance (ANOVA) test using the SPSS Statistical Package (Version 10.0), and where significant difference were indicated, means were tested using Least Significant Difference (LSD) test at the 5% level of significance (Zar, 1984).

Results

The physicochemical parameters of water were within the range for culture of Oreochromis niloticus (Table 2). The table shows that these values fall within the normal range for the warm water culture of O. niloticus. The summary of growth response and nutrient utilization parameters are shown in Table 3. There was a significant difference in mean weight gain of fish fed diets 1 and 2 on the hand, and diets 3 and 4 on the other; with the former being better than the latter. While the highest percentage weight gain was recorded in fish fed Diets 2 (75.00%), it was very closely followed by fish fed Diet 1 (74:16%) in which all the dietary oil inclusion is from fish oil. The carcass composition of O. niloticus at the beginning and at the end of the feeding trial are shown in Table 4. Carcass composition of fish at the end of the feeding trial was not significantly different from the initial composition. The viscerosomatic index (VSI) of the initial fish (prior to the feeding trial) was not significantly different from the values for all the other treatments except fish fed Diet 1 which had a significantly higher value. The gonadosomatic index (GSI) was not significantly different between treatments. But with the exception of diet 2, the hepatosomatic index

Table 1: Ingredient and proximate composition of diets with palm oil for the Nile tilania Oreochromis niloticus

Ingredients (g/100g DM)	Diets						
	Diet 1	Diet 2	Diet 3	Diet 4			
Fish meal (61% cp)	26	26	26	26			
Blood meal (82% cp)	17	17	17	17			
Starch	46	46	46	46			
Fish (cod liver) oil	6	4	2	0			
Palm oil	0	2	4	6			
Vitamin/mineral premix	3	3	3	3			
Carboxyl methyl cellulose	2	2	2	2			
Proximate							
Moisture (%)	8.30	9.15	13.02	12.92			
Ash (%)	9.93	9.61	9.47	11.11			
Crude protein (%)	28.45	28.79	28.01	28.01			
Crude lipid (%)	12.91	13.06	7.19	7.60			
Crude fibre (%)	0.20	0.27	0.34	0.27			
Nitrogen free extract (%)	40.55	39.12	41.68	40.11			
Gross Energy (Kcal/kg)	4504.92	4479.24	3987.37	3960.18			

(HSI) increased with increasing palm oil substitution. There was a significant difference in all the haematological parameters measured both between dietary treatments and between the control and dietary treatments (Table 5). Apart from fish fed diet 2 whose blood parameters did not follow the observed trend, all the fish fed other dietary treatments showed a common trend, namely the higher the palm oil substitution level, the higher the blood parameters. There was no significant difference between the initial (pre-treatment) values of haemoglobin, erythrocyte sedimentation rate. white blood cells and red blood cell and those of fish fed diet 4. MCHC values were not significantly different between all the treatments, but the MCV were significantly different between treatment groups, with value decreasing with increasing palm oil level in the diet. MCH values of the fish fed different diets presented a similar trend to the one observed for MCV.

Fish fed different diets did not present any significant difference in the mean score for either the sensory attributes of the raw fish (General appearance and colour) or organoleptic attributes of the cooked fish (texture, aroma, taste and juiciness) (Table 6). Not only was there no significant difference between sensory attributes of fish fed different dietary treatments, but even the overall mean score between treatments was not significantly different.

Discussion

The present study showed that feeding Nile tilapia diets containing palm oil had no negative effect on growth and nutrient utilization. There were no significant differences in growth rates among treatments, except fish fed diet 3 which performed poorer. Mortality was high generally, but decreased progressively with increasing palm oil substitution. Diet 4 with no cod liver (fish oil) performed best in term of survival (70%) and combined with its FCR, proved much better than the control diet (Diet 1). This result is in agreement with those of previous

studies which have shown that palm oil is a suitable dietary lipid and energy source for the red hybrid tilapia, Oreochromis sp. (Ng et al., 2001) and for some tropical catfish species (Lim et at., 2001; Ng et al., 2000, 2003). Tilapia, like other warm water fish, have a greater requirement for n-6 fatty acids found more in vegetable oil than n-3 fatty acid found in fish oil, for maximal growth (NRC, 1993). Kanazawa et al. (1980) reported depressed growth of tilapia with oil having high levels of n-3 PUFA. Takeuchi et al. (1983) could not obtain any enhanced growth of tilapia when the diet was supplemented with 18:3n-3 or n-3 HUFA. Hybrid red tilapia fed cod liver oil diet showed a slight depression in growth and feed efficiency ratio (Ng et al., 2001). The specific growth rate (SGR) obtained in this study ranged from 0.75±0.12 for fish fed Diet 3 to 1.00±0.03 for fish fed Diet 2. The SGR of fish fed Diet 1 was 0.99±0.06. only slightly inferior to the value for fish fed Diet 2 (1.00±0.03). The fish fed the diet without any fish oil inclusion (Diet 4) performed as well as those fed Diet 1.

The results of this study show that palm oil can be substituted for cod liver oil in tilapia diets without adverse effect on growth. In fact Diet 1 with no palm oil substitution showed slight depression in growth and feed efficiency. This might be due to the higher concentration of n-3 PUFA found in cod liver oil. This result agrees with the findings of Ng et al. (2001) who found slight depression in growth of hybrid tilapia. Because of the residual fish oil (about 2%) in the fishmeal component of the diet, palm oil was able to replace 100% of the added fish oil (Diet 4) with no significant reduction in growth rate or feed conversion rate. This result corroborates the findings of Bell et al. (2002) that Atlantic salmon fed a diet in which the fish oil was replaced 100% by palm oil showed no reduction in growth rate.

The feed conversion ratio (FCR) was not significantly different between treatments, except for diet 3 which performed significantly poorer that all the others (Table 3.3). The SGR and PER both presented a similar trend as that observed for the FCR. The not significant difference between FCR, SGR and PER of diets 1, 2 and 4 indicated that fish were able to digest the diets and convert the diets into body tissues with the same degree of efficiency. In the first two weeks of the experiment, growth was very slow (Fig. 1). It is probable that the fish were getting used to (acclimating) to the formulated diets. Thereafter, all the fish picked up and started utilizing the diets better.

The lipid, protein and ash contents of the carcass composition showed significant different between treatments, just as there were small differences in proximate composition between dietary groups (Table 1). The carcass crude protein of fish fed diets 1, 2 and 3 were not significantly different between treatments, but were significantly (p < 0.05) higher than those fed the 100% palm oil diet (diet 4) and those of the initial fish.

Table 2: Water Quality parameters of culture tanks during feeding trials of Oreochromis niloticus fed diets with palm oil.

Diet	Temp. range	Mean Temp.	pH range	Mean pH	Dissolved	Mean DO	
2.50	(°C)	(°C) ± S.E.	± S.E	(DO) (mg/l)	Oxygen	(mg/l)±S.E	
1	23.0 - 25.0	23.94±0.07 ^a	5.80 - 6.94	6.51±0.05 ^a	5.3 - 7.6	6.17±0.09°	
2	23.0 - 25.0	23.93±0.06 ^a	6.26 - 6.76	6.50±0.03 ^a	5.4 - 7.5	6.22±0.08°	
3	23.0 - 25.0	23.95±0.06°	5.92 - 6.95	6.57±0.04 ^a	5.2 - 7.8	6.19±0.11 ^a	
4	23.0 - 25.0	23.95±0.07°	5.91 - 6.89	6.56±0.03 ^a	5.1 - 7.7	6.20±0.10°	

Table 3: Growth performance of Oreochromis niloticus fed diets with palm oil for 56 days mean ± S.E. of three replicates.

	Diets				
	1	2	3	4	
Initial Weight	9.16±0.57ª	9.48±0.62°	9.01±1.75°	8.73±0.83°	
Final Weight	15.94±1.14°	16.57±0.93°	13.43±1.73°	14.70±1.58 ^b	
Mean Weight Gain	6.78±0.62°	7.09±0.34°	4.42±0.25°	5.97±0.79b	
Percentage Weight Gain	74.17±5.46 ^b	75.00±3.00 ^b	52.53±9.90°	68.03±4.58b	
Feed Conversion Ratio1	3.88±0.17 ^{a,b}	3.68±0.14°	5.22±0.77 [€]	4.18±0.25b	
Protein Efficiency Ratio ²	0.92±0.04b	0.95±0.04b	0.70±0.09°	0.86±0.05 ^{a,b}	
Specific Growth Rate ³	0.99±0.06b	1.00±0.03b	0.75±0.12°	0.93±0.05b	
Survival (%)	20.00±1.73°	23.33±1.53°	46.67±2.08b	70.00±1.73°	
Apparent Digestibility Coefficient of protein4	0.6601±0.07 ^{a,b}	0.8062±0.03b	0.5135±0.0 ^{1a}	0.6483±0.06 ^{a,b}	

FCR¹ (Feed conversion ratio) = TF/(Wf - Wi). TF is the average total feed fed to a fish.

PER² (Protein efficiency ratio) = Wet weight gain (g) / protein fed (g)

SGR3 (Specific growth rate) = 100 x (In Wf - In Wi)/rearing period (days), where Wf is Final weight and Wi is Initial weight.

ADC⁴ (Apparent digestibility coefficient of protein) = $10^{-2} 10^{-6}$ (% AIA in feeds x % protein in faeces) / (% AIA in faeces x % in protein in feed) (Halver *et al.*, 1993).

Table 4: Carcass composition VSI, GSI and HSI of *Oreochromis niloticus* (on a DM basis) fed diets with palm oil for 56 days, mean ± S.E. of three replicates.

Treat- ment	Moisture	Crude protein	Crude lipid	Ash	VSI	GSI	HSI
mone							
Initial	5.92±0.19 ^{b,c}	62.69±0.22°	5.40±0.69°	6.20±0.03b	10.71±1.69°	3.97±0.95°	0.87±0.08 ^{a,b}
Diet 1	6.40±0.40°	64.12±0.93b	5.72±0.53°	5.20±0.09 ^a	11.81±1.40 ^b	4.41±0.71b	1.04±0.48b
Diet 2	5.27±0.49b	63.74±0.23b	5.28±0.31°	5.20±0.09 ^a	9.61±0.37°	4.23±0.43b	0.50±0.01a
Diet 3	4.78±0.22a	63.46±0.15 ^b	5.62±0.51°	6.15±0.39b	9.76±0.67°	4.70±0.08°	1.68±0.05 [€]
Diet 4	5.90±0.15 ^{b,c}	62.65±0.18°	5.50±0.52°	6.19±0.41b	10.39±0.81°	3.93±0.42°	1.99±0.72°

The crude lipid was not significantly different between treatments. The ash content of fish fed diet 1 were significantly lower than those of fish fed other diets. The VSI of fish fed diet 1 had a significantly higher mean value than those of fish fed other diet types. It is probable that there was a higher lipid accumulation in the viscera which accounts for the significantly higher VSI value obtained with diet 1. The HSI of fish increased significantly higher across the treatment groups than that of the pre-treatment fish, the exception being fish fed diet 2 which had significantly lower HSI values than those of fish fed other diets. (Table 4).

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec *et al.*, 2000). Results of analysis of the hematological parameters of *Oreochromis niloticus* in this study (Table 5) showed that there was no significant difference (p < 0.05)) in the Hct values obtained for the different treatments. There was, however, a significant difference between the treatment values and the Hct value obtained for the control. The haematocrit (Hct) values of fish fed treatment diets increased with increasing palm oil substitution level.

The pre-treatment fish had significantly higher Hct values than the treated fish. The Haemoiglobin (Hb) content of fish fed different diet types increased with increasing palm oil substitution level The Hb values obtained in this study are very similar to that (3.7g/dl) reported by Haniffa and Vijayarani (1989). They are however much lower than the 7.0-9.8g/dL with a mean of 8.2g/dL reported by Hrubec et al. (2000); and the 8.0-8.9g/100ml reported by Fagbenro (1994). There was an inverse relationship established between the erythrocyte sedimentation rate (ESR) of fish and the level of palm oil in the diet, the higher the palm oil level the significantly lower the Esr. There was no significant difference in the white blood cells (WBC) of fish fed diets that had palm oil, no matter the level of inclusion. However, a significant difference existed between the WBC of fish fed diet 1 and all the other diets, including the WBC of the initial fish. It may be probable that n-3 PUFA in the codliver oil may have significantly reduced the white blood cells and therefore compromised the immune system. This perhaps may explain the very high mortality (80% recorded for diet 1 compared with 30% recorded for Diet 4) (Table 3). The mean cell haemoglobin (MCH)

Table 5: Haematology of *Oreochromis niloticus* fed diets with palm oil for 56 days, mean ± S.E. of three replicates.

	Tiacillatolog	gicai parametei						
Treat-	HCT (%)	НВ	ESR	WBC	RBC	MCH	MCHC	MCV (fl)
ment		(g dl ⁻¹)	(mm/hr)	(x10³/µI)	(x 10 ⁶ /µI)	(pg)	(g l ⁻¹)	
Initial	22.00±6.03°	7.40±2.00 ^b	11.67±1.20 ^{a,b}	5.30±1.40 ^b	2.37±0.64 ^b	31.27±0.09 ^{b,c}	337.3±3.20 ^{a,b}	92.65±0.19 ^{a,b,c}
Diet 1	11.33±0.33°	3.73±0.03 ^a	14.33±0.33°	2.57±0.03°	1.10±0.06 ^a	34.10±0.16°	329.7±11.10 ^a	103.58±0.60°
Diet 2	15.00±0.00 ^a	5.03±0.03a,b	12.00±0.00a,b	4.70±0.00b	1.80±0.00a,b	27.97±0.02°	335.3±2.30°	83.33±0.00a,b
Diet 3	13.33±0.33a,b	4.37±0.03°	13.00±0.00b	4.17±0.03ª,b	1.43±0.03ªb	30.47±0.06a,b	377.7±7.10°	93.17±0.39 ^{b,c}
Diet 4	17.33±0.33a,b	5.90±0.06a,b	10.67±0.33°	6.17±0.03b	2.13±0.07b	27.73±0.11a	340.7±9.50°	81.36±0.22a

Means with same superscript are not significantly different

Table 6: Sensory Evaluation of Oreochromis niloticus fed diets with palm oil for 56 days, means ± S.E. of four replicates.

Treat-	General	Colour	Texture	Aroma	Taste	Juiciness	Overall mean
ments	Appearance						for attributes
Diet 1	8.00±0.41b	8.25±0.50	7.75±0.25 ^b	6.75±0.48°	7.75±0.25 ^{a,b}	6.25±0.75°	7.46±0.44°
Diet 2	7.75±0.25 ^{a,b}	8.00±0.71	6.75±0.48°	7.25±0.25°	7.25±0.48°	7.50±0.50b	7.42±o.45°
Diet 3	7.25±0.75 ^a	8.25±0.25	7.00±0.41a	7.50±0.29b	8.00±0.41b	6.50±0.50°	7.42±0.44°
Diet 4	8.25±1.11b	7.00±0.41	8.00±0.71b	7.75±0.48 ^b	7.50±0.87 ^a	6.50±0.87°	7.50±0.74°

1. = the poorest, 9 = the best

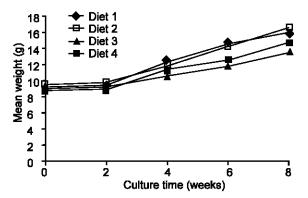


Fig. 1: Relationship between mean weight (mg) and culture time (weeks) in *Oreochromis niloticus* fed diets with palm oil for 56 days.

and mean cell volume (MCV) decreased significantly with increasing level of palm oil in the diet.

The use of palm oil did not affect flesh quality of *Oreochromis niloticus* in terms of general appearance and organoleptic properties. This result is consistent with that obtained by Hardy *et al.* (1987) and Bjerkeng *et al.* (1997) who used soybean meal in place of fishmeal and soybean oil in place of fish oil, respectively in diets of Atlantic salmon. However, Regost *et al.* (2003) found significant difference in the odour of fish fed diets containing soybean oil and fish fed 100% fish oil. Montero *et al.* (2005) did not find any significant difference in the growth and flesh quality of European sea bass fed diets with 60% soybean oil or linseed oil substitution for fish oil. Overall acceptability of fish fed the different dietary treatments was very favourable.

Conclusion: In summary, the results of this study show that palm oil can be used to replace dietary fish oil for Nile tilapia, without significantly compromising growth rates. Survival of fish fed diets with only palm oil was significantly higher than fish fed all the other treatments

that had codliver (fish) oil as either all or part of the lipid source. Carcass analysis did not show any significant difference from the control. The haematological parameters of fish fed diets with different levels of fish oil substitution were closer to the initial values and significantly different from fish fed diet with only fish oil. Palm oil can effectively replace cod liver (fish) oil in diets for Nile tilapia, *Oreochromis niloticus*

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