Fish oil replacement and alternative flaxseed oil and beef tallow in male Nile tilapia *(Oreochromis niloticus)* diets.

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Abstract: An experiment was performed to examine the effect of replacing partial or total of fish oil with flaxseed oil or beef tallow in male Nile tilapia were evaluated using nine isonitrogenous (31.4%), isolipidic (12.19) and isocaloric (18.85 MJ/ Vg) diets in 90 days trial. Dietary treatments consisted of : 1) Fo,containg 100% fish oil, 2) 100% flaxseed oil (Fxo), 3) 100% beeftallow (BT), 4) mixture 4) Mix. (50% Fo + 50 Fxo), 5) mix.2 (50% fish oil + 50% beef tallow) 6) Mix.3 (50% Fxo + 50% BT), 7) Mix.4 (25% Fo+ 31.5% Fxo + 37.5% BT), 8) Mix.5 (25% Fo + 75% Fxo). 9) Mix.6 (25% Fo + 75% BT). Growth performance weight was increase, specific growth rate, growth rates per metabolic weight unit appeared signficiantly (P <0.05) between treatments. Final body weight, weight gain to fish fed Mix. 4 was increase significantly (P<0.05) compared to other treatments. However fish fed the 100% BT and 50% BT and 25% BT had lower weight gain and feed efficiency. On contrast with fish fed Mix.4 and Mix. 1 a resulted improve in feed conversion ratio and feed conversion efficiency. Fillet proximate composition was similar for all fish treatments. However energy retention, resulted in increased significant (P< 0.05) with fish fed Mix.4. Replacing fish oil with different level from beef tallow (BT. Mix.2, Mix 4) resulted increasing in hepatosomatic index and total lipid in liver. Hematology parameters was similar respectively for all treatments. 25% fish oil + 37.5% flaxseed oil + 37.5% beef tallow in the diet male Nile tilapia was maintaining on increased growth performance and feed efficiency.

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1.Introduction:

Aquaculture is expected to meet much of the growing consumer demand for fish supply in the future, the substantial use of fish oils in this industry has raised concerns that the rapid expansion of the aquaculture industry will put more pressure on already declining marine resources (Tidwell and Allan , 2001).

Vegetable oils constitute promising candidates for fish oil replacement having steadily increasing production, with high availability and better economic value (Fountoulaki *et al.,* 2009).

The use of fish oil for aqua feed production is viewed as the most stringent obstacle to overcome for the sustainable development of the aquaculture sector (Naylor *et al.,*2009) . Several studies reported that vegetable oils can partially or fully replace fish oil in fish diets without compromising growth performance in a variety of species as long as their essential fatty acid requirements are met (Ng *et al.,*2003a , Turchini*et al.,*2003). NRC (1993) reported that flax oil consists of approximately 530g ALA/kg.The results by Mjoun *et al.* (2012) showed that partial or complete replacement of fish oil with flaxseed oil (mixtureoil: flaxseed oil, 1:1) and 100% flaxseed oil) were similar specific growth rate and feed efficiency compared to fish fed the fish oil (100%) diet.

Izquierdo *et al.*(2005) reported that substitution be vegetable oils of up to 60% fish oil in diets for gilthead seabream does not affect growth and feed utilization even after a long feeding.

Martins *et al.*(2009) found that the apparent digestibility of monoeroic fatty acids was lowest in groups fed poultry fat diet (88.0±1.3%) and highest with flaxseed diet (96.1±0.3%) through, flaxseed oil by itself is highly susceptible to oxidation, it can be mixed with a saturated animal fat to increase its stability – as saturated fatty acids behave as inert diluents in the oxidation process (Rosas- Romero and Morton , 1977) . Hardy *et al.*(1979 and Mugrditchian *et al.*(1981) reported that animal fat in combination with linseed oil was found to be a successful combination in Chinook salmon diets in a dry diet formulation.

Hertrampt and Piedad- Pascual(2000) and Turchini *et al.*(2003) showed that suitability of pork lard and poultry fat could be used as lipid sources for rainbow trout and brown trout.

On the other hand , Lim *et al* . (2009) appears that lipid levels ranging 5-12% are optimum in diets for tilapia . Though , Chou and Shiau (1996) reported that the optimal dietary lipid for maximal growth of hybrid tilapia (*Oreochromis niloticus X Oreocrhomis aureus*) is about 12% and 5% of dietary lipid appears to meet the fish requirement for this species , the same authors used mixed from different lipid source (corn oil : cod liver oil : lard , 1:1:1).

Therefore, the present study was designed to investigate the effects of partial and total substitution of fish oil with either flaxseed oil or beef tallow on growth performance , feed utilization and hematological parameters of male Nile tilapia (*Oreocrhomi niloticus*).

2. Materials and Methods

Experimental conditions

This experiment was conducted at El-kanater El-Khayria .Research station of the national institute of oceanography and fisheries, Egypt. Male Nile tilapia (*Oreochromi niloticus*) were obtained from a local fish farm . Each of the nine experimental diets was fed to two ponds, each containing 60 fish with an initial mean weight (100.16 ± 1.24g).were randomly distributed into a cement ponds consisting of 18 ($10m^3$ /unit) .

Fish were acclimated to the ponds and fed the commercial diets for two weeks prior to the beginning the trial. The experiment lasted for 90 days during the period from August to November 2012. Fish were acclimated to experimental conditions, while being fed commercial diet, for 2 weeks before the start of the experiment.

Experimental procedure:

Feeding rate was 3% of body weight. Fish were fed two times daily at 9:00 and 15:00.

Fish was bulk weighted biweekly and the daily ration was adjusted accordingly. Water temperature was 25.7± 3.1 C, pH was 7.4 – 8.1, NH_4+> 0.01 mg/L, and dissolved oxygen level was more than 5.9mg/l.

Analytical methods:

The test diets and fish fillets were analyzed for chemical composition following AOAC (1996) methods: dry matter by weight loss after 24 h in an oven at 105 ◦C for 16 h; crude protein (% × 6.25) determined using the Kjeldahl method, dietary lipids extracted was measured by ether extraction using Soxhlet method using Petroleum ether (40-60 ◦C boiling point), however total lipid in fish fillets and livers sample were measured according to Folch *et al.* (1957). Gross energy MJ /kg was calculated 1g crude protein = 23.6 K.J 1g crude lipid = 39.5 kj. 1g nitrogen free extract = 17.2 kj according to Thanuthong *et al.*(2011).

Lipids for fatty acid analysis:

Fatty acid analysis was performed on each experimental diet (Table.3)

Lipid Extraction:

Weigh 2-20g of the sample into a 205ml centrifuge bottle, add sufficient water to bring total water to 16 ml chloroform and macerate for 30 sec; add 20ml water and macerate age for 30 sec. Centrifuge the mixture for 10 min at 2000-2500 rpm. Draw off the lower chloroform layer and filter through a coarse filter paper into a dry weighed flask or beaker. Evaporate the chloroform to dryness (Pearson's chemical analysis of foods eighth edition 1981).

Methylation of lipid:

In a tube weigh 50 mg of lipid add 5 ml of methanolicsulphuric acid (1 ml cone sulphuric acid and 100 ml methanol) and 2 ml of benzene, close the tube well andplace in water bath at 90°c for an hour and half. Cool, add 8 ml water and 5 mlpetroleum ether shake strongly and separate out the ethereal layer in a day tube.

Evaporate to dryness

GC Conditions:

Device Model: HP (Hewlett Packard) 6890GC

Detector: FID (Flame lonization Detector)

Detector temperature: 250°c

Injector temperature: 220°c, injection volume 2 u,l, splitless mode

Column: HP-5(5% di phenyl, 95% dimethyl polysiloxane), 30m, 0.32mm ID, .25 u,mfilm thickness

Carrier gas: Nitrogen, gas flow: 1 ml/min

Oven Program:

Initial temp. 150°c for 2 min

Ramps	Rate °c/min	Final temp.	Hold time
1	10	200	
n	5	250	9 min

(Radwans,1978)

At the end of the feeding trial, and blood was collected from the caudal vein used as an anticoagulant for hematology (hemoglobin, haematocrit and redblood cell counting).

Haematocrit values (Ht) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuging for 15 min in a microhematocrit centrifuge Hemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomethaemoglobin according to Van kampen and Zilstra (1961).

Calculations:

Specific growth rate of weight (SGRW, % day^{-1})

$= (L_nW_f – L_nW_i)$ X 100 / t

Where,W_f and W_i are final and initial mean weight g.

Growth rates per metabolic weight unit.

GR_{MBW} calculated as

GR MBW (g $Kg^{-0.8}$ d^{-1}) = (W_f - W_I) / (W_{mean} /1000) 0.8/t

(weght gain) (WG%)

= 100 X (final body weight x final body protein initial body weight x initial body protein) X (total feed intake X dietary protein content).

Hepatosomatic index (HSI) = 100 X (liver weight) / (body weight)

Survival = final fish number – initial fish number .

Feed conversion ratio (FCR)

FCR = F 1 (W_f - W_i)

F1 = total feed intake during the experimental period .

Feed conversion efficiency (%)

FCE % = 100 × (final body weight – initial body weight) × (total feed in take)$^{-1}$

Feed in take of the fish as a percentage of body weight

FI_{perc} (% d-1) = Fl / W mean X 100

Feed intake of the fish per metabolic weight unit.

Fl_{MBW} (g kg$^{-0.8}$ d^{-1}) =

Fl / (W_{mean} /1000)$^{0.8}$

Where W_{mean} is the geometric mean body weight, which was

Calculated as

$W_{mean}(g) = \sqrt{wi \times w_{fi}}$

Wi and W_f are the initial and final average individual fish weight (g).

Statistical analysis :

The statistical analysis was performed using (SPSS 13.0) for windows.

Data are reported as mean values ± standard deviation. Homogeneity of variance was confirmed and comparison between means was by one- way ANOVA. Duncan's procedure was used for multiple comparison . Differences were regarded as significant when $p < 0.05$.

3.Results and discussion

The test diets were iso- nitrogenous 31.41% iso-lipidic 12.19% (Table .2) , and their fatty acids composition (Table3) reflected that of the added source lipids ,however, there were notable differences in the fatty acid profiles with respect to highly saturated fatty acids with both treat. BT and treat Mix.6, while both treat .FXO , and treat . Mix.5 were characterized by high levels of poly unsaturated fatty acids (Table 4).In the present study, male nile tilapia (*Oreochromis niloticus*) responded to feeding diets containing varying lipid sources (Table 4). the partial replacing of fish oil(75%) with alternative flaxseed oil with beef tallow (1:1) (mix.4) could be a good tool induced satisfying growth performance , its incorporated may be cover the essential fatty acids requirement , as fish oil are only dietary source of n-3 HUFA.(Sargent *et al. 2002* Xue *et al.,* 2006).

Analysis of variance for growth performance indicates that there is no significant differences among fish fed Mix.4 and fish fed only fish oil (FO) (Table 4) . Similar results as the present study were reported by Du *et al.*(2002) , who found that 42.7% replacing fish oil by soy bean and corn oil did not show significant difference on SGR. Xue *et al.*(2006) mentioned that 50% of alternative lipid sources replacement (10% of fish oil used in control diet) could be used effectively .

In Japanese sea bass feed, without negative effects on growth performances. The larger growth performances for fish fed mix.4 in comparison to male Nile tilapia exposed to the FO 100% diet (Table 4) . This increased growth is indirect conformity with the observations of Turchini *et al.*(2007) who described a positive stimulation of fish lipid metabolism in Murray cod, termed lipo- compensatory growth, when fish fed low n-3LC- PUFA diets were reverted to fish oil – based diet for a fatty acid restorative period.Chou and Shiau (1996) recommended the optimal dietary- level for maximal growth of tilapia when a mixed dietary lipid source was used .however ,Huange *et al.*(1998) found the growth performance of hybrid tilapia , *Oreochromis niloticus XO. aureus* fed the diet containing fish oil was the best among test groups (lard, soy oil, HUFA). They suggests those other fatty, acids such as linolenic acids, EPA and DHA in fish oil, may be important for this species as well. andFo. (100% fish oil) or between FXO (100% flaxseed oil).

Results presented in Table.5 ,fish fed Mix.5 (25% fish oil+75Flaxseed oil) and fish fed FO , growth performance was similar trends with treatments with obtained with Sagne *et al.*(2013) ,who found that the best growth rate and SGR were observed in Nile tilapia (*Oreochromis niloticus*) fed on diet containing a mixture of vegetable oils in equal proportions . this may be attributed to the fact that peanut oil and soybean oil enrich in linoleic acid (n-6) (13-4)% and 51.5% respectively. Though, Wing – Keong *et al.*(2011) reported that a novel dietary vegetable blend was able to totally replace fish oil in tilapia diets without negative impact on growth performance. In this connection, Corraz and Kaushik (2009) showed that the incorporation of vegetable oils in fish feed , replacing fish oil does not growth performance since the needs of essential fatty acids are covered from foundation fish meal (as source of fish oil which may be continued EFAS as in the diets . Though, Drew *et al.*(2007) found that complete replacement of fish oil with 65:35 mixture of Canola and linseed oils resulted significantly lower growth rates in rainbow trout fed a fish meal-free diet, but not in fish fed diets containing 10, 20 or 40% fish meal.

Furthermore , Medale and Kaushik (2008) found that total substitution of fish oil with vegetable oils (rapseed , linen) o a mixture of vegetable oils (55% repseed palm 30% +15% linen) does not change into the ingestion voluntary growth performance and feed efficiency .

Mourente *et al.*(2005) suggested that 60% of fish oil could be replaced by rapeseed , linseed and olive oils , without reduction on the growth rates in European sea bass (*Dicentrarchus labrax).*Tu *et al.*(2013) showed that flaxseed oil contained 18.64% linoleic acid and 51.61% linolenic acid of total fatty acids . Whereas fish fed the diet flaxseed oil preferentially oxidized oleic acid and linolenic acid (Wilson *et al.,*2007). The same authors found that salmon fed diet poultry fat preferentially oxidized 16:0 and perhaps some 14:0 , 16:1 n-7 and 20:5 n-3 for energy.

Chou and Shiau (1999) suggested that both n-3 and n-6 fatty acids are required for maximum growth of juvenile hybrid tilapia..Hsieh *et al.*(2007) appeared that weight gain and feed efficiency in fish fed a mixed diet (fish oil 7% + 5% corn oil) or higher than in those fed only a fish oil , or palmitoleic acid , or coconut oil diet. Though , Tu *et al.*(2013) concluded that 100% fish oil and fish meal –based substitution with linolenic acid (ALA) (rich vegetable oil) is not suitable for barramundi as the absence of fish oil and fish meal from the diet resulted in retardation in fish growth and decreases of all major n-3 LCPUFA in tissue. Li *et al.*(2013) found significantly declined with abalones were fed 100% linseed oil diet, may be related to the absence of dietary ARA and EPA, also they suggested that extremely high levels of LA or / and ALA might vanish the stimulating effect of EFA or even reduce the growth of abalone.

On the other hand ,Bell *et al.*(2001) appeared that when fish oil is replaced by terrestrial alternatives such as vegetable oils , the resultant decreased supply of dietary LC- PUFA has been reported to stimulate both the transcription rate and the actual activity (Francis *et al.,*2007) of the enzymes (Fatty acid denaturizes and elongases) involved in the fatty acid bioconversion pathway..Majoun *et al.*(2012) resulted that partial or complete replacement of fish oil with flaxseed oil 1:1; and 100% flaxseed oil were similar weight gain ,specific growth rate and feed efficiency fed the fish oil 100% diet.

Results presented in table.5, indicated that growth performance for fish / as the blend from fish oil, flaxseed oil and beef tallow in treat Mix(4) increased than fish fed flaxseed oil as only source lipid may influenced mainly high levels of LA and ALA in 100% linsed oil (FXO) , its may have consequences on the protein sparing effect using protein as energy instead of PUFA, and eventually on growth performance of abalone (Li *et al.,*2013) . Though , Mishra and Samantaray (2004) showed that dietary fatty acids are known to be energy provision for organisms through B- oxidation .

Moreover , PUFA (such as LA and ALA) is not preferred in this process than SFA and MUFA (Lim *et al.,*2001).

However ,Mjoun *et al.*(2012) found that partial or complete replacement of fish oil with flaxseed oil resulted in similar weight gain and specific growth rate. Kowalska*et al.*(2011) suggested that flaxseed oil can completely replace fish oil in the diet of yellow perch.

As given in table ,5 as affected by dietary different levels from sources of lipids , male Nile tilapia were fed diets containing 50% flaxseed oil and 50% beef tallow (Mix.3) from additive lipid , no significant difference in growth performance (WG, SGR , GR) comparatively with fish fed 100% fish oil as supplemented diet . (FO.) . These results could be in partial agreement with the findings of Mugrditechian *et al.*(1981) who examine linseed and animal fat as alternative lipid sources in dry diets for Chinook salmon(*Oncorhynchus tshawytscha*). They observed no significant differences in final weight of the fish fed the experimental diets after sixteen weeks of feeding. in this connection , YU *et al.*(1977a) reported success in the growth of rainbow trout fed a series of high energy diets in which one third to one half of the 22% hering oil was replaced by lard. Hardy *et al.*(1979) did not observe a significant difference in weight gain in salmon fed a diet in which one half of 8% linseed oil was replaced by tallow. noteworthy, no significant differences (*P*<0.05) in growth performance (SGRD GRMBW) evident between either treatment mix.2 , mix.3 or mix.6 (Table.5) may be to similar fatty acids composition respectively (Table 4) (Francis *et al.,*2009).

Wilson *et al.*(2007) show that replaced up to 75% of the supplemental anchovy oil in high energy extruded diets for Atlantic salmon post-smolts with either cold –pressed flaxseed oil , Canada oil or poultry fat without affecting their athletic health.Weight gain of the male Nile tilapia fed the 100% beef tallow (BT) comparable to those fed fish oil (FO) or blend of fish oil, flaxseed oil and beef tallow , (Table 4) , may be due to a deficiency in essential fatty acid (polyunsaturated) (Table.5) . Xu *et al.*(2007) found that terrestrial animal fat (park lard, beef tallow and poultry fat) contained lower PUFA and higher saturated fatty acids and mono-unsaturated fatty acids particularly 18: 1n-9. It has been suggested by (Chou and Shibau , 1996) that *tilapia zilli* requires approximately 1% n-6 fatty acids in the diet which can be met with linoleic acid (18: 2n-6) or arachidonic acid (20: 4n-6) (kanazawa *et al.*, 1980 ; Takeuchi *et al.*, 1983) . Stickney and Mcgeachin (1983a) indicated that growth of *Tilapia aurea* was not affected by dietary linoleic acid levels as high as 2% . Though , Wilson *et al.*(2007) suggested that when the fish oil removed from the experimental diet, which may have resulted in the fish being deficient in essential fatty acids .

Similar trends have been reported in Founto-ulaki *et al.*(2009), who suggested that the lower final weight of the palm oil dietary for gilthead sea bream (*Sparusaurate*) could be explained by the long duration of the experiment (24 weeks) which may have led to lower digestibility of the saturated and mono-unsaturated fatty acids especially 16:0 in which palm oil was the richest (Ng. *et al.*, 2003b) . it has been mentioned that saturated and mono-unsaturated fatty acids are preferred over PUFA for energy production in fish by the mitochondrial system (Henderson , 1996) . In this connection , YU and Sinnhyuber (1981) reported significantly lower growth rated of Coho salmon fed purified diets containing 4% fish oil and 12% beef tallow compared with fish fed a control die containing 16% fish oil. The same authors reported that the explanation for suppressed growth in fish fed the diet containing 12% beef tallow was that this ingredient impaired the diet quality.

In the present study, the poor in feed conversion ratio and lowest in feed conversion efficiency for fish fed beef tallow 100% as supplemented lipid (BT) and also with fish fed 75% beef tallow as supplemented lipid (mix.6) (Table6) may be to high concentration of saturated fatty acids (table 4) , Menoyo *et al.*(2007) found that lipid apparent digestibility coefficients was higher in diets containing vegetable oil (93-95%) than with animal lipid (90%) .

In Atlantic salmon ,though , the lowest in feed efficiency for treat BT and mix.6 , may be to high concentration of saturated fatty acids and decrease rations of PUFA/SFA (Table.4) which may have caused a reduce emulsion of dietary fats and lower lipolytic activity as suggest by Caballero *et al.*(2002). it demonstrates a relationship between fatty acid digestibility and melting points, which depends both on the length of the carbon chain and number of double bonds present (Ng *et al.*,(2004) and Francis *et al.*, (2007). Though , the formation of insoluble soaps in the intestine through the interaction of divalent cations with long chain free saturates and mooneyes is potentially another intervening factor (Osen *et al.*, 1998).

These results are in a partial agreement with the findings of. Mjoun*et al.*(2012) who show that partial ocomplete replacement of fish oil with flaxseed oil resulted in similar feed efficiency compared to fish fed the diet containing 100% fish oil as supplement lipid.

On the other hand , improvement feed conversion efficiency for male Nile tilapia fed mix.5 (25% fish oil + 75% flaxseed oil and FXO (100% flaxseed oil as supplement lipid) may to increased levels of plant oils are known to trigger hepatocyte fatty acyl desaturation / elongation activities (Bell *et al.*, 2001 and Tocher *et al.*, 2000).

As given in Table(6) fish fed mix.4 , mix.1 and FO had insignificant (p> 0.05) of feed intake , may to supplement fish oil (Hardy *et al.*, 1987) who found that fish fed the diet containing herring oil had the greatest differences in both flavor and texture attributes, when compared to fish fed the other diets. However,Boggio *et al.*(1985) showed no significant differences in sensory attributed when herring oil was replaced with a combination of swine fat and linseed oil. These results were in agreement with the male Nile tilapia fed mix.3 when compared feed in take for fed FO (P>0.05). However, Dupree *et al.*(1979) found that channel catfish fed diets containing menhaden oil had lower flavor rating than fish fed diets containing corn oil.

TU *et al.*(2013) observed for feed conversion ration was not different between fish fed the different alpha-linolenic acid (ALA. 18: 3n-3). Though, results presented in Table(6) are revealed that the differences in feed conversion ratio and feed conversion efficiency were not statistically different among both treat mix.2 (50% fish oil +50% beef tallow) and mix.6(25% fish oil and 75% beef tallow) . These results are in agreement with the findings of Mugrditchian *et al.*(1981) who reported that food conversion ratio on a dry weight basis was similar among fish (Chinook salmon) fed treat 50% salmon oil +50% beef suet and with treat 18.75% salmon oil+81.25% beef suet. Though , the same authors found also that fish fed diet 100% salmon oil had poorer food conversion indicating that perhaps the salmon oil was less nutritious may had a significantly higher thio-barbituric acid (BA) value than the other lipid sources and it is possible that , with the heat of pelleting , the TBA reactive materials reached with other nutrients in the diet such as proteins and vitamins , destroying some or making them biologically un availableto the fish. Li et al (2014) reported that dark barbell catfish (*Pelteo-bagrusvachelli*) fed 4% linseed oil showed higher feed efficiency ratio compared to those in other groups (0 and 2% linseed oil).

Ibeas *et al.*(1996) found that feed efficiency values were lowest in fish fed the diet with 10% beef tallow (lowest n-3 HUFA).

Twibell *et al.*(2012) observed no effect of dietary lipid source on feed intake in coho-salmon . however , self-selecting feed trials indicate rainbow trout prefer diets containing fish oil over those with plant oils (Pettersson *et al.*, 2009) with diets containing linseed oil being the most avoided and diets containing rapeseed oil the best accepted of the plant oils (Geurden *et al.*, 2005).In contrast, Moya- Falcon *et al.*(2005) found significantly lower feed intake in Atlantic salmon fed diets in which canola oil completely replaced fish oil.

Xue *et al.*(2006) examined the effects of replacement of fish oil with pork lard , beef tallow , poultry fat, soybean oil , corn oil and a mixed –fat (beef tallow 60% , soy oil 20% , fish oil 20%).On feed conversion ratio and feed intake , they found that feed conversion ratio and feed intake were not significantly different (P> 0.05).Proximate composition analysis of fish fillets revealed no significant differences between any of dietary treatments for the finishing phase(Table.6) however , significant differences were recorded for protein , lipid and energy retention in fillets fish which ranged from 16.44 ±1.13 to 22.67±1.02 ; 9.57±0.59 to 16.58±0.75 and 49.38±2.75 to 63.20±2.96 respectively .

Fish receiving the FXO had a significantly higher protein retention (%) in comparison to fish previously subjected to the BT; mix.6 and mix.2 dietary treatments.However, with respect to lipid and energy retention in fillets fish (%) fish previously fed the mix.4 and mix.1 treatments performed significantly better than fish continually fed the BT , mix.6 , FXO and Mix.3.

Data on the body composition of fish allows assessing the efficiency of transfer of nutrients from feed to fish and also, helps predict the overall nutritional status (Turichini *et al*,2009) and Sagne *et al.*, 2013 . In the present study ,alternative lipid source using would not have significantly effect on proximate composition of fillets for male Nile tilapia (Table. 6) .These results agree with findings of Thanuthong *et al* (2011) andTwibel *et al.*(2012) found no effects of dietary vegetable oil on carcass composition of salmonids.Though, France *et al.*(2011) showed that Nile tilapia did not present any significant difference in total lipid content with increasing in feed α-linolenic acid (ALA) content treatment 1.8% to treatment 31.9% was due to the added flaxseed oil (an ALA source) ; expected treatment feed 9.1% ALA . In this connection , results obtained by Tidwell *et al.*(2007) explained that fatty acid composition of the diet had no effect on the proximate composition of the test fish (largemouth bass). These finding are in accordance with those reported by Richard *et al.*(2006) revealed that total replacement of dietary fish oil by the blend of vegetable oil did not modify muscle lipid content of rainbow trout.

However, some studies have reported a significant increase in whole body lipids of salmonids fed diets containing vegetable oils(Karalazos*et al.*, 2011) . Tonial *et al.*(2009) reported that 45days is the shortest time period required for the inclusion of linseed oil in tilapia feeds to raise the nutritional value (n-6 to n-3 ratio of muscle tissue) of adult Nile tilpia . Also , with Nile tilapia Ng *et al.*(2003) observed that fish fed diets with linseed oil and fish oil had a lower percentage of total lipids in muscle (P<0.05) compared with fish fed diet with supplemented with olive, corn and soybean oil.Similar trends , the Nile tilapia fed diet containing 4% olive oil had the greatest lipid deposition in muscle compared to fish fed diet with fish and flaxseed oil (Menoyo *et al.*(2003).

On the other hand , from 16.5% (Mix.4) to 13.24 results presented in Table (7) indicated that lipid retention decreased significantly (p<0.05) (FXO) as the level of flaxseed oil in the diet increased (from 37.5%to 100%) respectively.

These results agree with finding of Francis *et al.*, (2007b) who found significantly differences (p<0.05) in fat deposition rate were apparent between mnurray cod (*Maccullochella peelii*peelii) receiving the fish oil and 100% vegetable oil diet.Similar trends have been reported in Chen *et al.*(2013) they showed increased dietary levels of linolenic acid (18:3n-3 , LNA) resulted in lower interoperation fat index and whole body lipid content of juvenile Nile tilapia (*Oreochroisniloticus*). the lower lipid retained in fish fed FXO, mix.1 and mix.5 (Table.1) (n-3 PUFA rich diets , Table.4). In the presented study , may be partly explained by Todorcevic *et al.*(2009) and Kjaer *et al.*(2008) who showed that both the higher incidence of fat cell death and the increase of B-oxidation capacities induced by n-3 PUFA, it inhibit transcription of lipogenic genes through suppressing the expression or proteolytic processing of sterol regulatory element binding protein (Takeuchi *et al.*, 2010; Xu *et al.*, 1999) which might be another factor causing the reduced fat content with the increasing dietary LNA levels(Chen *et al.*, 2013) . Furthermore, it was reported that n-3 PUFA increased muscle anabolic signaling activities and protein synthesis in order or even healthy young and middle aged adults(Smith *et al.*, 2011 a,b) . It's might be one of the reasons why fish protein deposition increased in fish fed FXO(100% flaxseed oil in diet) have increased dietary LNA(Table.1).

Sagne *et al.*(2013) found that the type of dietary lipid significantly affected the body composition of Nile tilapia (*Oreochromis niloticus*) .the same authors , showed that fish fed diet contained blend fish oil (32.9 / kg diet) and mix vegetable oil (16.9/kg diet) (peanut oil + soybean oil) had a higher body crude protein. these results (body crude protein) are in parties agreement with those of table(6) with fish fed Mix.5 (25%fish oil +75% flaxseed oil) in diet , compared (16.28% crude protein) to fish fed FO(100% fish oil in diet (16.14% fillets crude protein).

The lipid retention are given in table(6) tended to significantly decrease (P< 0.05) as the level of beef tallow of diet increased, indicating a lower rate of lipid retention with fish fed 100% beef tallow in diet(BT) and Mix .6 (Fish fed 75% beef tallow with 25% fish oil in diet) . Ibeas *et al.*(1996) examined influence of dietary n-3 highly unsaturated fatty acids levels by added different levels of beef tallow 10.8 and 7.3% in diets to obtained 0.19% , 1.1% and 1.5% n-3 HUFA in diets of *Sparusaurata* . The same authors found that lipid content in muscle was lowest in fish fed the diet with lowest n-3 HUFA content (fish fed 10% beef tallow indiets).In this connection, Senadheera *et al.*(2010.2011) clarified that linolenic acid was preferentially used for energy production and /o^2 bioconversion and the increased likelihood of a direct deposition of linoleic in it's " as fed" form into fish fillets . though , Thanuthong *et al.*(2011) reported that perhaps the most noteworthy result of this experiments was the effect that the increasing dietary (18 PUFA level had on the fillet fatty acid productive value of dietary fatty acids during the grow-out. Similar trends have been showed in Mugraditchian *et al.*(1981), they found that fish fed diets contained high levels of C18:3 from the linseed oil had higher levels of C18:4 W3 in their body lipid than did fish fed diets which contained no linseed oil, they indicates that fatty acid C18:3 in linseed oil is an intermediate in the elongation and desaturation process of conversation of C18:3W3 to C22:6W3 . However , the total saturated fatty acid (SFA) content of the fish was constant at 23% (22.9±0.7) (Takeuch and Watanabe, 1976, 1977). Regardless of increased the level of SFA in the diet may being metabolizable by the fish to keep the SFA levels in the body constant (YU *et al.*, 1977b) . Wilson *et al.*(2007) reported that salmon fed the diet flaxseed oil preferentially oxidized oleic acid and linolenic acid , however, fish fed diet poultry fat preferentially oxidized 16:0 and perhaps some 14:0 and 16: 1 n-7 for energy.

The results of present study (Table. 6) concluded that when fish fed 100% flaxseed oil (FXO) of diet or 50% flaxseed oil +50% fish oil had HIS similar (2.16 and 2.26 % , respectively).These finding are in accordance with those reported by Mjun*et al* . (2012) , who showed that partial or complete replacement of fish oil with flaxseed oil resulted in similar hepatosomatic index of yellow perch (*Percafla vescens*) .Li ,et. al (2014) found that HSI WAS highest (p<0.05) dark barble. catfish (*Pelteobagru svachellj*) fed on zero (0%) linseed oil compared to fish fed 2and 4% linseed oil diets. conversely,

Tu*et al.*(2013) observed that substituting fish oil and fish meal with blend of alpha-lindenic acid (ALA, 18:3n-3) which rich vegetable oil (14% w/w) and defatted poultry meal (34% w/w) in a formulated diet. Resulted HSl was not different between fish fed the different . ALA diets .though , XUE *et al.*(2006) reported that replacement of fish oil with park lard, beef tallow , pultry fat, soybean oil , corn oil and a mixed-fat (beef tallow 60%m soy oil 20%, fish oil 20%) on leptosomatic index in Japanese sea bass (*Lateolabraxjaponcius*) was not significantly different (p> 0.05) between fish fed the experimental diets.

a significant difference (p<0.05) in hepatosomatic index % between fish fed the various source lipid diets (Table 7) the results show higher HIS (2.83%) in fish being fed, the BT diet and following fish fed mix.6 (2.64%) as beef tallow 75% from total lipid in diet (Table.7).Another important observation in present study was the significant variability within treatment in HIS % (Table 7).However HIS was not significantly different (p>0.05) among fish fed mix.4 (2.37%) and fish fed 100% fish oil (HIS= 2.33%) respectively. similar trends have been reported by ibease*t al.*(1996) found that hepatosomatic index (HIS%) was higher in *Sparus aurata* fed 10% beef tallow in diet than other diet 8 and 7% beef tallow added in diets.

At the end of the 90 days , the survival rate did not differ significant between any of the dietary groups (Table5) . these results agree with findings of Xue *et al.*(2006) who showed no fish died during the growth period when they examined the effects of replacements of fish oil with port land , beef tallow poultry fat, soy bean oil , corn oil and a mixed –fat (beef tallow 60% , soy oil 20% and fish oil 20%).

Diets japans sea bass (*Lateolabrax japanicus*) . However , Takeuchi *et al.*(1990) reported that pagrus major (3.1g) fed a diet containing only 0.03% of n-3 HUFA began to show a high mortality and poor appetite after one week of feeding , by which are not agreement with survival rate in presented Table(5) may be due to increased HUFA in presented diets (Table 4) , also may be due to positive effect of n-3 fatty acids on the immune response of fish (Sheldon and Blazer , 1991; Ashton *et al.*1994). The same authors reported that high levels of dietary n-3 fatty acids . in the diet, increase the activity of head kidney macrophages of fish .However, in adequate levels of n-3 PUFA in the diet reduce antibody production (kiron *et al.*, 1995).

Significant differences (p>0.05) were found in the haematocrite value (Hct%) and the haemoglobin. However the number of circulating erythrocytes (RBC x 10^6/ mm^3) content among blood samples (Table.7) from fish fed the different experimental diets in the finishing period.Fish hematology is gaining increasing importance in fish culture to its importance in monitoring the health status of fish (Hrubec *et al.*, 2000).

Significant (p<0.05) replacing 50% (mix.1) , 75% (mix.5) and 100% (FXO) fish oil by flaxseed oil in diets did not affect Hct and Hb (Table.7). There was positive effect of increased beef tallow from 50% , 75% up to 100% in diets (from supplemented lipid on Hct and Hb .(Table.7).Similar results have been described for gilthead seabream (*Sparus aurata*) (Montero *et al.*, 2003) who should no significant differences (p> 0.05) were found in hematocrit value or the hemoglobin content among fish fed 100% fish oil (anchovy oil) and other diets contained vegetable oils(soybean oil , rapseed oil , linseed oil) to substitute for 60% of the anchovy oil used in diets.Though , haematology (in terms of hematocrit and haemoglobin) was not affected by dietary lipids as described by Kenari *et al.*(2011) .for brown trout , Green and Selivonchick (1990) for rainbow trout , (Dosanjh *et al.* (1984) for coho salmon.This is in contrast Twibell *et al.*(2011, 2012) who found that steel head (offspring of anadromous *O. mykiss*) fed a similar terrestrial protein / plant oil diet exhibited significantly lower (p<0.05).Haemoglobin and haematocrit concentration compared with fish fed a marine protein diet containing , fish oil . Though, Bell and Sargent (2003) reported significantly reduced hematocrit in salmon fed diets in which fish oil was replaced with a 1:1 mixture of linseed / rapseed oil.

Due to the high content of dietary fish oil (FO). the fish fed 100% fish oil showed significant higher (p<0.05) RBC number than other treatments (Table.7), though, RBC number increased in fish with increased supplemented fish oil (Table.7).This is in agreement with those data found for *Sparusaurata* by Montero *et al.*(2003) , who found that the number of circulating erythrocytes (RBC) was higher in fish fed diet fish oil when compared to fish fed diets 60% soybean oil and linseed oil. The same trend described by Green and Selivonchick (1990) for rainbow trout , who found that RBC number was higher in fish fed fish oil. based diet which could be related to a higher oxygen requirement due to a higher peroxisomal B- oxidation (Waagboo *et al.*, 1995).In agreement with this hypothesis , Grisdale – Helland *et al.*(2002) found a lower oxygen consumption in Atlantic salmon fed 50% soybean oil-contains diet when compared with 100% fish oil-containing diet.

Table. 1. Main ingredient and chemical compositions of the experimental diet.

Ingredient (% wet weight)	**Diet**
Fish meal (61.42% cp)	29.56
Meat and bone meal (47.53% cp)	08.52
Soybean meal (41.22% cp)	12.13
Corn yellow (7.32% cp)	17.69
Wheat bran (13.84% cp)	21.00
Vitamin and mineral supplement(1)	03.00
Vitamin E	00.10
Source of lipid(supplemented)*	08.00
Chemical composition (% wet weight)	
Moisture	9.25
Dry matter	90.75
Crude protein (cp)	31.41
Crude lipid (CL)	12.19
Ash	10.18
Fiber	8.62
Nitrogen free extract(NFE)	28.35
Gross energy (MJ/ kg dry weight)	18.85

(1)Vitamin and mineral premix (each1Kg contains: 4-8 M.I U vit.A; 0.8 M.I U. vit.D3; 1500 I U. vit .E; 0.8 g.vit .K; 4g.vit. B12; 4.09 vit .B2; 0.6 g.vit B8; 4.0g vit .pantothenioc acid ; 8.0 g. vit nicotinic acid ; 400 mg vit . folic acid ; 20mg vit. biotin;200g . choline chloride ; 4.0 g copper , 0.4 g iodine , 12g iron, 22g manganese ; 22g zinc and 6 mg selenium.

*Lipid sources employed in the experimental diets

(fish oil ,Flaxseed oil , Beef tallow,and Mixture)

Table 2.Experiment design

Diet group(1)	Composition of supplemental lipid (as percentage of supplemental lipid)		
	FOFish oil	FXOFlaxseed oil	BTBeef tallow
FO	100%	-	-
FXO	-	100%	-
BT	-	-	100%
Mix*1	50%	50%	-
Mix2	50%	-	50%
Mix3	--	50%	50%
Mix4	25%	37.5%	37.5%
Mix5	25%	75%	-
Mix6	25%	-	75%

(1)Diet abbreviations:FO- fish oil diet ; FXO – flaxseed oil ;BT- beef tallow ; mix – mixture of supplemental lipid. mix* - blend of fish oil and /or flaxseed oil and /or beef tallow.

Table .3 Fatty acid compositions (Percentage of total fatty acids)of experimental diets

Diet	Total saturated	Total monounsaturated	Total polyunsaturated
FO	34.6	27.00	38.3
FXO	10.98	24.27	64.41
BT	50.20	45.30	04.5
mix.1	22.79	25.64	51.36
mix.2	42.40	36.15	21.40
mix.3	30.59	34.79	34.46
mix.4	31.60	32.84	35.42
mix.5	16.89	24.96	57.89
mix.6	46.30	40.73	12.96

Values are means of three analysis.

Table .4.Growth performance of male Nile tilapia after being fed diets with various lipid source in the finishing period

Parameter	Experimental diets								
	FO	FXO	BT	mix.1	mix.2	mix.3	mix.4	mix.5	mix.6
Initial body weight(g)	98.35±3.71	99.68±4.79	102.31±3.12	100.16±3.74	101.33±4.01	100.72±3.46	100.46±2.33	98.67±3.47	99.80±2.95
Final body (weight(g)	195.26±6.82ab	191.91±7.55b	173.74±5.46d	199.72±6.94a	188.54±7.52bc c	193.46±6.29b	203.52±5.25a	193.24±6.19b	182.75±5.41c
Weight increase(g)	96.91±3.11ab	92.23±2.76b	71.42±2.34d	99.56±3.21a	87.21±3.51bc	92.74±2.83b	103.06±2.92a	94.57±2.72b	82.95±2.46c
Specific growth rate % /day	0.767±0.02a	0.728±0.04ab	0.588±0.03c	0.767±0.02a	0.690±0.03bc	0.725±0.04b	0.784±.02a	0.746±0.03ab	0.672±0.02c
Growth rated per metabolic weight unit (kg-0.08 d-1)	5.23±0.061a	4.99±0.58ab	3.98±0.71c	5.29±0.69a	4.72±0.47b	4.98±0.53ab	5.43±0.66a	5.12±0.43ab	4.57±0.58b
Survival number/60 fish	57	55	53	56	57	54	58	55	56

Values represent means ± standard deviation of two replicates. Mean in a row with different letter are significantly different (P< 0.05)

Table. 5. Feed intake and feed efficiency maleNile tilapia (*Oreachromisniloticus)*fed diets containing of different lipidsources(mean ±sd)

Parameter	Experimental diets								
	FO	FXO	BT	mix.1	mix.2	mix.3	mix.4	mix.5	mix.6
Feed intake(FI)g	235.89±11.8ab	234.38±13.9b	225.4±16.7c	240.7±10.9a	233.6±14.7b	237.4±12.8a	244.62±14.6a	235.08±10.5b	231.02±12.4bc
FI. d^{-1})	2.62/±0.23a	2.60±0.21ab	2.50±0.18b	2.67±23a	2.60±.19ab	2.64±0.25a	2.72±0.20a	2.61±0.18ab	2.57±0.22b
FI. perc.	1.89±0.10	1.88±1.25	1.88±.011	1.89±0.13	1.88±0.09	1.89±0.08	1.90±0.12	1.89±0.09	1.90±0.13
FI. mbw	12.74±1.03ab	12.66±1.25bc	12.53±1.30c	12.77±.34a	12.6±1.22bc	12.76±1.30a	12.89±1.53a	12.73±1.46ab	12.75±1.79ab
Feed conversion ratio	2.43±0.25c	2.54±0.30b	3.16±0.21a	2.42±0.18c	2.68±.32ab	2.56±0.27b	2.37±0.32c	2.49±0.26bc	2.79±0.18a
Feed conversion efficiency (%)	41.08±2.31a	39.35±2.07ab	31.68±2.18c	41.36±2.26a	37.33±1.75b	39.06±2.15ab	42.13±2.56a	40.23±1.95a	35.91±1.18bc
Protein efficiency ratio	1.31±0.04a	1.25±0.06ab	1.01±0.03b	1.32±0.04a	1.19±0.09b	1.24±0.09ab	1.34±0.07a	1.28±.08a	1.14±0.015b

values with different superscripts in each row significantly differ(*p*< 0.05) different letters(a,b and c) indicates that differences between the diets were significantly (*p*< 0.05) for tilapia.

Table.6.Proximate composition of fillets of Nile fed the different dietary treatments (g/100g of wet weight basis , mean ± sd, n=5) at the end of the experiment

	Moisture	Crude protein	Crude lipid	Ash	Gross energy(*)	Protein retention in filet	Lipid retentionin fillet	Energy retention in fillet	HS1%	Liver lipid(mgg
Initial	75.58±1.31	15.64±1.01	4.58±.73	4.13±0.47	16.92±1.35	-	-	-	-	-
FO	75.19±1.50	16.142±1.24	4. 61±.43	4.6±.31	22.53±1.28	21.77±0.83a	15.64±0.62ab	61.50±2.85ab	2.67±0.23b	7.04±0.94b
FXO	74.67±1.32	16.82±1.04	4.35±.29	4.14±0.39	22.12±1.41	22.67±1.02a	13.24±0.71bc	57.91±2.31c	2.16±0.19c	5.29±0.7c
BT	75.42±1.29	15.91±0.93	4.21±.46	4.49±.44	22.04±1.39	16.44±1.13c	9.57±0.59d	49.38±2.75d	3.25±0.21a	8.13±1.05a
mix.1	75.12±1.37	16.02±1.10	4.66±.35	4.23±.41	22.59±.74	21.59±0.97ab	16.08±0.82a	62.18±3.01a	2.45±0.15bc	5.80±0.82c
mix.2	75.17±1.20	16.18±0.86	4.28±.52	4.38±.34	22.19±1.82	19.98±1.11b	12.04±0.73c	56.07±2.05c	2.98±0.15a	7.69±1.0ab
mix.3	74.93±1.33	16.25±0.92	4.41±.37	4.42±.48	22.25±1.35	21.03±0.85ab	13.54±0.69bc	58.09±2.94bc	2.25±0.15bc	6.81±0.73bc
mix.4	75.03±1.42	16.14±1.03	4.69±.51	4.04±.42	22.67±1.4	22.30±1.07a	16.58±0.75a	63.20±29.6a	2.51±0.21b	6.73±0.88b
mix.5	74.71±1.11	16.28±0.95	4.47±.37	4.50±.51	22.17±1.91	21.71±0.95a	14.37±0.62b	59.01±2.43b	2.21±0.20c	5.23±0.79c
mix.6	75.25±2.03	16.11±0.89	4.23±0.45	4.43±.46	22.43±1.82	19.06±0.91b	11.22±0.71cd	55.32±2.66c	3.07±0.17a	7.82±1.01a

values with different superscripts in each column significantly differ (*p*<0.05).Different letters(a,b, c and d) indicate that different between diets were significant (*p*< 0.05)

(*)according to Thanuthong *et al* (2011)

Table .7 Hematological parameters of male Nile tilapia *Oreochromisniloticus*after being red diets with various lipid sources

Treatment	HCT(%)	HB(g/dl)	RBC(x 10^6 / mm^3)
FO	33.49±1.65a	6.95±0.78	2.105±0.065a
FXO	31.56±2.04b	6.57±0.56	1.832±0.05b
BT	35.64±2.11a	7.37±0.82	1.861±0.06b
mix.1	32.27±1.29ab	6.73±0.74	2.074±0.068a
mix.2	33.61±2.27a	7.03±0.68	2.036±0.057a
mix.3	32.11±1.98b	6.68±0.81	1.903±0.043ab
mix.4	32.73±1.57a	6.89±0.92	1.972±.052a
mix.5	31.83±2.24b	6.51±0.76	1.949±0.063a
mix.6	34.95±1.73a	7.26±0.59	1.895±0.058b

values with different superscripts in each column significantly differ (*p*<0.05). Different letters (a and b) indicate that different between the diets were significantly (p<0.05)

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