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Supplementation of Soy Based Feed with Linseed and its Effects on Growth and Fatty Acid Profile in Grass Carp (*Ctenopharyngodon idella*)

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ABSTRACT

Linseed is an economically important plant source of n-3 polyunsaturated alpha-linolenic acid (ALA) which can be used as supplemental dietary lipid in fish feed particularly carps. In developing countries where fish oil is not an ingredient of fish feed due to its high cost, the addition of economical plant oils rich in n-3 PUFA in fish diet can remarkably improve its quality. The present study evaluated the effect of the addition of linseed in fish feed on growth and fatty acids profile in grass carp (*Ctenopharyngodon idella*); an important commercial carp in Asia. The present study investigated the effects of three different diets (A, 5% linseed flour; B, 5 % LO; C, 2.5 % linseed flour + 2.5% LO) on growth and fatty acids profile ($C_{12:0}$– $C_{22:6}$) in grass carp (age = 12 months). The trial continued for 31 days at ambient temperature. The control group was fed with soybean based commercial diet. No significant effect of diet A and C was observed on fish growth and profile of n-3 and n-6 PUFA in total liver lipids. However, the treatment group fed with diet B showed a significant increase in K (1.75 ± 0.14 %), FCR (0.97) and HSI (0.62 ± 0.1%) as compared to those in the control group. A high profile of DHA ($C_{22:6}$) was observed in total liver lipids of fish in all three treatments groups (A: 0.31%, B: 0.41%, C: 0.40%) in comparison to the control group where DHA was not observed in the fatty acids profile. Similarly, high profiles of EPA ($C_{20:5}$) were observed in all three treatment groups (A: 0.56%, B: 1.64%, C: 1.35%) as compared to those in control group. Therefore, the present study indicates that linseed can be a suitable source of n-3 and n-6 PUFA in carp feed, improving the condition and flesh quality of fish.

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Authors' Contribution
SF, FM,HA, ZK, AL and SS designed and executed the study and analysed the data. ZA and HIG helped in performing fatty acids analysis while RSNJ provided technical assistance in writing the manuscript.

Key words
Linseed, Grass carp, α-linolenic acid, Growth, Fatty acids.

INTRODUCTION

Sustainable plant based aquafeeds or its supplementation has been an important area of aquaculture research. The global aquafeed industry currently relies mainly upon fish meal, the industry has been continuously looking for a fish feed formulation which is cost effective and have no harmful effects on growth and health of the fish (Hunters and Roberts, 2000). Health conscious consumers desire for high levels of EPA and DHA in their diet therefore maintaining the high level of these PUFAs in fish meat has always been major interest of industry (Schmidt *et al*., 2005). Fish meal has the highest level of EPA and DHA as compared to plant based feed. Due to high cost and low production of fish meal in developing Asian countries and sustainability issue, aquaculture feed manufacturing industry depend mostly upon plant protein and lipids sources particularly, soybean meal.

A number of studies have investigated the cost and quality benefits of replacing fish oil by C18 (α-linolenic acid) rich vegetable oils such as soybean, linseed and palm oil in many freshwater species. Linseed oil (LO) is the most distinct among all plant oils due to the highest proportion of ALA which can be converted to EPA and DHA by fresh water fish species. Using LO in aquafeed has certain limitations such as its oxidation (Boran *et al*., 2006). This problem can be resolved by the addition of antioxidants such as vitamin E and tert-butyl hydroxytoluene (BHT) which reduce lipid peroxidation (Chaiyapechara *et al*., 2003; Lukaszewicz *et al*., 2004).

Various studies have reported no negative effect of LO substituted diet on growth in different fish species including Atlantic salmon (*Salmo salar*; Menoyo *et al*., 2005), rainbow trout (*Oncorhynchus mykiss*; Turchini and

Abbreviations

ALA, alpha-linolenic acid; BHT, butylated hydroxytoluene; DHA, decosahexanoic acid; EPA, eicosapentanoic acid; FCR, feed conversion ratio; HIS, hepatosomatic index; K, condition factor; n-3, omega-3; n-6, omega-6; NFE, nitrogen free extract; LA, linoleic acid; LO, linseed oil; PUFA, polyunsaturated fatty acids; SBM, soybean meal; SGR, specific growth rate; %WG, percentage weight gain.

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Francis, 2009), tilapia (*Oreochromis niloticus*; Karapanagiotidis *et al*., 2007), seabream (*Pagrus pagrus*; Piedecausa *et al*., 2007), Senegalese sole (Benítez-Dorta et al., 2013), Murray cod (*Maccullochella peelii*; Francis *et al*., 2006), rohu (*Labeo rohita*; Latif *et al*., 2008) and GIFT tilapia (*Oreochromis niloticus*; Li *et al*., 2016). However, the addition of vegetable oils results in increasing the levels of 18:3n-3, 18:2n-6 and 18:1n-9 PUFAs and decreasing levels of EPA and DHA in European seabass (*Dicentrarchus labrax*) (Mourente and Bell, 2006), rainbow trout (Bell *et al*., 2002; Masiha *et al*., 2013) and tilapia (Peng *et al*., 2016).

Soybean has been widely used as rich plant protein source in aquaculture feeds that supports healthy and efficient fish growth. The cost of soybean mean (SBM) is significantly less (344 USD/MT) than most animal sourced aquaculture feed components (Fishmeal: 1475 USD/MT) thus maintaining the sustainability in aquaculture operations. However, alpha linolenic acid (ALA) content in soybean is 6.8% as compared to distinctively high proportion of 53.3% in LO (NRC, 1993). The deficiency of ALA in SBM can be improved through the addition of linseed meal or LO, improving it as a better plant source aquaculture feed component both in proportion of protein and omega-3 fatty acids.

Linseed is cultivated all over the world. The highest areas of linseed production (94%) in the world are in Asia, Europe and America, cultivating 49.25%, 22.57% and 22.42%, respectively. The average yield ranges from 242 kg/ha (Belarus) to 3918 kg/ha (Sweden) (Popovic *et al*., 2016). Therefore, it is an easily available commodity for partial supplementation of aquaculture feed at a cheaper price (~ 400 USD/MT) and easy market access.

Considering the above mentioned significance of linseed in improvement of EPA profile and DHA in fish, the present study investigated the effects of addition of the linseed meal and LO in soy based fish feed in grass carp. The main objective of this study was to improve the profile of ALA in SBM by supplementation of linseed to enhance its significance in the local aquaculture feed market. Grass carp was selected as an animal model due to its importance in the major carp aquaculture industry. Global production of this specie was recorded as 55,37,793 tonnes (FAO, 2016) and considered as a major commodity in the Asian aquaculture industry particularly in China. Therefore, it is important to study the effect of linseed supplementation in this species for the purpose of improving growth and flesh quality especially for the Asian region.

MATERIALS AND METHODS

Growth experiment

The present study was conducted after the approval of animal ethical committee of Department of Zoology, Lahore College for Women University (LCWU) (RERC/ZOO/2016-28, 30). A total of 64 fish (body weight: 82.22 ± 10.00 g, body length: 17.37 ± 0.55 cm) were obtained from a commercial fish farm (Himalaya Hatchery, Sheikhupura) and transferred to aquaria (92 cm × 44 cm × 42 cm) located at the Aquaculture Facility, Lahore College For Women University on April 4, 2017. Fish were divided into one control (n= 16) and three treatment groups (n= 16 in each group). Each group had two replicates. Water quality parameters were maintained at pH 6.8- 7.2, chlorine < 0.02 mg/L, total ammonia < 2 mg/L, nitrite < 1 mg/L, nitrate < 80 mg/L. Fish were fed at the rate of 2% of their body weight three times a day. Fish were acclimatized for three days under ambient conditions. Trials continued for a period of 36 days (April 5 - May 5, 2017).

Experimental diets

Fish in the control group were fed with soy based commercial feed (Oryza Organics, Pakistan). Treatment diets were prepared by supplementing commercial feed with 5% linseed flour (Diet A), 5% linseed oil (Diet B), and combination diet (2.5% linseed flour + 2.5% linseed oil) (Diet C). Vitamin E (7.5 g/kg) and butylated hydroxytoluene (BHT) (12.5 g/kg) were used as antioxidants.

Calculations

Total body length (± 1 mm) and total body weight (±1 g) of fish were measured after every week. %WG, K, SGR and FCR were measured each week by using the following formulae:

$$\%WG = 100\ [(Wf - Wi) / Wi]$$

$$K = W/ (L)^3 \times 100$$

$$SGR\ (\%/day) = 100 \times [(lnW1 - ln\ W0)/t]$$

$$FCR = food\ consumed\ (g) / weight\ gain\ (g)$$

All fish were killed at final sampling using an overdose of anesthetic through means of transfer into a 50-L tank containing 20 ml/l of clove oil. Liver weight (±1 g) was measured to calculate HSI using the following formula:

$$HSI = [liver\ weight\ (g) / total\ body\ weight\ (g)] \times 100$$

Proximate analysis

Proximate analysis of prepared feed and soy based commercial feed was performed by utilizing the lab facility of Applied Chemistry Research Center, PCSIR Lahore. The level of moisture in the diets was estimated by drying at 100 ± 2°C until their weight was constant for 24 h. The level of crude protein (N × 6.25) was determined by using the Kjeldahl method. The total ash in the diets was assessed after the combustion at 550 ± 25°C over-night and crude fat from the diets samples was extracted with a Soxhlet extractor. Feed ingredients and composition of control and experimental diet is given in Table I.

Table I.- Ingredient (%) and proximate composition (% dry matter) of the control and experimental diets.

Ingredients (%)	Control* SBM	Treatments* Linseed flour		Linseed oil
Ground wheat	14.30	8.60		8.60
Soybean meal	25.40	40.00		20.00
Linseed oil	_	4.00		8.00
Wheat starch	2.00	2.00		2.00
Carboxy methyl cellulose	28.09	10		10
Mineral premix[a]	0.30	1.50		1.50
Vitamin premix[b]	0.30	2.00		2.00
Soybean hulls	15.40	-		-
Proximate composition (%)	**Control**	**T 1**	**T 2**	**T 3**
Crude protein	30.00	30.15	30.20	30.25
Crude lipids	5.50	13.10	17.65	15.75
Crude fiber	6.50	2.91	2.00	2.74
Ash	7.80	5.00	5.00	4.00
Moisture	10.00	9.60	8.00	9.40
NFE[c]	40.20	39.24	37.51	37.86

*Each treatment and control had two replicates. n was 16 in each group. a, contains (mg/Kg food): Mg, Zn, Fe, Cu, CO and I; b, contains (mg/Kg food) E, K, niacin, thiamine, riboflavin, folacin, pyridoxine and biotin; c, NFE: nitrogen free extract calculated by difference (100-moisture-ash-crude protein-crude lipid-crude fibers).

Estimation of fatty acid

Total liver lipids were extracted according to Bligh and Dyer (1959) method. Fatty acids profile ($C_{12:0}$–$C_{22:6}$) in total liver lipids were determined by Gas Chromatography Flame Ionization Detector (GC-FID).

Statistical analysis

Statistical analysis was performed following Peng *et al*. (2017) procedure. One-way ANOVA was performed to analyze the variance between weekly values of control and each treatment group. Paired sample t-test was carried out to compare the weekly values of the control group with those of the treatment groups. P<0.05 indicated significant differences between means.

RESULTS

Weekly mean values of K (condition factor) in control group were found to be insignificant at (P > 0.05) different throughout the trial ($F_{3, 33}$ = 1.82) (Fig. 1). Similarly, no significant difference (P > 0.05) was observed between weekly means of K in treatment A ($F_{3, 33}$ = 0.62). On the other hand, a significant difference (P < 0.05) was observed in weekly mean values of K in both treatment B ($F_{3, 34}$ = 4.43) and C ($F_{3, 31}$ = 3.41).

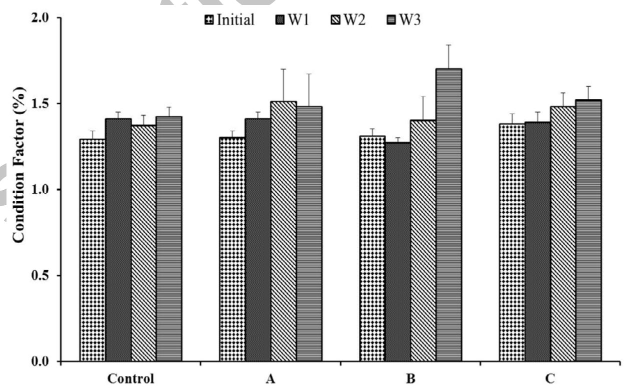

Fig. 1. Weekly (Mean ± SE) variations in condition factor in control and treatment groups of grass carp (*Ctenopharyngodon idella*). Diet of treatment group A, B and C contained 5% of linseed flour, 5 % linseed oil, combination (2.5 % linseed flour + 2.5 % linseed oil), respectively. Trial continued for 36 days (April 5 - May 5, 2017). Control group was fed with soybean feed. Initial: Pre-treatment value, W1: first week post-treatment, W2: second week post-treatment, W3: third week post-treatment

Table II.- Weekly (mean ± SE) variations in specific growth rate (%) and feed conversion ratio in control and treatment groups of of grass carp (*Ctenopharyngodon idella*) fed with three different diets (A, 5% linseed flour; B, 5 % linseed oil; C, 2.5 % linseed flour + 2.5% linseed oil) over the period of 36 days (April 5 - May, 5, 2017). Control group was fed with soybean feed.

Sample point	Specific growth rate (%)				Feed conversion ratio			
	Control	A	B	C	Control	A	B	C
Week 1	0.12 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	1.24	1.29	1.29	1.37
Week 2	0.18 ± 0.01	0.23 ± 0.02	0.21 ± 0.03	0.22 ± 0.01	1.28	1.35	1.26	1.25
Week 3	0.21 ± 0.02	0.26 ± 0.04	0.23 ± 0.01	0.25 ± 0.02	1.31	1.30	1.31	1.34

During the final sample point, HSI was found to be 0.59 ± 0.11 %, 0.62 ± 0.1 %, 0.52 ± 0.03 % and 0.55 ± 0.1 % in control, treatment A, B and C group, respectively. SGR and FCR in treatment groups were found to be insignificant at ($P > 0.05$) different from those in the control group (Table II). Levels of α-Linolenic acid were found to be higher in control (4.46 %) in comparison to treatment groups (Table III). Fish in treatment B showed the highest proportion of EPA ($C_{22:5}$) (1.64 %) as compared to treatment A, C and control group. DHA ($C_{22:6}$) was observed only in treatments.

Table III.- Comparison of fatty acid profile (%) in total liver lipids of grass carp (*Ctenopharyngodon idella*) fed with three different diets (A, 5% linseed flour; B, 5 % linseed oil; C, 2.5 % linseed flour + 2.5% linseed oil) over the period of 36 days (April 5 - May, 5, 2017). Control group was fed with soybean feed.

Carbon No.	Fatty acids	Control	A	B	C
$C_{12:0}$	Lauric acid	1.34	1.32	1.30	-
$C_{14:0}$	Myristic acid	0.44	0.47	0.15	0.76
$C_{16:0}$	Palmitic acid	26.10	28.79	38.69	33.66
$C_{16:1}$	Palmitoleic acid	1.02	0.40	0.31	0.39
$C_{17:0}$	Heptadecanoic acid	-	0.73	1.30	0.52
$C_{18:0}$	Stearic acid	5.49	0.91	1.20	0.50
$C_{18:1}$	Oleic acid	36.50	39.89	39.11	39.09
$C_{18:2}$	Linoleic acid	19.87	24.85	12.71	17.23
$C_{18:3}$	α-Linolenic acid	4.46	0.57	1.07	1.73
$C_{18:3}$	β-Linolenic acid	2.15	0.36	-	2.31
$C_{20:0}$	Arachidic acid	1.10	0.32	0.35	0.73
$C_{20:1}$	Condolic acid	0.74	0.33	1.13	1.22
$C_{20:2}$	Eicosadionic acid	-	-	0.24	-
$C_{20:3}$	Eicosatrionic acid	-	-	-	-
$C_{20:5}$	Eicosapentanoic acid	0.79	0.56	1.64	1.35
$C_{22:0}$	Decosanoic acid	-	0.20	0.22	0.23
$C_{22:6}$	Decosahexanoic acid	-	0.31	0.41	0.40

DISCUSSION

In the present study, supplementation of linseed (flour and oil) in fish feed in treatments group showed higher levels of EPA and DHA in comparison to non-supplemented soy based feed in control group. The highest levels of C16:0, C18:1n and n3 LC-PUFAs, EPA (20:5n-3), and DHA (22:6n-3) were observed in the LO group. This finding was in agreement with previous studies on common carp (*Cyprinus carpio*; Zhelyazkov *et al.*, 2014; Zupan *et al.*, 2016), rainbow trout (Masiha *et al.*, 2013), Senegalese sole (Benítez *et al.*, 2013), Nile tilapia (Omolo *et al.*, 2017) and juvenile turbot (Peng *et al.*, 2017). This finding was in contrast with silver barb fingerlings (Nayak *et al.*, 2017). In the present study it was observed that dietary total proportion of LA and ALA was higher in the control group but lower in the treatment groups. However, higher levels of EPA and DHA levels found in treatment groups may be attributable to enzymes Δ6 and Δ5 fatty acyl desaturase (FAD) and elongase, involved in the biosynthesis of LC-PUFAs (EPA and DHA) from the catalysis of the C18 substrates LA/ALA. In fish, both LA and ALA are considered essential due to their incapability to biosynthesized these acids de novo. It is well known that freshwater fish species have a high dietary requirement for n-3 and n-6 fatty acids in comparison to marine fish species, predominantly in the form of α-linolenic and linoleic acids (Turchini and Francis, 2009). In present study, high DHA content in liver samples of treatment groups indicated the conversion of some of ALA into n-3 LC PUFA. It suggests that carp has a capacity to desaturate and elongate ALA to EPA and DHA. Similar observation has also been reported in other freshwater fishes, tilapia (Li *et al.*, 2016), common carp (Zupan *et al.*, 2016) and trout (Turchini and Francis, 2009).

Although a superior profile of EPA and DHA were observed in the LO group however, higher crude lipids were also observed. Lipid deposition in fish fed with higher lipids proportion in their diet has been observed in a number of species including, common carp (Zupan *et al.*, 2016), rainbow trout (Masiha *et al.*, 2013), brown trout (*Salmo trutta*) (Arslan *et al.*, 2012), Senegalese sole (Reis *et al.*, 2014) and turbot (*Scophthalmus maximus*) (Mourente *et al.*, 2005). The health benefits of a diet rich in EPA and DHA are well documented in fetal development, Alzheimer's disease, diabetes, cardiovascular disease, inflammatory disorders and immune dysfunction (Swanson *et al.*, 2012; Soni *et al.*, 2016; Bechoua *et al.*, 2003; Bell *et al.*, 2004). Adiposity due to the addition of LO in soy based feed can be improved by further adjusting LO according to market requirements. In contrast to the improvement in EPA and DHA profiles in treatment groups, SGR and FCR did not show any increase over the study period. Similar results were observed in juvenile tench (Zakes *et al.*, 2010) and turbot (Altundag *et al.*, 2014; Peng *et al.*, 2017). In contrast to this, previous study on common carp showed significant increase in growth rate by increasing the concentration of linseed oil indicated that concentration of LO influence on the growth performance (Zupan *et al.*, 2016). The SGR results of present study could possibly be due to the short duration of study trials and the concentration of added linseed (flour, oil). The results of the present study do not agree with some reports that by increasing the dietary lipid content, the growth of the fish may improve (Pei *et al*., 2004; El-Marakby, 2006). On the other hand, it has also reported that a high dietary lipid level (> 7%) could reduce fish growth (Pei *et al*., 2004; El-Marakby, 2006; Zupan *et al*., 2016). It could be due to the reduction in feed intake, low ability to digest and absorb high lipid and fatty acid imbalance in the diet. From previous studies it has been noticed that fish are especially sensitive to the quality of feed and the presence of dietary anti-nutritional factors (NRC, 1993). One of the major problems in using the alternative vegetable protein and lipid sources in aquaculture feeds is their palatability that is mainly related to feed taste. Therefore, the determination of feed ingredients is an important condition for evaluation of their potential use in fish nutrition. The results of the present study showed that the use of linseed in experimental fish diets did not result in low growth rates and stunted growth; therefore, it is a suitable feed component for carp.

This study also shows that replacing fish oil with vegetable oils consistently results in major changes in fatty acid profile of fish. In this and previous studies considering n-3 LC-PUFAs such as EPA and DHA is important as these acids are extremely beneficial to human health and the final quality of fish. These fatty acids as well as arachidonic acid (ARA), another essential fatty acid, are normally expected to be found abundantly in fish. However, there is concern that by partially or totally replacing the fish oil in fish diets with vegetable oil, the amount of healthy fatty acids in the fish meat may be reduced and this concern may have more validity in marine fish.

CONCLUSION

In conclusion, 5% LO supplementation of commercial extruded soy based feed for carps, increased the profile of EPA and DHA. However, no significant increase in SGR and FCR could be observed in the treatment group which might be related to short duration of trial. The present study showed that the linseed oil could be successfully used as a supplement component for carp feed. Their addition to the extruded feed positively influenced the growth while producing no negative effect on the survival rate of the fish and FCR.

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Statement of conflict of interest

The authors declare no conflict of interest.

REFERENCES

Altundag, M.S., Tiril, S.U. and Ozdemir, A., 2014. Effects of sunflower oil supplementation in diet on growth performance and body fatty acid composition of turbot (*Psetta maxima*). *Aquacult. Int*., **22**: 597-605. https://doi.org/10.1007/s10499-013-9686-x

Arslan, M., Sirkecioglu, N., Bayir, A., Arslan, H. and Aras, M., 2012. The influence of substitution of dietary fish oil with different vegetable oils on performance and fatty acid composition of brown trout (*Salmo trutta*). *Fish. aquat. Sci*., **12**: 575-583. https://doi.org/10.4194/1303-2712-v12_3_04

Bechoua, S., Dubois, M., Vericel, E., Chapuy, P., Lagarde, M. and Prigent, A.F., 2003. Influence of very low dietary intake of marine oil on some functional aspects of immune cells in healthy elderly people. *Br. J. Nutr*., **89**: 523-531. https://doi.org/10.1079/BJN2002805

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R. and Porter, A., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. *J. Nutr*., **132**: 222-230. https://doi.org/10.1093/jn/132.2.222

Benítez-Dorta, V., Caballero, M.J., Izquierdo, M., Manchado, M., Infante, C., Zamorano, M.J. and Montero, D., 2013. Total substitution of fish oil by vegetable oils in Senegalese sole (*Solea senegalensis*) diets: Effects on fish performance, biochemical composition, and expression of some glucocorticoid receptor-related gene. *Fish Physiol. Biochem*., **39**: 335-349. https://doi.org/10.1007/s10695-012-9703-4

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.,* **37**: 911–917.

Boran, G., Karacam, H. and Boran, B., 2006. Changes in the quality of fish oils due to storage temperature and time. *Fd. Chem*., **98**: 693-698. https://doi.org/10.1016/j.foodchem.2005.06.041

Chaiyapechara, S., Casten, M.T., Hardy, R. and Dong, R.M., 2003. Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Onchorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. *Aquaculture*, **219**: 715-738. https://doi.org/10.1016/S0044-8486(03)00025-5

El-Marakby, M., 2006. Effect of dietary sources and levels of lipids on growth performance and feed utilization of fry Nile tilapia, *Oreochromis niloticus*

(L.). *Fish. aquat. Sci.*, **1**: 117-125. https://doi.org/10.3923/jfas.2006.117.125

FAO, 2016. *The state of world fisheries and aquaculture. Contributing to food security and nutrition for all*. Food and Agriculture Organization, Rome, pp. 200.

Francis, D.S., Turchini, G.M., Jones, P.L. and de Silva, S.S., 2006. Effects of dietary oil source on growth and fillet fatty acid composition of Murray cod (*Maccullochella peelii peelii*). *Aquaculture*, **253**: 547-556. https://doi.org/10.1016/j.aquaculture.2005.08.008

Hunter, B.J. and Roberts, D.C.K., 2000. Potential impact of the fat composition of farmed fish on human health. *Nutr. Res*., **20**: 1047-1058. https://doi.org/10.1016/S0271-5317(00)00181-0

Karapanagiotidis, I.T., Bell, M.V., Little, D.C. and Yakupitiyage, A., 2007. Replacement of dietary fish oils by alpha-linolenic acid-rich oils lowers omega 3 content in tilapia flesh. *Lipids*, **42**: 547-559. https://doi.org/10.1007/s11745-007-3057-1

Latif, K.A., Alam, M.T., Sayeed, M.A., Afzal, H.M., Sultana, S. and Hossain, A.M., 2008. Comparative study on the effects of low cost oil seed cakes and fish meal as dietary protein sources for *Labeo rohita* (Hamilton) fingerling. *J. Zool*., **27**: 25-30. https://doi.org/10.3329/ujzru.v27i0.1949

Li, F.J., Lin, X., Lin, S.M., Chen, W.Y. and Guan, Y., 2016. Effects of dietary fish oil substitution with linseed oil on growth, muscle fatty acid and metabolism of tilapia (*Oreochromis niloticus*). *Aquacult. Nutr*., **22**: 499-508. https://doi.org/10.1111/anu.12270

Lukaszewicz, M., Szopa, J. and Krasowska, A., 2004. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. *Fd. Chem*., **88**: 225-231. https://doi.org/10.1016/j.foodchem.2003.12.042

Masiha, A., Ebrahimi, E., Soofiani, M.N. and Kadivar, M., 2013. Effect of dietary vegetable oils on the growth performance and fatty acid composition of fingerlings of Rainbow trout (*Oncorhynchus mykiss*). *Fd. Sci. Tech*., **1**: 21-29. https://doi.org/10.1186/2193-1801-2-1

Menoyo, D., López-Bote, C.J., Obach, A. and Bautista, J.M., 2005. Effect of dietary fish oil substitution with linseed oil on the performance, tissue fatty acid profile, metabolism, and oxidative stability of Atlantic salmon. *J. Anim. Sci*., **83**: 2853-2862. https://doi.org/10.2527/2005.83122853x

Mourente, G. and Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comp. Biochem. Physiol. B*, **14**: 389-399. https://doi.org/10.1016/j.cbpb.2006.08.012

Mourente, G., Good, J.E. and Bell, J.G., 2005. Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): Effects on flesh fatty acids composition, plasma prostaglandins E2 and F2a, immune function and effectiveness of fish oil finishing diet. *Aquacult. Nutr*., **11**: 25-40. https://doi.org/10.1111/j.1365-2095.2004.00320.x

Nayak, M., Saha, A., Pradhan, A., Samanta, M. and Giri, S.S., 2017. Dietary fish oil replacement by linseed oil: Effect on growth, nutrient utilization, tissue fatty acid composition and desaturase gene expression in silver barb (*Puntius gonionotus*) fingerlings. *Comp. Biochem. Physiol*., **205**: 1-12. https://doi.org/10.1016/j.cbpb.2016.11.009

NRC, 1993. *Nutrient requirements of fish*. National Research Council, National Academy Press, Washington, DC, USA.

Omolo, K.M., Onyango, A., Magoma, G., Munguti, J. and Ogila, K., 2017. Dietary effect of varying linseed oil compositions on growth response, survival and polyunsaturated fatty acid levels in Tilapia (*Oreochromis niloticus*). *Fish. Aquacult*., **9**: 31-41. https://doi.org/10.5897/IJFA2017.0621

Pei, Z., Xie, S., Lei, W., Zhu, X. and Yang, Y., 2004. Comparative study on the effect of dietary lipid level on growth and feed utilization for Gibel carp (*Carassius auratus* Gibelio) and Chinese longsnout catfish (*Leoiocassis longirostris* Günther). *Aquacult. Nutr*., **10**: 209-216. https://doi.org/10.1111/j.1365-2095.2004.00291.x

Peng, M., Xua, W., Tana, P., Maia, K.D.J., Zhoua, H., Zhanga, Y., Nianb, R., Macqc, B. and Aia, Q., 2017. Effect of dietary fatty acid composition on growth, fatty acids composition and hepatic lipid metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed diets with required n3 LC-PUFAs. *Aquaculture*, **479**: 591-600. https://doi.org/10.1016/j.aquaculture.2017.06.032

Peng, X., Li, F. and Lin, S., 2016. Effects of total replacement of fish oil on growth performance, lipid metabolism and antioxidant capacity in tilapia (*Oreochromis niloticus*). *Auacult. Int*., **24**: 145-156. https://doi.org/10.1007/s10499-015-9914-7

Piedecausa, M.A., Mazón, M.J., García, G.B. and Hernández, M.D., 2007. Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus *puntazzo*). *Aquaculture*, **263**: 211-219. https://doi.org/10.1016/j.aquaculture.2006.09.039

Popovic, V., Sikora, V., Tatić, M., Filipović, V., Terzić, D., Janjić, S. and BrdarJokanović, M., 2016. *Analysis of production of linseed (*Linum usitatissimum *L.) in the world*. 20th International Eco-Conference, 9th International Eco-Conference on Safe Food, pp. 119-127

Reis, B., Cabral, M.E., Telmo, J.R.F., Cunha, C.M., Beatriz, M.P.P.O., Cunha, M.L. and Luísa, M.P.V., 2014. Long-term feeding of vegetable oils to Senegalese sole until market size: Effects on growth and flesh quality. Recovery of fatty acid profiles by a fish oil finishing diet. *Aquaculture*, **434**: 425-433. https://doi.org/10.1016/j.aquaculture.2014.09.002

Schmidt, E.B., Arnesen, H., de Caterina, R., Rasmussen, L.H. and Kristensen, S.D., 2005. Marine n-3 polyunsaturated fatty acids and coronary heart disease. Part I. Background, epidemiology, animal data, effects on risk factors and safety. *Thrombosis Res*., **115**: 163-170. https://doi.org/10.1016/j.thromres.2004.09.006

Soni, P.R., Katoch, M., Kumar, A. and Verma, P., 2016. Flaxseed – composition and its health benefits. *Res. Environ. Life Sci*., **9**: 310-316.

Swanson, D., Block, R. and Mousa, S.A., 2012. Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Adv. Nutr*., **3**: 1-7. https://doi.org/10.3945/an.111.000893

Turchini, G.M. and Francis, D.S., 2009. Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets. *Br. J. Nutr*., **102**: 69-81. https://doi.org/10.1017/S0007114508137874

Zakes, Z., Jankowska, B., Jarmołowicz, S., Zmijewski, T., Partyka, K. and Zakes, D.K., 2010. Effects of different dietary fatty acids profiles on the growth performance and body composition of juvenile tench (*Tinca tinca* L.). *Fish Biol. Fish*., **20**: 389-401. https://doi.org/10.1007/s11160-009-9146-x

Zhelyazkov, G., Staykov, Y. and Nikolov, G., 2014. Effect of linseed and sunflower oil supplementation in the diet on the growth performance in carp (*Cyprinus carpio* L.), cultivated in a recirculating system. *Res. Environ. Life Sci*., **6**: 263-266.

Zupan, B., Jubojevic, D., Pelic, M., Cirkovic, M., Oordevic, O., Bogut, I. and Res, V.S., 2016. Common carp response to the different concentration of linseed oil in diet. *Slov. Vet. Res*., **53**: 19-28.