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***Research Article***

# STUDY ON THE EFFICACY OF FISH OIL REPLACEMENT WITH ALTERNATIVE LIPID SOURCES IN FISH FEED

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## ABSTRACT
Fish oil (FO) is the major source of lipid and is supplemented at very high levels in fish diets. In consideration of economical and environmental concerns, FO substitution in aquaculture with alternative dietary lipid sources is the focus of many fish nutritionists. The present study was conducted to identify fatty acid composition of three vegetables oils *viz.* canola oil (CO), soybean oil (SBO) and sunflower oil (SFO) and two animal fats *viz.* poultry fat (PF) and goat fat (GF) in order to find out suitable alternative dietary lipid source rich in essential fatty acids to be used in fish feed formulation in order to reduce cost of fish feed production. SBO was found to be rich in total polyunsaturated fatty acids (PUFAs) (58.84±1.58%) followed by SFO (52.78±0.12%), CO (28.98±0.47%), PF (24.34±0.03%) and GF (4.60±0.05%). The study revealed that fish oil can partially be replaced with SBO in fish feed formulation.

***Keywords:*** *Fatty Acids, Gas Chromatograph, Fish Oil, Vegetable Oils, Animal Fats*

## INTRODUCTION
Fish oil (FO) is the major source of lipid and is supplemented at very high levels in fish diets, particularly in diets of carnivorous fish (Martinez-Lorens *et al.*, 2007). Fish oil is rich in highly unsaturated fatty acids of the n-3 series (n-3 HUFA) including eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA), which are beneficial to human health (Martino *et al.*, 2002; Turchini *et al.*, 2009) and cultured fish. However, in the near future, the FO resources may not be enough to cover the increasing demand for oils in aquafeeds due to stagnating fisheries catches, which has resulted in higher FO prices (Turchini *et al.*, 2009; Glencross 2009; Asdari *et al.*, 2011). The challenge for fish nutritionists is to reduce the utilization of fish oil in aquafeed formulations while ensuring that appropriate amounts of n-3 long chain polyunsaturated fatty acids (n-3LCPUFA) are present in final product (Turchini *et al.*, 2009).
Vegetable oils (VOs) are rich in C18 polyunsaturated fatty acids (C18 PUFA) but lack the n-3 highly unsaturated fatty acids (n-3 HUFA) such as EPA and DHA which are abundant in FOs (Mourente *et al.*, 2005; Mourente and Bell 2006; Turchini *et al.*, 2009; Asdari *et al.*, 2011); Moreover, the world production of VOs is continuously increasing (McKevith 2005; Imanpoor *et al.*, 2011). Some freshwater fish can elongate and desaturate FAs with 18 carbons, specifically linolenic acid to PUFA with 20-22 carbons of the *n*-3 series. This ability to synthesize EPA and DHA from linolenic acid allows the formulation of diets containing less expensive plant oils/animal fats. Fishes are theoretically capable of biosynthesizing EPA and DHA via the desaturation and elongation of α-linolenic acid (18:3n-3) found in some vegetable oils. Researchers discovered that some fish species possess natural capacity to bio-convert dietary C18 PUFA precursors to HUFA, to increase their tissues content of especially n-3 HUFA (Sargent *et al.*, 2002; Kaushik, 2004). Consistent with this observation, Tocher (2003) specifically hypothesized that freshwater fish that are capable of this bio-conversion must express activity of enzymes necessary for process to occur. Important among these enzymes are fatty acyl desaturase and elongase (FAD and FAE).
Therefore, VOs are the promising candidates to replace FO because of their easy availability, relative price stability and fatty acid profiles. Hence, successful replacement of FO with VOs would decrease both dependence on FO as an ingredient in fish diets and the associated costs (Turchini *et al.*, 2003; Izquierdo *et al.*, 2005; Xue *et al.*, 2006; Mourente and Bell 2006; Huang *et al.*, 2007; Piedecausa *et al.*, 2007). According to the expressions of Blanchard *et al.*, (2008), n-3 polyunsaturated fatty acids (n-3 PUFA) are needed for growth of many fish species, while effects of n-6 polyunsaturated fatty acids (n-6 PUFA) on

increasing growth are variable among species. Studies indicated that the essential fatty acids (EFAs) requirements of fish can only be met by supplying the correct concentrations and ratios of both n-6 and n-3 fatty acids. According to report of Bransden *et al.*, (2003), the use of VOs in aquafeeds can be effective in modifying the n-3/n-6 ratio in fish meat and improve the immune system and disease resistance of fish. Thus the present study was conducted to identify fatty acid composition of different vegetables oils and animal fats obtained from the market, in order to find out suitable alternative dietary lipid source rich in essential fatty acids to be used in fish feed formulation in order to reduce the cost of fish feed production.

## MATERIALS AND METHODS

Three vegetable oils *viz.* soybean oil (SBO), canola oil (CO) and sunflower oil (SFO) and two animal fats *viz.*, poultry fat (PF) and goat fat (GF) were procured from local market for their fatty acid analysis.

**Transesterification of samples:** Ethyl esters of the lipid samples were prepared by taking 2 ml of the petroleum ether containing lipids in a test tube to which 1.5ml of sodium ethylate was added. It was then allowed to stand for 30 minutes followed by addition of 1.5ml of 8% sodium chloride solution. Contents in the tube were mixed well and kept undisturbed for another 30 minutes. As soon as the two layers got separated, the upper layer (petroleum ether layer) was transferred to another test tube with a dropper.

**Gas chromatography:** Two µl of the upper layer was then injected into the oven (using Hamilton microsyringe) of M/s Nucon Engineers AIMIL Gas Chromatograph (solid state), model Nucon series 5700/5765 equipped with flame ionization detector fitted with SS column 1/8" outer diameter x 2m length, packed with 15% D.E.G.S on CHROMOSORB W.H.P, 80-100 mesh size. The conditions for the separation were oven temperature 200°C, injector temperature 230°C, detector temperature 240°C, hydrogen flow 30ml/min, air flow 300ml/min and nitrogen flow 40ml/min. Identification of peaks was done by comparison of their retention time with those of standard fatty acyl esters (M P Biomedicals Inc. USA). Relative concentration of fatty acids was calculated by use of an automatic integrator-Windows based AIMIL Ltd., DASTA - 710 Gas Chromatograph Datastation software, version WinAcds 7.1. The estimations were done in triplicates.

**Statistical analysis:** The data were subjected to the Analysis of Variance (ANOVA) with the help of STATGRAPH and Microsoft Excels statistical packages.

## RESULTS AND DISCUSSION

Alternative dietary lipid sources collected from local market were analyzed using gas chromatograph for their fatty acid (FA) compositions. Figure 1 shows the trend of fatty acid in three vegetable oils and two animal fats.

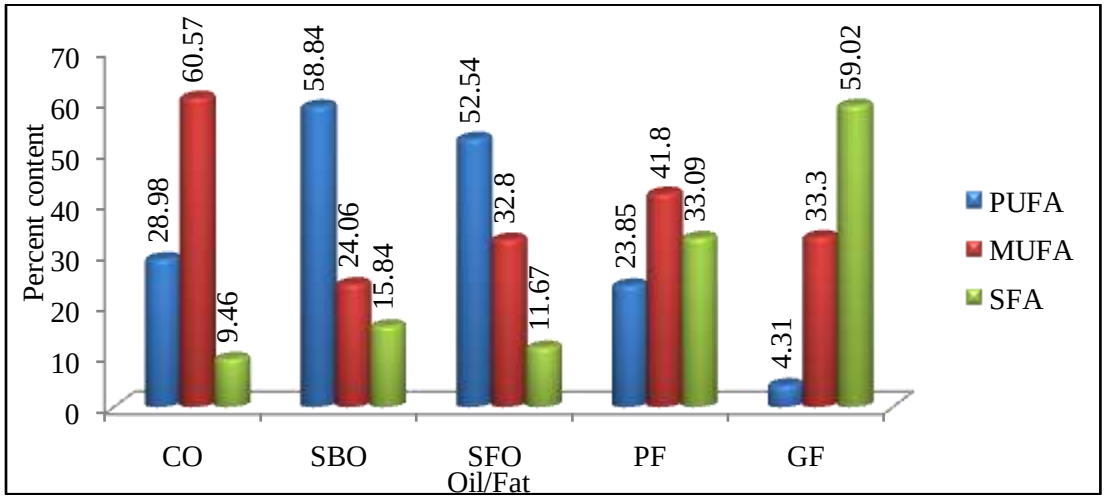


**Figure 1: Fatty acids in three vegetable oils and two animal fats**
**(CO: canola oil, SBO: soybean oil, SFO: sunflower oil, PF: poultry fat, GF: goat fat)**

Table 1 shows the polyunsaturated fatty acids (PUFAs) of alternative dietary lipid sources. Amongst the n-3 PUFAs, only linolenic acid (LNA) was present and amongst n-6 PUFAs, linoleic acid (LA) was found predominantly and arachidonic acid was present only in traces. LNA was significantly more in vegetable oils as compared to animal fats. Percent LNA was significantly more in CO (9.86±0.55%) followed by SBO (7.37±0.20%) and SFO (5.23±0.12%). However, among animal fats there was non-significant difference between PF (1.39±0.04%) and GF (1.15±0.01%). Estimated content of LNA in CO and SBO is almost similar with the results of Ziambia *et al.*, 2007 who reported 8.37 and 7.6% of LNA in CO and SBO, respectively but the results were different for SFO, as they reported only 0.28% of linolenic acid. Whereas, Hassankiadeh *et al.*, 2013 reported 5.31, 4.02 and 4.05% of LNA in CO, SBO and SFO, respectively. Major n-3 PUFAs *viz.* EPA and DHA have not been detected in the present study in any of the alternative dietary lipids.

Amongst n-6 PUFAs, there was a significant difference in the content of linoleic acid of different alternative dietary lipid sources. It was maximum in soybean oil (51.13±1.52) followed by sunflower oil (47.31±0.16), poultry fat (22.46±0.05%), canola oil (18.88±0.94) and goat fat (3.16±0.05). Percentage of linoleic acid in soybean oil is almost similar with that recorded by Chowdhury *et al.*, 2007 (52.18%), Ziambia *et al.*, 2007 (56.02%) and Hassankiadeh *et al.*, 2013 (49.15%). Amount of LA recorded in sunflower is also similar to those recorded by Chowdhury *et al.*, 2007 (46.02%) and Hassankiadeh *et al.*, 2013 (47.39%) but is different from that recorded by Ziambia *et al.*, 2007 who reported 71.17% of linoleic acid. Linoleic acid content recorded in canola oil is almost similar to those observed by Ziambia *et al.*, 2007 (20.12%) but is different from Hassankiadeh *et al.*, 2013 (37.80%).

**Table 1: Polyunsaturated fatty acids (PUFAs) of alternative dietary lipid sources**

| Fatty acids | Canola oil | Soybean oil | Sunflower oil | Poultry fat | Goat fat |
|---|---|---|---|---|---|
| **n-3 PUFAs** | | | | | |
| **Linolenic acid (C18:3 n-3)** | 9.86±0.55$^{d}$ | 7.37±0.20$^{c}$ | 5.23±0.12$^{b}$ | 1.39±0.04$^{a}$ | 1.15±0.01$^{a}$ |
| **Total n-3** | 9.86±0.55$^{d}$ | 7.37±0.20$^{c}$ | 5.23±0.12$^{b}$ | 1.39±0.04$^{a}$ | 1.15±0.01$^{a}$ |
| **n-6 PUFAs** | | | | | |
| **Linoleic acid (C18:2 n-6)** | 18.88±0.94$^{b}$ | 51.13±1.52$^{e}$ | 47.31±0.16$^{d}$ | 22.46±0.05$^{c}$ | 3.16±0.05$^{a}$ |
| **Arachidonic acid (C20:4 n-6)** | 0.26±0.03$^{a}$ | 0.33±0.03$^{b}$ | 0.22±0.03$^{a}$ | 0.47±0.04$^{c}$ | 0.22±0.01$^{a}$ |
| **Total n-6 PUFAs** | 19.12±0.90$^{b}$ | 51.46±1.50$^{e}$ | 47.54±0.17$^{d}$ | 22.94±0.07$^{c}$ | 3.39±0.04$^{a}$ |
| **n-3/n-6 Ratio** | 0.51±0.05$^{d}$ | 0.14±0.004$^{b}$ | 0.11±0.001$^{ab}$ | 0.06±0.001$^{a}$ | 0.35±0.01$^{c}$ |
| **Total PUFAs** | 28.98±0.47$^{c}$ | 58.84±1.58$^{e}$ | 52.78±0.12$^{d}$ | 24.34±0.03$^{b}$ | 4.60±0.05$^{a}$ |

*Only major PUFAs have been included. Values are mean±S.E and are presented as %age of total lipids. Values with different superscripts in a row differ significantly ($p<0.05$)*

Soybean oil was found to be rich in total PUFAs (58.84±1.58%) followed by sunflower oil (52.78±0.12%), canola oil (28.98±0.47%), poultry fat (24.34±0.03%) and goat fat (4.60±0.05%), respectively. Total PUFAs recorded in soybean oil in the present study is almost similar to those reported by Chowdhury *et al.*, 2007 and Ziambia *et al.*, 2007 i.e. 57.86% and 63.7%, respectively. Total PUFAs recorded in sunflower oil is similar to the values obtained by Hassankiadeh *et al.*, 2013 (53.32%) but different from the results obtained by Ziambia *et al.*, 2007 (71.71%). On the contrary, total PUFAs recorded in canola oil is similar to the values obtained by Ziambia *et al.*, 2007 (28.60%) but different from the values obtained by Hassankiadeh *et al.*, 2013 (43.82%).

The trend of n-3/n-6 ratio of alternative lipid sources is shown in Table1 and Figure 2. It was maximum in canola oil i.e. 0.51 and minimum was observed in poultry fat i.e. 0.06. The n-3/n-6 ratio recorded by Hassankiadeh *et al.*, 2013 in canola oil, soybean oil and sunflower oil was 0.15, 0.09 and 0.12, respectively.

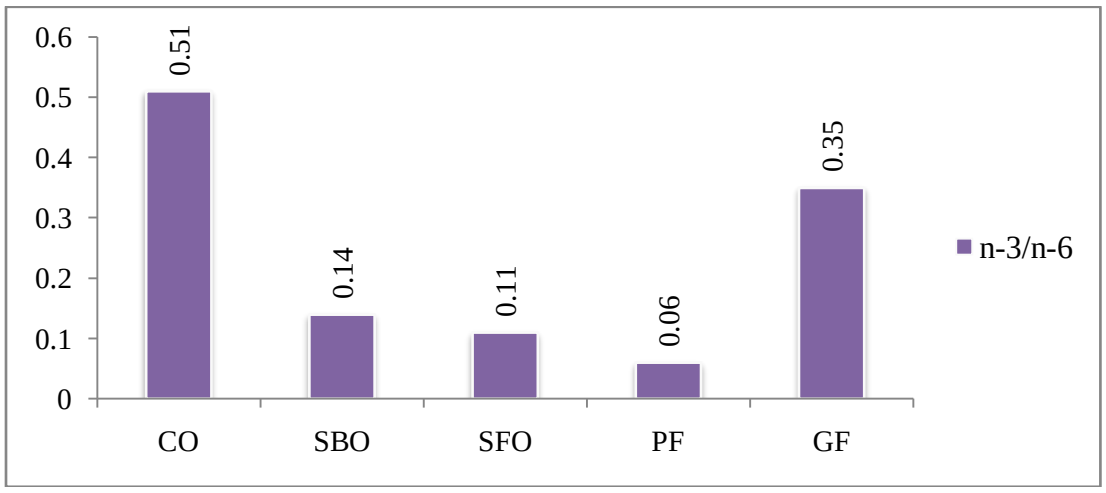


**Figure 2: Trend of n-3/n-6 ratio of alternative lipid sources**
**(CO: canola oil, SBO: soybean oil, SFO: sunflower oil, PF: poultry fat, GF: goat fat)**

Oleic acid (OA) was the predominant MUFA present in all the alternative dietary lipids (Table 2). There was significant difference in percent content of oleic acid among different alternative dietary lipid sources studied in present investigation. Canola oil contained the highest percentage of MUFAs (60.57±0.14) followed by poultry fat (41.80±0.42), goat fat (33.30±1.13), sunflower oil (32.80±0.99) and soybean oil (24.06±0.53). Earlier studies also reported oleic acid as the major MUFA in vegetable oils (Chowdhury *et al.*, 2007, Ziambia *et al.*, 2007 and Hassankiadeh *et al.*, 2013). Oleic acid content of canola oil estimated in present study is almost similar to those reported by Ziambia *et al.*, 2007 (62.41%). Similarly soybean oil has almost the same content of oleic acid as reported by Chowdhury *et al.*, 2007 (23.28%).

**Table 2: Monounsaturated fatty acids (MUFAs) of alternative dietary lipid sources**

| Fatty Acid | Canola oil | Soybean oil | Sunflower oil | Poultry fat | Goat fat |
|---|---|---|---|---|---|
| **Palmitoleic acid (C16:1 n-7)** | 0.04±0.002$^{a}$ | 0.08±0.00$^{a}$ | 0.15±0.01$^{b}$ | _ | _ |
| **Oleic acid (C18:1 n-9)** | 60.53±0.14$^{d}$ | 24.58±0.53$^{a}$ | 32.65±1.00$^{b}$ | 41.80±0.42$^{c}$ | 32.00±0.20$^{b}$ |
| **Total MUFAs** | 60.57±0.14$^{d}$ | 24.66±0.53$^{a}$ | 32.80±0.99$^{b}$ | 41.80±0.42$^{c}$ | 32.00±0.20$^{b}$ |

*Values are mean±S.E and are presented as %age of total lipids. Values with different superscripts in a row differ significantly ($p<0.05$)*

There was significant difference in percent content of the saturated fatty acids (SFAs) of the alternative dietary lipids taken during present investigation. (Table 3). Canola oil contained the lowest percentage of SFAs (9.46±0.16) followed by sunflower oil (11.67±0.27), soybean oil (15.84±0.65), poultry fat (33.09±0.18) and goat fat (59.02±0.41). The total SFAs recorded in soybean oil in the present study is almost similar to those recorded by Chowdhury *et al.*, 2007, Zambiazi *et al.*, 2007 and Hassankiadeh *et al.*, 2013. Zambiazi *et al.*, 2007 recorded almost similar total SFAs (6.98%) in canola oil as with that recorded in the present study. Palmitic acid was found to be the predominant SFA in CO, SBO, SFO and PF while stearic acid was found to be the predominant SFA in GF.

SFAs/ PUFAs was estimated to be maximum (12.83±0.14) in goat fat and minimum (0.22±0.00) in SFO. The ratio of SFAs/ UFAs was maximum (1.56±0.04) in GF and minimum (0.10±0.00) in CO. The trend in the ratio of SFAs/ PUFAs and SFAs/ UFAs were statistically significant (Table 3).

**Table 3: Saturated fatty acids (SFAs) of alternative dietary lipid sources**

| Fatty Acid | Canola oil | Soybean oil | Sunflower oil | Poultry fat | Goat fat |
|---|---|---|---|---|---|
| **Capric acid (C10:0)** | 0.21±0.003$^{c}$ | 0.15±0.005$^{b}$ | 0.32±0.01$^{d}$ | _ | 0.07±0.01$^{a}$ |
| **Undecyclic acid (C11:0)** | _ | 0.12±0.01$^{a}$ | 0.13±0.01$^{a}$ | _ | _ |
| **Myristic acid (C14:0)** | _ | 0.03±0.003$^{a}$ | 0.29±0.23$^{b}$ | 0.48±0.02$^{b}$ | 2.62±0.08$^{c}$ |
| **Pentadecanoic acid (C15:0)** | 3.20±0.18$^{d}$ | 1.47±0.26$^{c}$ | 0.33±0.09$^{a}$ | 0.12±0.02$^{a}$ | 1.07±0.00$^{b}$ |
| **Palmitic acid (C16:0)** | 4.47±0.10$^{a}$ | 10.82±0.30$^{c}$ | 8.63±0.14$^{b}$ | 28.01±0.15$^{e}$ | 21.81±0.17$^{d}$ |
| **Stearic acid (C18:0)** | 1.58±0.04$^{a}$ | 3.23±0.46$^{b}$ | 1.94±0.04$^{a}$ | 4.47±0.07$^{c}$ | 33.43±0.35$^{d}$ |
| **Total SFAs** | 9.46±0.16$^{a}$ | 15.84±0.65$^{c}$ | 11.67±0.27$^{b}$ | 33.09±0.18$^{d}$ | 59.02±0.41$^{e}$ |
| **SFAs/ PUFAs** | 0.32±0.001$^{a}$ | 0.26±0.01$^{a}$ | 0.22±0.005$^{a}$ | 1.35±0.008$^{b}$ | 12.83±0.14$^{c}$ |
| **SFAs/ UFAs** | 0.10±0.001$^{a}$ | 0.18±0.01$^{b}$ | 0.13±0.003$^{a}$ | 0.50±0.00$^{c}$ | 1.56±0.04$^{d}$ |

*Values are mean±S.E and are presented as %age of total lipids. Values with different superscripts in a row differ significantly ($p<0.05$)*

According to the expressions of Blanchard *et al.,* (2008), n-3 polyunsaturated fatty acids (n-3 PUFA) are needed for growth of many fish species, while effects of n-6 polyunsaturated fatty acids (n-6 PUFA) on increasing growth are variable among species. Studies indicated that the essential fatty acids (EFAs) requirements of fish can only be met by supplying the correct concentrations and ratios of both n-6 and n-3 fatty acids. According to the report of Bransden *et al.,* (2003), the use of VOs in aquafeeds can be effective in modifying the n-3/n-6 ratio in fish meat and improve the immune system and disease resistance of fish. Canola oil is also rich in oleic acid (18:1n-9) (Grant *et al.,* 2008) and shows a good balance between 18:1n-9, linoleic acid (18:2n-6; LA) and linolenic acid (18:3n-3; LNA) (Huang *et al.,* 2007). However, unlike FO, canola oil is free of n-3 HUFA and arachidonic acid. El-Husseiny *et al.,* (2010) reported that the suitable ratio of n-6/n-3 is more essential than the specific amounts of these FAs in Nile tilapia diets. The main problem in the replacement of animal oil with vegetable oils is the difference in their composition (Almaida-Pagan *et al.,* 2007).

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