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Evaluation of dietary fish oil:soybean oil ratios on growth performance feed utilization, haematological indices and body fatty acid composition of Keeled mullet, *Liza carinata* fingerlings

Ahmed M. Aboseif [*], Ashraf M.S.-A. Goda
National Institute of Oceanography and Fisheries (NIOF), Egypt
[*]Corresponding Author: Ahmed.Aboseaf@Gmail.com

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ABSTRACT

Dietary fat is a vital component of fish nutrition. Fish oil (FO) is considered the best source of fat due to its balanced fatty acid content. This prompted nutritionists to consider reducing the reliance on fish oil in fish diets by substituting it for cheaper and more readily available vegetable oils. This study was conducted to investigate the effect of different dietary ratios of fish oil: soybean oil (SO). Four isonitrogenous (30.99% crude protein) and isolipidic (16.92%) diets were formulated to obtain four different dietary ratios of FO: SO .The formulated diets contained the tested oils, either as the sole source of (FO, FO_{100}: SO_0 and SO, FO_0: SO_{100}), or various combinations of FO and SO (FO_{75}: SO_{25} and FO_{50}: SO_{50}). One hundred and eighty *Liza carinata* fingerlings with an initial weight of 1.54 ± 0.18 g were distributed into 12 plastic tanks (55L each, triplicate per treatment). The experiment was conducted for 60-days. The best significant ($P < 0.05$) FCR and protein efficiency ratio (PER) values were reported for FO_{75}: SO_{25} diet, compared to the other experimental treatments. The results observed that the higher FO content (FO_{100}: SO_0 and FO_{75}: SO_{25} diets) had higher n-3 FA content such as 20:5n3 EPA and 22:6n-3 DHA than the diet with higher SO ratios (FO_{50}: SO_{50} and FO_0: SO_{100}). The results of the present study suggested that *Liza carinata* fingerlings fed diet FO_{75}: SO_{25} for 60-days enhanced growth performance, diet utilization efficiency, FCR, and hematological indices. The body fatty acid (FA) profile in the experimental fish groups reflected the FA profile of their respective diets.

INTRODUCTION

During the past three decades, aquaculture has contributed significantly and effectively to the nutritional strategies developed to bridge the gap between supply and demand for animal protein from seafood, but this development must be sustainable (Aboseif *et al.*, 2022 a,b; Flefil *et al.*, 2021). Fats are important in the aquaculture feed industry, as they are the main source of essential FA and provide energy for fish (Wang *et al.*, 2022; Zorlu *et al.*, 2022). Fish oil (FO) is the primary ideal lipid source in aquatic feeds which is high in n-3 long-chain polyunsaturated fatty acids (n-3LC-PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid DHA, 22:6n-3 (Fukada *et al.*, 2020; He *et* *al.,* 2021; Gia *et al.,* 2021). Additionally, palatability-enhancing substances and essential fish nutrients were found in fish oil, making FO the primary source of fat for fish nutrition (Qin *et al.,* 2022). With the rise of global fish consumption, FO resources are gradually becoming scarce from capture fisheries (Wenning, 2020), thus the use of fishmeal and FO in aquaculture has shown a declining trend. As aquaculture continues to grow worldwide, the gap between the availability and demand for ingredients will continue to grow (Seong *et al*, 2020; Dupont-Cyr *et al.,* 2022). Hence the research interest in alternative fat sources is increasing to ensure the reduction of the amount of FO in aqua-feeds and to achieve long-term development of the aqua-feed industry. (Watson *et al*., 2020; Katsik *et al*.,2021; Liu *et al.,* 2022).

Recently, the global production of vegetable oils (VO) increased about 100 times more than that of FO. Therefore, VO is the cheapest alternative to replace fish oil in aquatic feeds (Tseng and Lin, 2020; Syamsunarno *et al.,*2021). However, in commercial aqua-feed formulations, the results vary when VOs are employed, which is mostly attributed to the inconsistent fatty acid quality of those oils including poly-unsaturated fatty acids such as C18 FA : oleic acid (C18:1n-9); linolic acid (C18:2n-6); αlinolenic acid (C18:3n-3) (Alvarez *et al.,* 2020; Mu *et al*., 2020; Gou *et al.,*2021; Sanchez-Moya *et al.,* 2020). Considerable and successful efforts are made to replace FO in aqua feeds. Soybean oil has attracted special interest in aqua feeds among the selected fat sources due to the advantage of stable production and economical price (Casu et al., 2019, Xu et al., 2021). Fish species mostly reported a rise in tissue n-6 polyunsaturated fatty acids. The high value for PUFAs in SO have been the reason for its successful use as a lipid source in fish diets such as turbot, (*Scophthalmus Maximuss*) (Peng *et al.,* 2014), Blunt snout bream (*Megalobrama amblycephala)* (Li *et al*., 2016); pond loach (*Misgurnus anguillicaudatus)* (Li *et al.,* 2017), large yellow croaker (*Larimichthys crocea*) (Mu *et al*., 2018) and a cyprinid (*Onychostoma macrolepis*) (Gou *et al*., 2021).

Mullets are characterized by high consumer demand thanks to their acceptable quality and price. Therefore, mullets are of commercial importance in the Egyptian market (Abd El-Ghaffar *et al.,* 2020; Mohamed Wafeek *et al.,* 2021). Keeled mullet, *Liza carinata* is found and cultivated mainly in farms in Ismailia and Suez governorates. *Liza carinata* has a high degree of tolerance to high salinity. Although the growth rate of Keeled-fry mullet is not particularly high when compared to the main species of mullet. Keeled mullet Liza (Valenciennes, 1826) contributed about 18% of the total catch from the Gulf of Suez in Egypt over the past 10 years (Ganam et al., 2021; Abdel Ghaffar et al., 2020). Generally, the degree of consumer demand is high in the market - and therefore is characterized by high prices. To our knowledge, the information on the nutritional requirements of Keeled mullet is scarce. Therefore, this study was conducted to investigate the effect of different dietary ratios of fish oil (FO): soybean oil (SO) on growth performance, feed utilization, hematological variables, the fatty acid composition of Keeled mullet, *Liza carinata* fingerlings.

MATERIALS AND METHODS

2.1 Experimental fish and culture technique:

This study was conducted in Shakshuk research station , National Institute of Oceanography and Fisheries (NIOF), Fayoum Governorate, Egypt. Keeled mullet, *Liza carinata* fingerlings, with an initial weight of 1.54 ± 0.18 g/ fish, Keeled mullet were obtained from a private farm in Suez Governorate Egypt. Fish were acclimated to the experimental conditions for 2 weeks and were fed a commercial diet (30% crude protein and 18 MJ gross energy /kg) at a level of 5% of body weight per day. The daily ratio was divided into two equal amounts and offered on two time a day (09:00 Am and 1:00 Pm).
One hundred and eighty *Liza carinata* fingerlings with an intial weight of 1.54 ± 0.18 g were distributed into 12 plastic tanks (55L each) treplicate per treatments. *Liza carinata* were stocked at a density of 15 fish per tank. Water quality was maintain in the ideal range for *Liza carinata*, under natural light 12:12-hr light: dark schedule during the trial. The measurements of water temperature, dissolved oxygen, pH, and ammonia were taken daily. The experiment was conducted for 60-days.

2.2 Experimental diets:

Four isonitrogenous (30.99% crude protein), isolipidic (16.92%) and isocaloric (20 MJ GE kg^{-1}) diets were formulated to obtain four different dietary ratios of FO: SO. The proximate analyses of the diets is presented in Table 1. The formulated diets contained the tested oils either as: sole source of (FO, FO_{100}: SO_0 and SO, FO_0: SO_{100}), or various combinations of FO and SO (FO_{75}: SO_{25} and FO_{50}: SO_{50}).
The experimental diets were processed by blending the dry ingredients into a homogeneous mixture and so passing the mixed feed through a laboratory pellet mill (a California Pellet Mill, made in USA). The resulting moist pellets were dried at 40°C for two days. The diets were stored in plastic bags in arefrigerator (−4°C) until use.

2.3 Growth performance and feed utilization:

The mean final body weight (FBW) per tank is decided by dividing the total weight in each tank by the number of fish. Survival (S%), weight gain (WG), specific rate of growth (SGR), protein efficiency ratio (PER), protein productive value (PPV), energy retention (ER) and feed conversion ratio (FCR) were determined according to Davies *et al.* (2020).

Table 1. Ingredients formulation and proximate composition of the experimental diets (g/kg).

Ingredients	$FO_{100}: SO_0$	$FO_{75}: SO_{25}$	$FO_{50}: SO_{50}$	$FO_0: SO_{100}$
Meat meal (60% CP)[1]	90.00	90.00	90.00	90.00
Soybean meal (4٤% CP)[٢]	375.00	375.00	375.00	375.00
Corn gluten meal (6٠% CP)[٣]	90.00	90.00	90.00	90.00
Wheat meddling (14% CP)[4]	227.40	227.40	227.40	227.40
Yallow corn[5]	110.00	110.00	110.00	110.00
Sodium chloride[6]	5.00	5.00	5.00	5.00
Fish oil (FO)[7]	100.00	75.00	50.00	0.00
Soybean oil (SO)[8]	0.00	25.00	50.00	100.00
Vitamin C[9]	0.50	0.50	0.50	0.50
Mineral and vitamin premix[10]	2.00	2.00	2.00	2.00
Anti-Oxidant[11]	0.10	0.10	0.10	0.10
Analysed composition (dry matter basis)				
Dry matter, DM%	91.59	91.59	91.59	91.59
Crude protein, CP%	30.99	30.99	30.99	30.99
Crude lipid, CL%	16.92	16.92	16.92	16.92
Nitrogen free extract NFE%	40.52	40.52	40.52	40.52
Crude fibre, CF%	5.00	5.00	5.00	5.00
Ash, %	6.58	6.58	6.58	6.58
Gross energy (MJ/kg)	٢١.٨٢	٢١.٨٢	٢١.٨٢	٢١.٨٢
FO/SO ratio	100:0	75:25	50:50	0:١00

[1] Local meat meal Horus co, Egypt, [2] Imported soy bean from Argentina, [3] Imported corn gluten meal from Argentina, [4] Local wheat meddling; Elmasrya co, Egypt, [5] Imported corn gluten meal from Argentina, [6] local sodium chloride , Egypt, [7] Imported fish oil from Indian, [8] Imported soy bean oil from Argentina, [9] vitamin C Ascorbic Acid (Chem-Lab NV, Belgium- www.chem-lab.be), [10] Vitamin and mineral Premix, AGREVET for manufacturing vitamins and feed additives, Egypt, [11] Anti-Oxidant (Hadox dry) Hameco Agro – Netherlands.

Equations:

WG = Final body weight (g) - Initial body weight (g); SGR = (ln FBW - ln IBW)/t ×100; where: FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t = time in days; FCR = Feed intake (g)/weight gain (g); PER = weight gain (g)/protein intake (g); PPV = (protein gain (g)/protein intake (g)) ×100; ER = (energy gain (kJ)/energy intake (kJ)) ×100 and Survival (S%)= (Final fish number/ Initial fish number)*100.

2.4 Proximate and fatty acid composition:

Following the guidelines of reference, the approximate composition of feed and fish samples was determined (AOAC, 2012). To evaluate the dry matter, samples were baked in an oven at 105°C to a constant weight. Using Kjeltec TM 8400 Auto Sample Systems (Foss Tecator AB, Sweden), the Kjeldahl technique (N 6.25) was used to determine the CP. Crude lipid was resolute by the soxhlet extraction method by using soxhlet Avanti 2050 (Foss Tecator AB). Ash was measured within the residues of samples burned in an exceedingly muffle furnace at 550°C for 8 h. Before compositional analysis, the fish samples were homogenised, autoclaved at 121°C for 20 minutes, and then dried at 65°C for 24 hours. The determination of fatty acid composition in diets and fish bodies was extracted using the chloroform-methanol method (Mu *et al.*, 2018; Gao *et al*., 2020).

2.5 Haematological parameters:

Blood samples were taken after the experiment for analysis. Before blood sampling, test fish were starved for 24 hours.Blood samples were taken from the fish's tail using 1.5–2 ml sterile tubes with EDTA (2.5 mg/ml) added. Allan (2006) approach was used to analyze blood parameters.

2.6. Statistical analysis:

After establishing the normality and homogeneity of variance using the Kolmogorov-Smirnov test and Levene's test in SPSS Statistics 22.0, the numerous differences between treatments were examined using analysis of variance (ANOVA) and Student-Neuman-Keuls multiple comparison tests (SPSS 22.0, Michigan Avenue, Chicago, IL, USA). Before statistical analysis, data that was reported as ratios or percentages underwent data transformation. To evaluate the significance of linear or quadratic models to describe the response of the variable to dietary FO: SO ratio levels, orthogonal polynomial contrasts were frequently used. The results were presented as the mean ± standard error of the mean (SEM).

Ethical approval

The National Institute of Oceanography and Fisheries (NIOF), acting through the Fish and Welfare Ethical Assessment Body, subjected the experiment to moral review and approval. The National Institute of Oceanography and Fisheries (NIOF), Egypt, carried out this study by the modified Animals Scientific Procedures Act, which implemented EU Directive 2010/63. Data accessibility declaration on reasonable request, the corresponding author will provide the information supporting the study's conclusions.

RESULTS

3.1. Growth performance and feed efficiency:

Growth performance , feed efficiency of keeled mullet *Liza carinata* fingerlings fed with different diets, are shown in Tables 2 and 3. The results observed a significant (P<0.05) increase in final weight (FBW), weight gain (WG), specific growth rate (SGR) and survival (S%) for fish fed the FO_{75}: SO_{25} diet. The best significant (P<0.05) FCR and PER values was reported for FO_{75}: SO_{25} diet compared to the other experimental treatments. The fish fed the FO_0: SO_{100} diet recorded the highest significant (P<0.05) values of PPV and ER compared with the other fish groups.

Table 2**.** Growth performance of *Liza carinata* fingerlings fed the experimental diets for 60-days.

Treatments	IBW (g/fish)	FBW (g/fish)	WG (g/fish)	SGR (%/day)	S (%)
FO_{100}: SO_0	1.54±0.03	15.94±0.59 ^{d}	14.41±0.60^{d}	3.90±0.08^{d}	91.11±3.85^{b}
FO_{75}: SO_{25}	1.53±0.00	23.05±0.36^{a}	21.52±0.36^{a}	4.52±0.02^{a}	95.55±3.85^{a}
FO_{50}: SO_{50}	1.56±0.03	18.57±0.80^{b}	17.02±0.82^{b}	4.12±0.09^{b}	86.66±6.66^{c}
FO_0: SO_{100}	1.54±0.02	16.93±0.36^{c}	15.39±0.35^{c}	4.00±0.04^{c}	88.88±3.84^{c}

*Values (mean ± SE) in the same column having different superscripts letter are significantly (P<0.05).

Table 3**.** Feed efficiency of *Liza carinata* fingerlings fed the experimental diets for 60-days.

Treatments	FI (g/fish)	FCR	PER	PPV (%)	ER (%)
FO_{100}: SO_0	26.67±1.74	1.85±0.05^{b}	1.55±0.04^{b}	14.46±0.24^{c}	15.16±0.75^{c}
FO_{75}: SO_{25}	37.59±1.28	1.75±0.03^{c}	1.64±0.03^{a}	14.31±0.49^{c}	14.11±0.54^{d}
FO_{50}: SO_{50}	31.84±0.78	1.87±0.07^{b}	1.53±0.06^{b}	16.47±1.20^{b}	15.58±1.13^{bc}
FO_0: SO_{100}	29.85±0.32	1.94±0.03^{a}	1.47±0.02^{c}	20.50±2.90^{a}	19.84±3.00^{a}

*Values (mean ± SE) in the same column having different superscripts letter are significantly (P<0.05).

3.2. Proximate body composition in fish:

There was no significant effect of the tested diets (P > 0.05) on the dry matter and crude protein content of whole fish body among all tested groups Table 4. Conversely, whole body crude lipid; gross energy recorded highest significant (P<0.05) values for the fish fed either FO_{75}: SO_{25} or FO_0: SO_{100} diets compared to other experimental fish groups (P<0.05).

Table ٤. Proximate composition of *Liza carinata* fingerlings fed the experimental diets for 60-days.

Treatments	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)	Gross energy (KJ/100g)
FO_{100}: SO_0	21.18±0.36	64.55±1.08	16.94 ± 0.94^{c}	19.22 ± 0.49^{a}	219.58 ± 5.49^{b}
FO_{75}: SO_{25}	20.00±0.97	63.98±1.07	19.29 ± 0.81^{a}	15.71 ± 0.67^{b}	227.51 ± 5.01^{a}
FO_{50}: SO_{50}	20.12±0.69	64.41±1.15	16.64 ± 0.80^{c}	19.24 ± 0.94^{a}	218.06 ± 7.3^{c}
FO_0: SO_{100}	18.98±1.22	64.02±1.18	18.30 ± 0.55^{ab}	15.98 ± 0.87^{b}	223.70 ± 4.29^{ab}

*Values (mean ± SE) in the same column having different superscripts letter are significantly (P<0.05).

3.3. Fatty acid composition:

FA composition of the experimental diets was shown in Table (5). FO_{100}: SO_0 diet had higher contents of ω3 fatty acids such as 20:5n 3 and 22:6n-3, whereas the diet contained lower content of highly unsaturated fatty acids (HUFA) total saturated fatty acids (SFA) and total monounsaturated fatty acids (MUFA) and better contents of ω6 fatty acids such as polyunsaturated fatty acid (PUFA).

Fatty acid composition of the body of keeled mullet *Liza carinata* fingerlings fed different diets is shown in Table 5. The results showed that the body fatty acid (FA) composition within the experimental fish groups was reflected the FA composition of their respective diets. The higher FO content (FO_{100}: SO_0) diet had higher n-3 FA content such as 20:5n3 EPA and 22:6n-3 DHA than the diet with higher SO level. Whereas, the higher SO content (FO_0: SO_{100}) in the diet had a higher n-6 FA content such as C18:2n-6, and C18:3n-6 compared to the diet with a higher level of FO. On the contrary, the total SFA, total MUFA, and n-3:n-6 fatty acid ratio of fish fed the FO_0:SO_{100} diet were significantly decreased compared to FO_{100}:SO_0 diet.

The effect of different experimental diets on the in fish body fatty acid contents of ∑n-3, ∑n-6, ∑PUFA and ∑MUFA are shown in Figure (1). The results showed that highly positive correlation was recoreded between the body FA of ∑n-3 (R^2= 0.99) , ∑n-6 (R^2=

0.99), ∑PUFA (R^2= 0.99) and ∑MUFA (R^2= 0.99) and the same FA groups composition of their respective diets.

Table 5**.** Effects of different dietary FA on body fatty acid composition of keeled mullet *Liza carinata* fingerlings.

Fatty acid	FO_{100}: SO_0	FO_{75}: SO_{25}	FO_{50}: SO_{50}	FO_0: SO_{100}
C16:0	27.14±1.09 ^{a}	20.35±1.26 ^{b}	18.51±0.83 ^{c}	17.63±0.49^{c}
C16:1n7	3.18±0.51 ^{a}	2.56±0.37 ^{ab}	2.32±0.27 ^{b}	2.45±0.38^{ab}
C17:0	1.35±0.36^{a}	0.76±0.11 ^{b}	0.50±0.09 ^{c}	0.35±0.05 ^{c}
C17:1	1.80±0.36 ^{a}	0.55±0.11 ^{b}	0.36±0.05 ^{b}	0.21±0.05 ^{b}
C18:0	16.42±1.24 ^{a}	15.29±0.27 ^{a}	12.94±1.34 ^{b}	10.96±0.80 ^{c}
C18:1n-9 (OA)	13.77±0.56 ^{d}	16.79±0.87 ^{c}	21.41±0.31^{b}	27.88±1.56 ^{a}
C18:2n- 6 (LA)	13.96±0.33 ^{d}	17.21±1.72 ^{c}	21.22±0.47 ^{b}	27.63±1.56 ^{a}
C18:3n- 3(LNA)	0.29±0.02 ^{d}	0.38±0.06 ^{c}	0.96±0.02 ^{b}	1.79±0.10 ^{a}
C20:0	1.54±0.11 ^{a}	0.66±0.02 ^{b}	0.52±0.03 ^{b}	0.87±0.033^{b}
C20:2n6	2.35±0.23 ^{a}	1.38±0.12 ^{b}	0.73±0.17 ^{c}	0.14±0.00 ^{d}
C20:4n-6 (ARA)	0.28±0.05 ^{b}	0.50±0.11 ^{b}	1.03±0.03^{ab}	1.62±0.16 a
C20:5n-3(EPA)	0.07±0.00 ^{a}	0.05±0.00 ^{b}	0.04±0.00 ^{c}	0.03±0.00 ^{c}
C22:0	1.27±0.11 ^{a}	0.87±0.04 ^{b}	0.60±0.03 ^{c}	0.17±0.01 ^{d}
C22:5n6	1.79±0.20 ^{b}	1.24±0.12^{b}	0.95±0.05^{a}	0.17±0.01^{ab}
C22:6n-3 (DHA)	2.42± 0.35 ^{a}	1.48±0.03^{b}	0.68±0.20 ^{c}	0.14±0.04 ^{d}
C23:0	0.34 ±0.02^{a}	0.17±0.07 ^{c}	0.24 ±0.02^{bc}	0.28±0.03 ^{ab}
C24:0	0.46±0.06 ^{a}	0.11±0.00 ^{c}	0.12±0.01 ^{c}	0.22±0.02 ^{b}
∑Saturates fatty acids (SFA)	48.51±1.54^{a}	38.19±1.51 ^{b}	33.42±2.05 ^{c}	30.47±1.34 ^{c}
∑ Monounsaturated FA (MUFA)	18.75±1.12 ^{bc}	19.90±0.91^{bc}	24.08±0.58 ^{b}	30.54±1.19 ^{a}
∑n-3 (PUFA)	2.70±0.36 ^{a}	1.86±0.09 ^{b}	1.63±0.02 ^{b}	1.92±0.06 ^{b}
∑n-6	16.79±0.48 ^{d}	19.68±1.75^{c}	23.83±0.65 ^{b}	30.93±1.50^{a}
∑ PUFA	55.16±1.19 ^{c}	56.71±1.56 ^{c}	62.28±1.60 ^{b}	73.98±1.13^{a}
∑ HUFA	6.27±0.27^{a}	3.87±0.23 ^{b}	2.58±0.17 ^{c}	1.52±0.12 ^{d}
∑n-3/∑n-6	0.16 ±0.01 ^{a}	0.09 ±0.00 ^{b}	0.07 ±0.00^{c}	0.06 ±0.00 ^{c}

$^{a-e}$ Values in the same row with different superscripts indicate significant differences ($P < 0.05$).

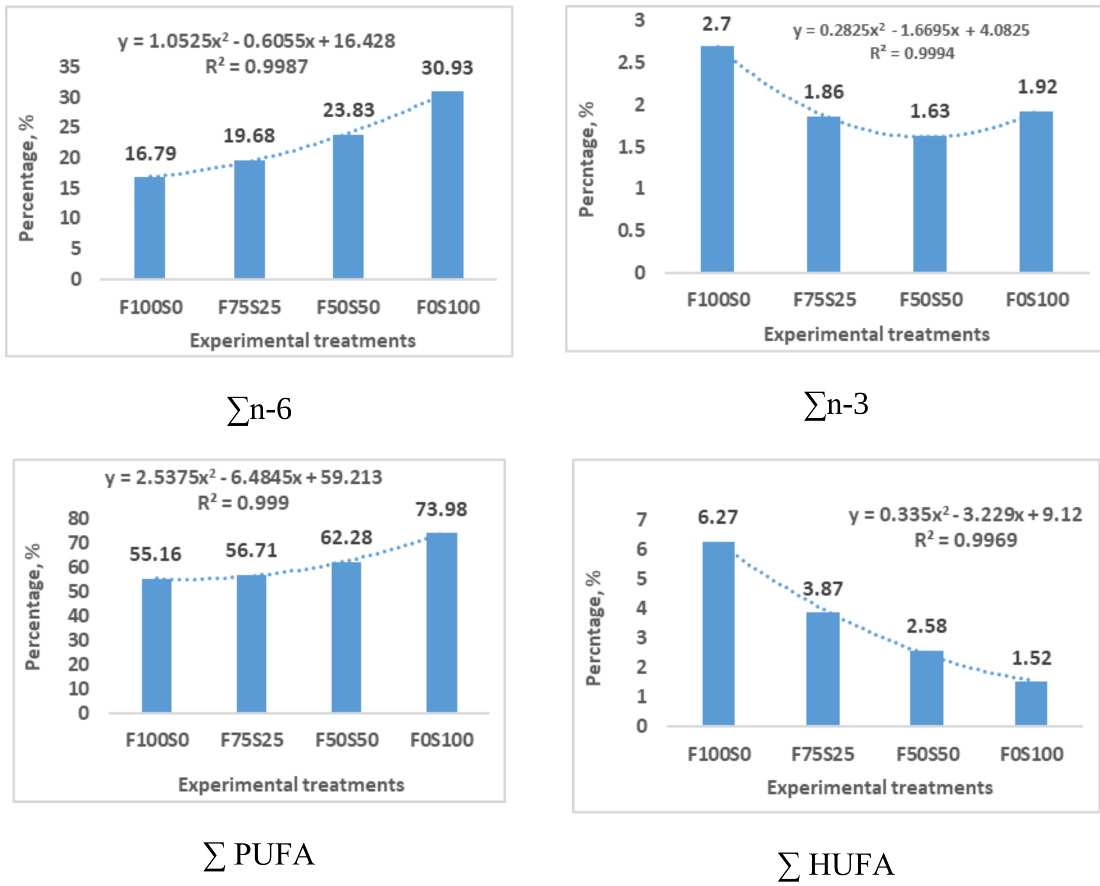

Figure 1. Show the correlation between the fatty acied content of different experimental diets (∑n-3, ∑n-6, ∑ PUFA and ∑ HUFA) and the body fatty acid contents. Total highly unsaturated fatty acids (HUFA); Total poly unsaturated fatty acids (PUFA); Total n-3 fatty acids and Total n-6 fatty acids.

3.4. Haematological parameters

Haematological parameters of keeled mullet *Liza carinata* fingerlings fed different diets is shown in Tables 6,7. Haemoglobin (Hb), Haematocrit (Hct, %), and Mean Corpuscular volume (MCV) showed haighly significant ($P>0.05$) value for fish fed FO_{50}: SO_{50} diet compared to other treatments ($P<0.05$). White blood cell (RBCs) and Red cell distribution width as standard deviation (RDW-SD) recorded highest significant ($P>0.05$) values for ish fed FO_0: SO_{100} diet. Whereas, WBCs, Platelets (PLT), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) counts recored the highest values for fish fed FO_{100}: SO_0 diet. RDW-CV values showed unsignificantly for all treatments ($P>0.05$).

Table 6**.** Haematological performance of *Liza carinata* fingerlings fed the experimental diets for 60-days.

Treatments	WBC (10^3/ mm^3)	RBC (10^6/ mm^3)	Hb (g/dl)	Ht (%)	PLT (10^3/ mm^3)
FO_{100}: SO_0	450.07±4.68^a	1.38±0.23^c	4.36±0.25^c	13.07±1.74^c	170.0±6.65^a
FO_{75}: SO_{25}	415.67±1.19^b	1.76±0.05^b	4.40±0.10^{bc}	20.83±1.20^b	120.0±1.0^b
FO_{50}: SO_{50}	278.85±17.24^c	1.73±0.11^b	5.10±0.26^a	23.10±1.80^a	94.33±4.51^c
FO_0: SO_{100}	219.11±0.71^d	1.91±0.09^a	4.63±0.15^b	21.50±0.62^b	64.66±3.78^d

* Values (mean ± S.E.) in the same column having different superscripts letter are significantly (P<0.05). **WBCs = White blood cell, RBCs = Red blood cell, Hb= Haemoglobin (g/dl), Ht= Haematocrit (%), PLT = Platelets (10^3× mm^3).

Table 7**.** Red blood cell indices, of *Liza carinata* fingerlings fed the experimental diets for 60-days.

Treatments	MCH (pg)	MCV (fL)	MCHC (g/dl)	RDW-SD (fL)	RDW-CV (%)
FO_{100}: SO_0	34.76±1.75^a	116.50±2.62^c	31.10±0.70^a	48.87±0.65^b	13.40±1.41
FO_{75}: SO_{25}	23.07±0.42^{bc}	117.70±6.10^b	23.07±0.42^b	50.10±2.03^b	13.07±0.81
FO_{50}: SO_{50}	22.20±0.66^c	123.43±1.36^a	22.00±0.66^b	41.27±0.32^c	11.16±0.55
FO_0: SO_{100}	23.53±0.51^{bc}	120.43±0.81^b	23.53±0.51^b	54.37±1.89^a	12.73±1.72

* Values (mean ± S.E.) in the same column having different superscripts letter are significantly (P<0.05). **MCH = Mean Corpuscular Haemoglobin (pg), MCV= Mean Corpuscular volume (fL), MCHC = Mean Corpuscular Haemoglobin Concentration, RDW-SD (fL) = Red cell distribution width as standard deviation, RDW-CV (%) =Red cell distribution width as coefficient of variation.

DISCUSSION

4.1. Growth performance

Because VO is cheap, it is often used as a substitute for FO in many proportions and has been generally accepted as having no significant negative impact on fish farming (Yıldız *et al*., 2018). Freshwater fish can be rated up to 100% compared to marine fish and has been determined as 60% as a vegetable oil alternative to fish oil (Dernekbas *et al*., 2021). Within the present study, low values for WG, FBW, SGR, FI, and PPV were detected within the groups fed by FO, based-similar results were also reported by Yıldız *et al*. (2018) and Dernekbaşı *et al*. (2021) in rainbow trout (*Oncorhynchus mykiss*); Yu *et al*. (2018, 2019) in Manchurian trout, *Brachymystax lenok*. However, compared to FO_{100}: SO_0 diet (100 g FO /kg), fish-fed diets containing up to 250 g/kg SO demonstrated good growth performance, and this can be attributed to the fact that *Liza carinata* need in the diet a portion of fatty acids in the form of n-6. Simlair results observed for groupers and some other fish (Yan *et al.,* 2020) when fish-fed diets containing SO and other VOs either alone or mixed as complete substitutes (Alvarez *et al.,* 2020; Sanchez Moya *et al.,* 2020; Wang *et al.,* 2018). In this study, as the amount of soy oil in the diet increases, a trend of growth performance and feed utilisation that initially increases and then drops is seen. The increasing feed intake, FCR, FBW, and the SGR at the optimum ratio of FO: SO tested (FO_{75}: SO_{25} diet) compared to the FO_{100}: SO_0 diet it is amenable to the best dietary fatty acid profile meet the nutritional requirements of *Liza carinata*.

To satisfy fish nutrition requirements should still be evaluated soy oil inclusion level, with and without individual FA supplementations DHA, ARA, and EPA. In contrast, some studies have shown results in poor growth in marine and freshwater fishes that primary dietary inclusion levels of SO (Chen *et al*., 2020; Jin, Mu *et al*., 2018). Similarly, Wang *et al*., (2022) showed no significant effects between the FO diet and VO diet on WG, SGR, and PER for *Rhynchocypris lagowskii*. Similar results are reported that no negative effects on growth performance were observed because of the substitute of dietary FO with VO of some fish species blunt snout bream, (*Megalobrama amblycephala),* red hybrid tilapia (*Oreochromis sp)*, rainbow trout (*Oncorhynchus mikyss)* and marine fish European sea bass (*Dicentrarchus labrax)* , Japanese Seabass (*Lateolabrax japonicus*) (Roy *et al.*, 2020; Torrecillas *et al.*, 2017). During the current study, the growth performance and deformation of the *Liza carinata* were gradually impeded by the increase of SO in the diet, and this may be due to the lower EPA and DHA in the diets as a result of the increase SO (Mzengereza *et al*., 2021). Various species, growth stages, and dietary ingredients in feed formulations tested were could reason for these differences in results (Qin *et al*., 2022; Gudid *et al*., 2020).

4.2. Proximate composition

The results of the current study showed that FO and SO had no effect on the body content of both dry matter and crude protein in *Liza carinata*.These results are compatible with, Sajedkhanian et al. (2021) in replacing fish oil with plant oil in juveniles of the *Salmo caspius* diet. Masiha *et al.* (2015) and Nikzad *et al.* (2013) studied replacing fish oil with plant oil and reported that, adding vegetable oils had no impact on the carcass's chemical composition in young beluga sturgeon, *Huso huso* and trout diet. On the other hand, the results of the present study showed that the crude fat contents were significantly higher within the whole fish group fed on SO diets compared to the FO_{100}:SO_0 group. The dietary C18:1n-9 and C18:2n- 6 levels varied significantly in response to dietary SO inclusion levels. At high dietary SO including, the body lipid accumulation more compared to other fish gropus. Similarly the replacement of SO for FO increased tissue fat content in grass carp *Ctenopharyngodon Idella* (Liu *et al.,* 2022); large yellow croaker (Du *et al.,* 2017) and seabream (Izquierdo *et al.,* 2015) .

An imbalance, between hepatic lipid synthesis, transport in tissue cells and b-oxidation, is another cause of excessive lipid build-up (Viscarra and Sul, 2020; Qin *et al*., 2022), resulting in the lipid metabolic syndrome of the liver (Marchix *et al.,* 2020). On the contrary, lipid retention within the liver is unaffected by SO inclusion levels during a few studies (Li *et al*., 2019). These results combined imply that SO supplementation may affect lipid retention in the various fish species differently. Additionally, n-3 PUFAs may prevent the synthesis of fatty acids and lessen the buildup of tissue fat (Liu *et al*., 2022; Qin *et al.,* 2022).

4.3. Effects of dietary soy oil on the body fatty acid profile of *Liza carinata* fingerlings:

The liver converts feed fats into fatty acids, which are then transported through the blood to tissues in lipoproteins form. Fatty acids have a major role in the deposition of fats and the formation of fish tissues. Consequently, the fatty acid content of fish tissues typically reflects the diet (Liu *et al.,* 2022; Qin *et al*., 2022). In the current study, soybean oil had a major effect in increasing n-6 chain polyunsaturated fatty acids, while saturated fatty acids and polyunsaturated fatty acids (n-3) decreased. The fatty acid composition of the fish body was known to vary depending on the dietary fatty acid composition because this was observed in other fish species (Emre *et al.,* 2016; González-Félix *et al.*, 2016). Along with raising SO, the inclusion level in the diet also increased the body's levels of (C18:1n-9) and (C18:2n-6) while lowering the levels of other FO. Accordingly, numerous investigations utilising either vegetable oils alone or their combination have revealed similar effects on fatty acids profile (Emre *et al.*, 2016; Monge-Ortiz *et al*., 2018; Qin *et al.,* 2022). Interestingly, the impact of the incremental dietary SO level and the shifting trend of the n-3/n-6 PUFA ratio was dependent on the content of dietary fatty acids.

Another important thing to keep in mind while formulating feed is the n-3/n-6 PUFA ratio (Jin *et al.*, 2019; Ma *et al*., 2019; Qin *et al.*, 2022). The highest concentration of an n-3/n-6 PUFA in feed for healthy growth of *Liza carinata* in the current study was produced by a ratio found in FO_{75}: SO_{25} diet (0.09) showing that n-3/n-6 PUFA dietary ratio maintains good growth even with the lowest n-3/n-6, which was also reported for sea bream (Qin *et al.*, 2022 and Jin *et al*., 2019). This was in agreement with Emre *et al.* (2016), who found that replacing dietary FO with vegetable oil raised the levels of linolic acid and omega-6 in the muscles (Emre *et al.,* 2016). In particular, the amount of $\sum$ HUFA in fish reduced as dietary SO increased, showing that replacing dietary FO with VO significantly decreased the amount of $\sum$ HUFA in fish. The fatty acids profile in muscle also changed as a result of dietary oil, and levels of ARA, EPA, and DHA were decreased (Xu *et al.*, 2015; Emre *et al.,* 2016).

4.4. Effects of dietary FO: SO ratios on the haematological performance of *Liza carinata* fingerlings:

To maintain physiological processes that lead to healthy growth, immunological responses, and disease resistance in fish, a dietary lipid is crucial. In all cells, fatty acids have a variety of tasks. Therefore, any changes to the fatty component of the diet may have an impact on fish health (Mu *et al*., 2020). One of the most common measures of fish general health problems is haematological indices. Prior research has demonstrated the beneficial benefits of the FO: SO ratio on fish immunological responses, haematological, and antioxidative responses in both marine and freshwater fish (Reda *et al.*, 2020; Agh *et al.*, 2020). Our results indicated that after feeding keeled mullet *Liza carinata* fingerlings for 60 days, increasing the dietary SO content at the expense of FO had no adverse impact on haematological markers. Despite the significant difference in the values of blood parameters, they were all within the safe limit values. Reda *et al.* (2020),reported that switching from FO to plant oil had no adverse effects on *O. niloticus's* haematological markers. Additionally, there were appreciable differences in RBC, MCV, and RDW-SD (fL) values across diets containing varying levels of FO; however, SO addition to feeding increased values of RBC, MCV, and RDW-SD (fL) in keeled mullet. Kenari *et al.*, (2011) showed little variations in haematological markers in salmon trout caspius fed diets with FO (tuna) or canola, Furthermore, the addition of linseed oil to the diet for 12 weeks improved the MCHC levels in *Ictalurus punctatus* (Thompson *et al.*, 2015). According to these findings, channel catfish-fed diets supplemented with 2 per cent soyabean oil and fish oil did not substantially differ in MCHC, Hct, and Hgb (Ofori-Mensah *et al.*,2020). This may indicate that the diets provided the keeled mullet fingerlings needed by the HUFA to maintain the subjects' great health state.

CONCLUSION

The findings of this study showed that keeled mullet, *Liza carinata* fingerlings had increased growth performance and feed utilisation when fed a diet with a FO_{75}: SO_{25} ratio. The efficient use of soy oil in feed formulations can significantly improve performance, lower costs, and increase production efficiency. However, as dietary SO inclusion levels increased, n-3 PUFA, EPA, DHA, and n-3/n-6 PUFA tissue concentrations declined. or keeled mullet, Liza carinata feeding practises, to our knowledge more study is required to determine the relationship between the incremental dietary FO: SO ratio and lipid metabolism.

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الملخص العربي

تقييم نسب زيت السمك:زيت فول الصويا الغذائي على أداء النمو والكفائة الغذائية، مؤشرات الدم، وتكوين الأحماض الدهنية في الجسم لإصبعيات السهيلية.

أحمد محمد أبوسيف و أشرف محمد عبد السميع جودة

المعهد القومي لعلوم البحار و المصايد - مصر

الدهون الغذائية عنصر حيوي لتغذية الأسماك. يعتبر زيت السمك (FO) أفضل مصدر للدهون بسبب محتواه المتوازن من الأحماض الدهنية. ومع ذلك ، فقد تم تقييد استخدام FO بسبب ارتفاع سعره وانخفاضه المستمر وعدم كفاية انتاجه. دفع هذا خبراء التغذية إلى التفكير في تقليل الاعتماد على زيت السمك في وجبات الأسماك عن طريق استبدال الزيوت النباتية الأرخص والأكثر توافراً. أجريت هذه الدراسة لمعرفة تأثير محتوى العليقة من النسب الغذائية المختلفة لزيت السمك: زيت فول الصويا (SO) على أداء النمو ، الاستفادة من الغذاء ، ومعايير الدم، وتكوين الأحماض الدهنية لإصبعيات سمك السهيلى ، *Liza carinata*. تمت تركيب أربعة علائق غذائية متساوية فى محتواها الكلى من النيتروجين (٣٠.٩٩٪ بروتين خام) والليبيدات (١٦.٩٢٪) وتحتوى على أربع نسب غذائية مختلفة من زيت الصويا : زيت السمك . احتوت العلائق على الزيوت المختبرة إما كمصدر وحيد لـ زيت السمك، FO_{100}: SO_0 و زيت الصويا، (FO_0: SO_{100}) ، أو توليفات مختلفة من زيت السمك و زيت الصويا (FO_{75}: SO_{25} و FO_{50}: SO_{50}). وزعت مائة وثمانين من إصبعيات السهيلى *Liza carinata* بوزن ابتدائي ١.٥٤ ± ٠.١٨ جم في ١٢ خزانًا بلاستيكيًا (٥٥ لترًا لكل معاملة ، ٣ مكرارت لكل معاملة). وأجريت التجربة لمدة ٦٠ يومًا. أشارت النتائج إلى أن التغذية على العليقة FO_{75}: SO_{25} أدى إلى زيادة معنوية في أداء النمو وتحسين التحويل الغذائي (FCR). تم تسجيل أفضل قيم معنوية لمعامل التحويل الغذائى FCR ($P < 0.05$) ونسبة كفاءة البروتين (PER) للاسماك التى تغذت على العليقة FO_{75}: SO_{25} مقارنة بباقى العلائق المختبرة. سجلت الأسماك التي تم تغذيتها على العليقة FO_0: SO_{100} أعلى قيم معنوية ($P < 0.05$) للقيمة الإنتاجية للبروتين (PPV) ، والطاقة المحتجزة بالجسم (ER) مقارنة بمجموعة الأسماك الأخرى. انعكس تكوين الأحماض الدهنية في الجسم (FA) داخل مجموعات الأسماك التجريبية على محتوى الاحماض الدهنية للعلائق المختبرة. لاحظت النتائج أن محتوى زيت السمك الأعلى فى العلائق (FO_{100}: SO_0 و FO_{75}: SO_{25}) كان يحتوي على محتوى أعلى منالاحماض الدهنية من نوع n-3 مثل 20:5n3 EPA و 22:6n-3 DHA مقارنة بالعلائق الذي تحتوي على نسب أعلى من زيت الصويا (FO_{50}: SO_{50} و FO_0: SO_{100}). في حين أن المحتوى الأعلى من زيت الصويا في العلائق كان يحتوي على محتوى أعلى منالاحماض الدهنية n-6 مثل C18: 2n-6 و C18: 3n-6 مقارنة بالعلائق التي تحتوي على مستوى أعلى من زيت السمك. أشارت نتائج الدراسة الحالية إلى أن تغذية إصبعيات السهيلى *Liza carinata* على العليقة FO_{75}: SO_{25} لمدة ٦٠ يومًا ، أدى إلى تحسين أداء النمو ، وكفاءة استخدام الاستفادة من الغذاء وارتفاع معدل التحويل الغذائى ومؤشرات معايير الدم. محتوى الجسم من الأحماض الدهنية داخل مجموعات الأسماك التجريبية كان انعكاس واضح لمحتوى العلائق من الاحماض الدهنية.