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Effect of various lipid sources on the growth and survival of juveniles of Oscar, *Astronotus ocellatus*

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Abstract
Effects of Various Lipid Sources on growth and survival of Oscar, *Astronotus Ocellatus* Juveniles was evaluated. Each diet was prepared to contain 40% protein and a lipid source. In experiment, the juveniles (Length 1.8 ± 0.04 cm; Weight 0.03 ± 0.01g) were fed six isoenergetic and isonitrogenous diets viz., sardine oil + linseed oil (T_1), sardine oil + soybean oil (T_2), sardine oil + mustard oil (T_3), cod liver oil + linseed oil (T_4), cod liver oil + soybean oil (T_5) and cod liver oil + mustard oil (T_6) for a period of 60 days. The experiment was designed as per completely randomize design with four replicates for each combination of oil sources. Among the experimental diets, the diet incorporated with sardine oil + mustard oil showed significantly higher growth such as length gain (220.25 ± 0.11), weight gain (440.96 ± 0.14) and specific growth rate (2.80 ± 0.04) and survival. The feed utilization such as protein efficiency ratio (20.04 ± 0.61) and lipid efficiency ratio (114.53 ± 0.35) were significantly higher than other diets, whereas feed conversion ratio (0.13 ± 0.01) significantly lower than other diets.

Keywords: Oscar, lipid sources, growth and survival

Introduction
In India, various ornamental fishes such as goldfish, koi carp, barbs, mollies, swordtails, guppies, angel fish, tetras, zebra-danios, oscar, discus, flowerhorn, arowana, etc., are reared popularly. Among these, cichlids are most interesting and popular aquarium fishes due to their social life and interesting behaviors. Oscar, *Astronotus ocellatus* belongs to family Cichlidae have different varieties like tiger, albino, peacock, marbled, red eye, velvet, lutino and veil tail, etc [1, 2]. Tiger Oscar has velvet black colour with brown shading and 1-2 orange-yellow colour spots on the lateral side of the body as well as at the base of the caudal peduncle [3].

Feed is one of the most essential operational inputs for rearing of fish. The quality, quantity and cost of feed are of paramount importance to the success of fish rearing operations. Nutritional requirements of fish species are the basic prerequisite to formulate nutritionally balanced and low cost feed. Oscar is an omnivorous fish, feeds on wide variety of diet including bloodworms, brine shrimp, snails, small fishes, insect larvae, pieces of mussel, beef heart, goat heart and also accept the compounded feeds [2].

Among the essential nutrients, lipids are important source of energy. Essential fatty acids and phospholipids provide a vehicle for absorption of fat soluble sterols and vitamins. In addition to their function of prostaglandin synthesis, lipids also plays vital role in the structure of cell and cellular membrane and serve as the precursors for several hormones. Lipids are highly digestible in fish and are reported to spare protein. However, excess dietary lipids suppresses *de novo* fatty acid synthesis and reduces ability of fish to digest and assimilate, resulting in reduced growth [4]. Source of lipid also play an important role in growth and survival of fishes [5]. Therefore, knowledge of dietary requirement of lipid is essential for achieving optimum growth and survival of fish [6, 7].

As in other vertebrates, fish cannot synthesize n-3 (Linolenic) and n-6 (Linoleic) polyunsaturated fatty acids (PUFA) but fish require these two essential fatty acids which have to be provided from exogenous sources. Freshwater fish in general, requires either dietary 18:2 n-6 (Linoleic) or 18:3 n-3 (Linolenic) acids or both [8]. The n-3 PUFA level was high and n-6 PUFA level was low in fish oil as compared to vegetable oils [9].

Fish oil is widely used in the production of fish feed because of its high content in polyunsaturated fatty acids (PUFA) that are essential to biological structure and normal function of cell membranes [10].

However, their high un-saturation makes them sensitive to lipid per-oxidation [11]. and excess PUFA intake could induce adverse effect on fish growth [12, 13]. Many studies regarding the combination of animal oil and plant oil in the diets have carried out, and in general the combination of animal oil and plant oil appears to be possible when the essential fatty acid (EFA) requirement are satisfied [10, 14-17]. In addition to lipid sources, dietary lipid levels must also be evaluated carefully. Within certain limits, increasing the dietary lipid level improves the utilization of feed [18, 19] and protects somehow against the metabolism of protein in energy [4, 20]. However, some studies showed that high fat diet lead to induced metabolic impairments [21] abnormal oxidative status [22] and also altered fatty acid of the muscle tissue [23, 24]. This study was undertaken to evaluate the combination of fish oil and plant oil on growth and survival of Oscar, *A. ocellatus* by comparing the effect of different dietary lipid sources.

Materials and Methods

Experimental fish

Juveniles of Oscar, *Astronotus ocellatus* were obtained from Srushti Aquaculture Farm, Pen, Raigad, Maharashtra, India and were maintained in plastic pool (550 L). The juveniles were acclimatized to experimental condition for two weeks during which fishes were fed diets containing 40% protein and 7% lipid. The juveniles were fed at the 8% of body weight per day. The daily ration were divided into three installments of 50%, 25% and 25% and given at 08.00, 12.00 and 18.00 h, respectively. The faeces and uneaten feed were removed from plastic pool by using siphoning tube every morning.

Experimental diets

The six isoenergetic and isonitrogenous semi-purified diets containing 7% crude lipid and 40% crude protein were prepared using different sources of lipid *viz*. sardine oil + linseed oil (T_1), sardine oil + soybean oil (T_2), sardine oil + mustard oil (T_3), cod liver oil + linseed oil (T_4), cod liver oil + soybean oil (T_5) and cod liver oil + mustard oil (T_6). Diets were formulated using casein, gelatin and fish powder as protein sources, dextrin as carbohydrate source, α-cellulose as filler and carboxymethyl cellulose as a binder while vitamin and mineral premix were also added into the diet at 3 and 2% levels, respectively. The ingredients used, proximate composition and gross energy of the experimental diets are given in Table 1.

Table 1: Ingredients used and proximate composition of experimental diets (g kg^{-1} DM)

Ingredients (g kg^{-1} DM)	Diets					
	T_1	T_2	T_3	T_4	T_5	T_6
Fish powder[a]	200.0	200.0	200.0	200.0	200.0	200.0
Casein[b]	248.0	248.0	248.0	248.0	248.0	248.0
Dextrin[b]	323.4	323.4	323.4	323.4	323.4	323.4
Gelatin[b]	30.0	30.0	30.0	30.0	30.0	30.0
CMC[be]	15.0	15.0	15.0	15.0	15.0	15.0
Cellulose[b]	77.6	77.6	77.6	77.6	77.6	77.6
Vitamin mixture[c]	30.0	30.0	30.0	30.0	30.0	30.0
Mineral mixture[d]	20.0	20.0	20.0	20.0	20.0	20.0
Sardine oil	28.0	28.0	28.0	-	-	-
Cod liver oil	-	-	-	28.0	28.0	28.0
Linseed oil	28.0	-	-	28.0	-	-
Soybean oil	-	28.0	-	-	28.0	-
Mustard oil	-	-	28.0	-	-	28.0
Proximate Composition (g kg^{-1} DM except for moisture)						
Moisture	68	69.9	68	69.7	70	71
Crude protein	403.6	403.6	403.6	403.6	403.6	403.6
Crude lipid	68.6	69.9	70	69.6	70	69.7
Crude ash	45.1	46	45.3	45.4	48.3	45.4
Crude fiber	40	40	41	40	40	42
NFE[f]	442.7	440.5	440.1	441.4	438.1	439.3
Gross energy (MJ kg^{-1})[g]	20.17	20.18	20.18	20.18	20.14	20.15

a. Fish powder (Moisture: 9.84, Crude protein: 70.68%, Crude lipid: 7.55%, Crude ash: 7.07%, Crude Fiber: 1.72%, NFE: 3.14, Gross energy: 20.52),
b. Obtained from Himedia, India,
c. Becosules capsules, product of Pfizer Ltd., India: Vitamin: Thiamine mononitrate IP – 10 mg, Riboflavina IP – 10 mg, Pyridoxine Hydrochloride IP – 3 mg, Vitamin B_{12} IP - 15 mcg, Niacinamide IP – 100 mg, Calcium Pantothenate IP – 50 mg, Folic acid IP - 1.5 mg, Biotin USP -100 mcg, Ascorbic acid IP – 150 mg.
d. Agrimin, product of Virbac Animal Health India Pvt. Ltd.; Mineral: Cobalt – 150 mg, Copper – 1200 mg, Iodine – 325 mg, Iron – 5000 mg, Magnesium – 6000 mg, Manganese – 1500 mg, Potassium – 100 mg, Selenium – 10 mg, Sodium - 5.9 mg, Sulphur - 0.922%, Zinc – 9600 mg, DL-Methionine – 1920 mg, L-lysine mono-hydrochloride - 4400 mg, Calcium - 24%, Phosphorous - 12%.
e. Carboxy Methyl Cellulose,
f. Nitrogen-free extract (Calculated by the difference)**,**
g. Gross energy, calculated based on 23.9, 39.8 and 17.6 MJ kg^{-1} for protein, lipid and nitrogen free extract (NFE), respectively (Schulz *et al*., 2007)
DM- dry matter

Experimental Design

Experiment was designed as per completely randomize design (CRD) with four replicates for six dietary treatments. Fish (Total length, 1.8 ± 0.04 cm and weight, 0.03 ± 0.01 g) were randomly assigned to each circular plastic tub (35 L capacity) at the rate of 30 fishes per tub. Aerators were provided in each tub for continuous aeration. Feeding was carried out till satiation three times daily 08.00 h 12.00 h and 18.00 h. The feeding trial lasted for 60 days. Twenty-five per cent of water from each tubs was replaced with freshwater every day to maintained water quality.

Chemical analysis

Proximate composition

Proximate composition of all diets was carried out according to the method [25]. Moisture was analysed by gravimetric analysis following oven drying to constant weight at 105 ^{0}C. The nitrogen content was derived by using KEL PLUS-CLASSIC DX. Crude protein was calculated by multiplying nitrogen content by a constant 6.25. Crude lipid content was determined by using SOCS PLUS with petroleum ether. Ash content was determined gravimetrically by burning in Muffle furnace at 550^{0}C for 6 hours. Crude fibre content was determined by using FIBRA PLUS with 1.25% sulphuric acid and sodium hydroxide wash further ashing the sample in Muffle furnace at 550 ^{0}C for 2 hours. The nitrogen free extract was determined by difference.

Water parameters such as temperature, dissolved oxygen, pH, free carbon-dioxide and total alkalinity were recorded by using standard methods [26].

Growth and survival

At the end of experiments fishes were weighed using mono-pan electronic (Sartorius, BS 224s) balance with an accuracy of 0.01 mg. Total length of fish was measured from tip of mouth to tip of caudal fin with the help of foot rule having a least count of 0.5 mm. The length and weight of fishes were recorded at starting, at end of experiments and after fifteen days interval. The fishes were counted for estimation of survival percentage.

Data collected during the experiments was used for estimating the following growth parameters and survival using standard formulas [27].

Length gain

$$\text{Length gain (\%)} = \frac{(\text{Final length} - \text{Initial length})}{\text{Initial length}} \text{ X } 100$$

Weight gain

$$\text{Weight gain (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \text{X } 100$$

Specific growth rate (SGR %)

$$\text{Specific Growth Rate (\%)} = \frac{(\ln Wt - \ln Wo)}{dt} \text{X } 100$$

Where, W_t = Final weight

W_o = Initial weight

dt = Rearing period in days

Survival (%)

$$\text{Survival (\%)} = \frac{\text{Final count}}{\text{Initial count}} \text{X } 100$$

Feed utilization was estimated by using the following formulas

Feed Conversion Ratio

$$\text{Feed Conversion Ratio} = \frac{\text{Total feed intake}}{\text{wet weight gain (g)}}$$

Protein efficiency ratio

$$\text{Protein Efficiency Ratio} = \frac{\text{wet weight gain (g)}}{\text{Crude protein intake (g)}}$$

Lipid Efficiency Ratio

$$\text{Lipid Efficency Ratio} = \frac{\text{wet weight gain (g)}}{\text{Crude lipid intake (g)}}$$

Statistical analysis

Analysis of variance was conducted using the general linear model procedure of the SAS 9.3 computer software (SAS College of Fisheries, Shirgaon, Ratnagiri, Maharashtra, India). Data were expressed as the mean ± SE of four replicates. Length gain %, weight gain %, specific growth rate, feed conversation ratio, protein efficiency ratio, lipid efficiency ratio and survival were analyzed using one-way ANOVA (Analysis of Variance) and SNK (Student Newman-Keuls) multiple-range test to determine significant difference (P< 0.05) among the treatments means. A second order polynomial regression model between final body weight and dietary lipid level was used for estimation of dietary lipid levels that promoting maximum somatic weight gain of juveniles of *A. ocellatus* [28- 32].

Results

Growth

Length gain

The maximum length gain of 220.25 ± 0.11% was observed in diet T_3 whereas diet T_2 showed minimum length gain of 115.23 ± 0.13% (Fig. 1). One way ANOVA showed significant difference (P< 0.05) in the length gain of juveniles of *A. ocellatus*. Student's Newman Keul multiple range test (SNK) indicated that length gain of juveniles fed T_3 diet was significantly higher (P< 0.05) than that of juveniles fed other diets However, there was no significant difference (P> 0.05) in length gain of juvenile fed diets T_1, T_2, T_4, T_5, and T_6.

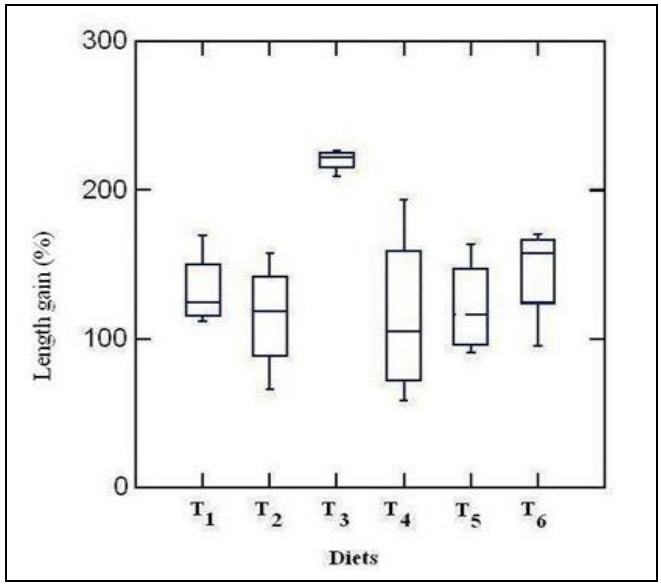

Fig 1: Length gain of juvenile *A. ocellatus* fed with different diets for 60 days

Weight gain

The maximum weight gain of 440.96 ± 0.14 % was observed

in the juveniles fed diet T_3 while diet T_2 showed minimum average weight gain of 97.69 ± 0.03 % (Fig. 2). One-way ANOVA revealed significant difference ($P< 0.05$) in the weight gain of juveniles *A. ocellatus*. Student's Newman Keul multiple range test (SNK) revealed that the average weight gain of fishes fed T_3 diet was significantly higher ($P< 0.05$) than that of fishes fed other diets. However, there was no significant difference ($P> 0.05$) in weight gain of juvenile fed diets T_1, T_4, T_5, and T_6.

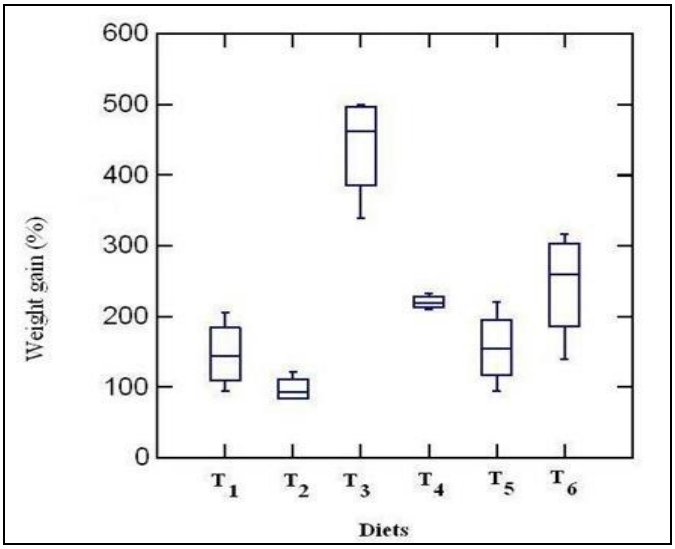

Fig 2: Weight gain of juveniles *A. ocellatus* fed with different diets for 60 days.

Specific growth rate (SGR)

The juveniles fed diet T_3 showed the maximum specific growth rate of 2.80 ± 0.04 whereas diet T_2 showed minimum specific growth rate of 1.13 ± 0.03 (Fig. 3). One-way ANOVA showed that there is significant difference ($P< 0.05$) in the specific growth rate of juveniles of *A. ocellatus* fed with different diets. The Student Newman Kuel's multiple range test (SNK) showed significantly higher (P<0.05) specific growth rate of fish fed diet T3 than the fishes fed other diets (Table 2). However, there was no significant difference ($P> 0.05$) in specific growth rate of juvenile fed diets T_1, T_4, T_5, and T_6.

Table 2: Specific growth rates of juvenile *A. ocellatus* fed with different dietary lipid sources.

Replicates	Diets					
	T_1	T_2	T_3	T_4	T_5	T_6
Specific growth rate (SGR)						
R_1	1.35	1.01	2.97	1.89	1.94	1.99
R_2	1.86	1.16	2.78	1.95	1.66	2.38
R_3	1.61	1.33	2.47	2.01	1.11	1.46
R_4	1.11	1.01	2.99	1.91	1.45	2.26
Mean	1.48^{bc}	1.13^{c}	2.80^{a}	1.94^{b}	1.54^{bc}	2.02^{b}
(±) S.E.	± 0.06	± 0.03	± 0.04	± 0.01	± 0.06	± 0.07

Values mean ± SE in the same row with different superscripts are significantly different from each other using SNK test (P<0.05)

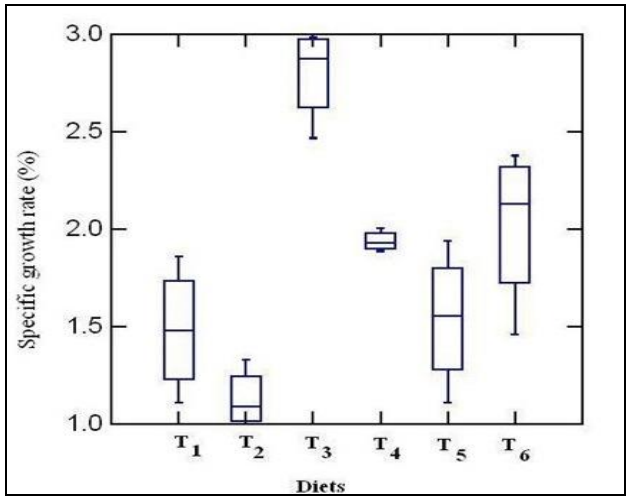

Fig 3: Specific growth rate of juveniles *A. ocellatus* fed with different diets for 60 days.

Survival

The maximum survival (77.55 ± 0.16) was recorded for juveniles fed diet T_3, while juveniles fed diet T_6 showed minimum survival (41.67 ± 0.16) given in table 3 and Fig. 4. One-way ANOVA showed significant difference ($P< 0.05$) in the survival of juveniles of *A. ocellatus*. Student's Newman Keul multiple range test (SNK) indicated that survival of juveniles fed diet T_6 was significantly lower ($P< 0.05$) than that of juveniles fed otherdiets However, there was no significant difference ($P>0.05$) in survival percentage of juveniles fed diets T_1, T_2, T_3, T_4, and T_5.

Table 3: Survival of juvenile *A. ocellatus* fed with different dietary lipid sources.

Replicates	Diets					
	T_1	T_2	T_3	T_4	T_5	T_6
Survival						
R_1	70.00	66.67	70.00	50.00	66.67	40.00
R_2	53.33	70.00	80.00	80.00	76.67	60.00
R_3	66.67	56.67	86.67	73.33	46.67	30.00
R_4	60.00	56.67	73.33	70.00	66.67	36.67
Mean	62.50^{a}	62.50^{a}	77.55^{a}	68.33^{a}	64.17^{a}	41.67^{b}
(±) S.E.	0.018	0.19	0.16	0.11	0.15	0.16

Values mean ± SE in the same row with different superscripts are significantly different from each other using SNK test (P<0.05)

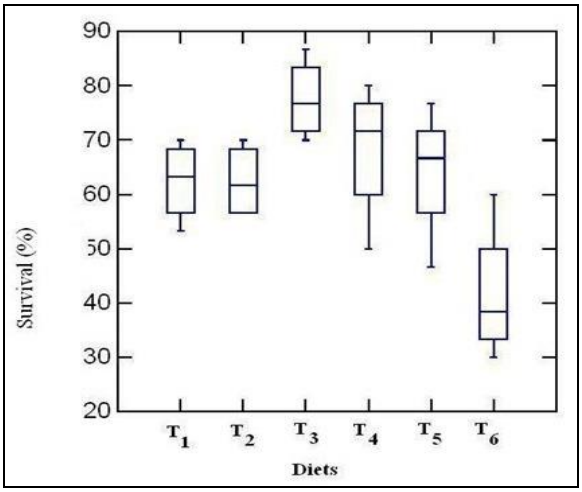

Fig 4: Survival of juveniles *A. ocellatus* fed with different diets for 60 days

Feed utilization

Feed conversion ratio (FCR)

The better feed conversion ratio (0.13 ± 0.01) for juveniles was found in diet T_3 and the highest value of feed conversion ratio (0.59 ± 0.02) was observed in diet T_2 (Table 4 & Fig. 5). One-way ANOVA showed significant difference ($P< 0.05$) in the feed conversion ratio of juveniles of *A. ocellatus* fed with different diets. The Student Newman Kuel's multiple range test (SNK) analysis revealed that the feed conversion ratio of fish fed diet T3 significantly differed (P<0.05) from fish fed other diets. However, there was no significant difference ($P> 0.05$) in feed conversion ratio of juvenile fed diets T_1, T_4, T_5, and T_6.

Table 4: Feed conversion ratios of juvenile *A. ocellatus* fed with different dietary lipid sources.

Replicates	Diets					
	T_1	T_2	T_3	T_4	T_5	T_6
Food conversion ratio (FCR)						
R_1	0.46	0.67	0.11	0.28	0.26	0.25
R_2	0.28	0.55	0.13	0.27	0.34	0.18
R_3	0.36	0.46	0.16	0.25	0.61	0.41
R_4	0.61	0.67	0.11	0.27	0.42	0.20
Mean	0.43^{ab}	0.59^{a}	0.13^{c}	0.27^{bc}	0.41^{ab}	0.26^{bc}
(±) S.E.	± 0.03	± 0.02	± 0.01	± 0.01	± 0.03	± 0.02

Values mean ± SE in the same row with different superscripts are significantly different from each other using SNK test (P<0.05)

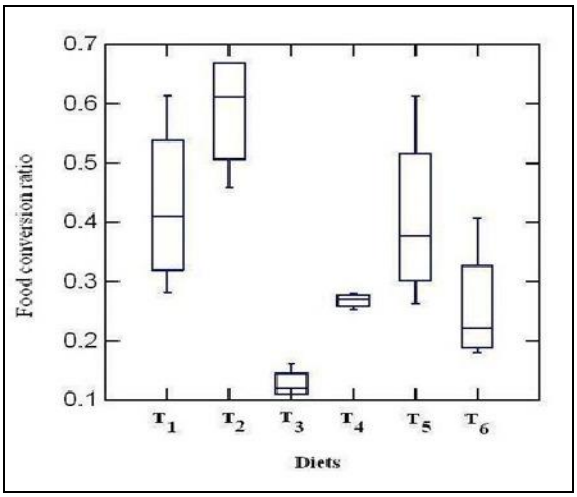

Fig 5: Feed conversion ratio of juveniles *A. ocellatus* fed with different diets for 60 days.

Protein efficiency ratio (PER)

The protein efficiency ratio of *A. ocellatus* fed with different diets was shown in Table 13. The highest value of protein efficiency ratio (20.04 ± 0.61) of juveniles of *A. ocellatus* was found in diet T_3 whereas the lowest value of protein efficiency ratio (4.36 ± 0.15) was observed in diet T_2 (Fig. 6). One-way ANOVA showed significant difference ($P< 0.05$) in the protein efficiency ratio of juveniles of *A. ocellatus* fed different diets for 60 days (Table 14). According to Student Newman Kuel's multiple range test (SNK) it was observed that protein efficiency ratio of fishes fed diet T3 was significantly higher (P<0.05) compared to that of other diets, whereas no significant difference ($P> 0.05$) in protein efficiency ratio of juvenile fed diets T_1, T_4, T_5, and T_6 (Table 5).

Table 5: Protein efficiency ratio of juvenile *A. ocellatus* fed with different dietary lipid sources.

Replicates	Diets					
	T_1	T_2	T_3	T_4	T_5	T_6
Protein efficiency ratio (PER)						
R_1	5.40	3.74	22.39	8.92	9.48	10.12
R_2	8.86	4.51	19.61	9.42	7.39	13.89
R_3	6.99	5.46	15.45	9.89	4.08	6.14
R_4	4.07	3.74	22.73	9.10	5.98	12.67
Mean	6.33^{bc}	4.36^{c}	20.04^{a}	9.33^{b}	6.73^{bc}	10.71^{b}
(±) S.E.	± 0.38	± 0.15	± 0.61	± 0.08	± 0.42	± 0.63

Values mean ± SE in the same row with different superscripts are significantly different from each other using SNK test (P<0.05)

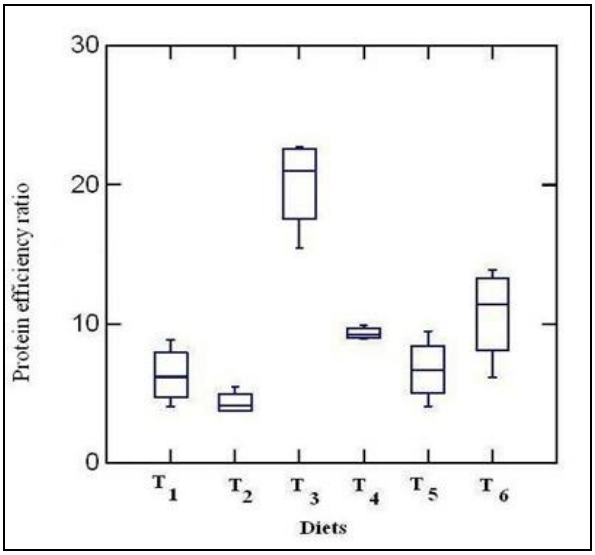

Fig 6: Protein efficiency ratio of juveniles *A. ocellatus* fed with different diets for 60 days**.**

Lipid efficiency ratio (LER)

The lipid efficiency ratio of juveniles of *A. ocellatus* was shown in Table 6. The highest value of lipid efficiency ratio (114.53 ± 0.35) was found in diet T_3 where the lowest value of lipid efficiency ratio (24.92 ± 0.09) was found in diet T_2 (Fig.7).

One-way ANOVA showed significant difference ($P< 0.05$) in the lipid efficiency ratio of juveniles of *A. ocellatus* fed with different diet. The Student Newman -Keul's test (SNK) revealed significant higher ($P<0.05$) lipid efficiency ratio of the fish fed diet T3 than the other diets. However there was no significant difference ($P> 0.05$) in lipid efficiency ratio of juvenile fed diets T_1, T_4, T_5, and T_6.

Table 6: Lipid efficiency ratio of juvenile *A. ocellatus* fed with different dietary lipid sources.

Replicates	Diets					
	T_1	T_2	T_3	T_4	T_5	T_6
Lipid efficiency ratio (LER)						
R_1	30.84	21.37	127.95	50.96	54.19	57.85
R_2	50.63	25.76	112.06	53.81	42.22	79.37
R_3	39.94	31.18	88.26	56.50	23.30	35.09
R_4	23.26	21.37	129.87	52.01	34.17	72.40
Mean	36.17^{bc}	24.92^{c}	114.53^{a}	53.32^{b}	38.47^{bc}	61.18^{b}
(±) S.E.	± 0.22	± 0.09	± 0.35	± 0.04	± 0.24	± 0.36

Values mean ± SE in the same row with different superscripts are significantly different from each other using SNK test (P<0.05)

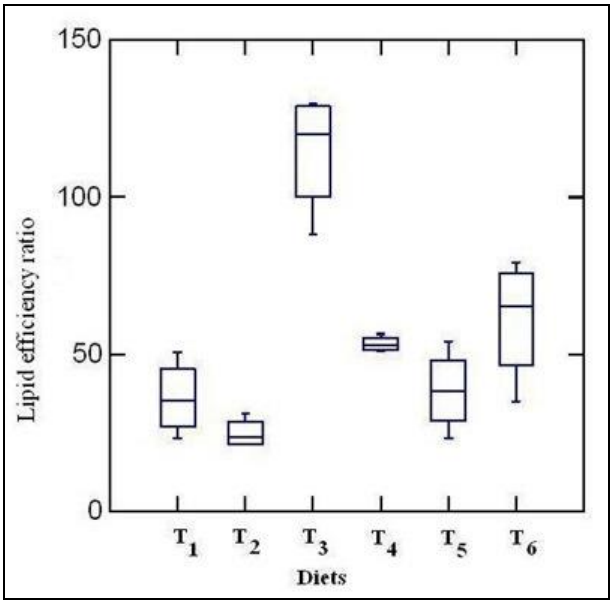

Fig 7: Lipid efficiency ratio of juveniles *A. ocellatus* fed with different diets for 60 days

Water parameters

Water parameters such as water temperature (26.14 ± 0.18 ^{0}c), pH (6.61 ± 0.03), dissolve oxygen (5.51 ± 0.10 mg L^{-1}), free carbon dioxide (1.81 ± 0.23 mg L^{-1}) and total alkalinity (40.24 ± 0.61 mg L^{-1}) recorded during rearing of *A. ocellatus* for a period of 60 days are given in Table 7.

Table 7: Water parameters during rearing of the juveniles of *A. ocellatus* for a period of 60 days

Water parameters	Ranges	Mean values (±SE)
Temperature (°C)	25 - 27	26.14 ± 0.18
pH	6 - 7	6.61 ± 0.03
Dissolved oxygen (mg L^{-1})	4 – 6	5.51 ± 0.10
Free carbon dioxide (mg L^{-1})	1 – 2	1.81 ± 0.23
Total alkalinity (mg L^{-1} as $CaCO_3$)	36 - 43	40.24 ± 0.61

Discussion

The result of present experiment showed significantly higher growth (length gain, weight gain and specific growth rate) and survival of the juveniles of *A. ocellatus* fed a diet T_3, incorporated with sardine oil and mustard oil in 1:1 (w/w) proportion as compared to other oil sources tested. Similarly the lowest values of feed conversion ratio along with maximum values of protein efficiency ratio and lipid efficiency ratio were observed with diet T_3, containing sardine oil and mustard oil. The probable reason for better growth, survival and feed efficiency may be attributed to variation in fatty acid profile of oils used. The literature reviewed with respect to fatty acid composition of different oil shows that high content of monounsaturated fatty acid and n-3, n-6 highly unsaturated fatty acid in sardine oil [33] as compared to cod liver oil [34]. The higher level of monounsaturated fatty acid in mustard oil [9] as compared to that in linseed oil and soybean oil [35] may resulted in higher growth and improved feed efficiency as monounsaturated fatty acid are readily digested and absorbed. The results of the present study are difficult to compare with those of other studies because of difference in fish species and oil sources used.

Conclusion

Results of this study, suggests that juvenile *A. ocellatus* fed diets containing vegetable oil (mustard oil) and fish oil (sardine oil) in 1:1 ratio showed better growth and feed utilization during rearing for 60 days in laboratory condition.

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