Effect of *Linum usitatissimum* and *Sesamum indicum* Extract as Growth Promotors a Diets of Fingrlings of *Ctenpharyngodon idella*

Sajid Ali[1], Muhammad Ashraf[1], Arshad Javid[1], Noor Khan[1], Hafeez ur Rehman[1], Sumaira Abbas[1], Ehsan Mehmood[2]

Abstract

A 90-day feeding trial was designed to investigate the effects of dietary supplementation of *Linum usitatissimum* seed extract and *Sesamum indicum* seed extract on hematological parameters and growth of fingerlings *Ctenpharyngodon idella*. Fish were housed in cemented rectangular tanks at stocking density of 130 mg L^{-1} of water. There were three treatments and a control group. Control diet contained 32% protein and was composed of conventional ingredients. 1 % of the total ingredients were replaced with *Linum usitatissimum* seed extract in treatment 1 and 2% with *Sesamum indicum* seed extract in treatment 2 respectively. Treatment 3 composed of 96% of control diet + 2% *Linum usitatissimum* and 2% *Sesamum indicum* seed extract. Feed was offered to fish @ 2% of its body weight. Initial and final weight of *Ctenpharyngodon idella* fingerlings were 7.33, 15.98g in control, 7.14 , 17.38 g in T_1, 7.27 , 16.49 g in T_2 and 7.30, 16.86 g in T_3 respectively. All treatments showed better growth than control but treatment composed of linseed oil in diet showed significantly higher growth. Hematological studies showed increased levels of Hemoglobin, Platelets, TLC, RBCs, HCT, WBCs, and MCH in treatments while the level of Lymphocytes & MCV decreased as compared to control diet. Fish fed diet with 2% linseed extract in feed showed highest weight gain (10.24g) best F.C.R (1.28) and good hematological results suggesting that *Linum usitatissimum* seed extract is better option to include in future feed formulations for *Ctenopharyngodon idella*.

Keywords: *Ctenopharyngodon idella*, *Linum usitatissimum*, *Sesamum indicum*, *growth*, *hematology*.

Introduction

Animal protein is an integral part of human food. Fish among other animals can be a cheaper and quality source of protein but its production is continuously declining in wild waters and is in low production in captivity. It’s well managed

[1]Faculty of Fisheries & Wildlife, University of Veterinary & Animal Sciences, UVAS Lahore, Ravi Campus Pattoki. Email: muhammad.ashraf@uvas.edu.pk
[2]Department of Fisheries Punjab, Lahore.

https://doi.org/ 10.58932/MULH0004

culture under controlled conditions can help in acheiving this shortage which is not possible without availability of cost effective feeds (Mokolensang , Yamasaki , & Onoue , 2003). Fish feeds demand fish meal due to its balanced amino acid profile and essential fatty acid contents. Lin seed or flax (*Linum usitatissimum*) is the only plant based oil that contains the higher ratio of omega-3 and omega- 6 fatty acids can replace fish meal an expensive feed ingredient to some extent.

Ctenpharyngodon idella is an herbivorous, freshwater fish species with comparatively better growth and taste. 20 cm young fish stocked spring can reach up to 45 cm by fall. The maximum length of 1.4 m and the maximum weight of 40 kg has been observed. According to one study, they live an average of five to 9 years, with the oldest surviving 11 years. They eat up to three times their own body weight daily. When the preferred food of the grass carp is not available, this fish feeds on terrestrial vegetation hanging over the surface of the water or offered from outsie. Moving toward intensive farming and for increasing the fish production, need balanced food. The quantity and quality of feed consumed have a pronounced effect on growth rate, feed conversion efficiency and chemical composition of fish (Hasan, Moniruzzaman, & Omar Farooque, 1990) and(Jena, Aravindakshan, 1998).

Whole flax seed, a terrestrial plant famous for its oil, contains 41% fats, 28% dietary fiber, 21% protein, and 3% carbohydrates having sufficient magnesium, potassium, zinc, and B vitamins with soluble (25%) and insoluble fiber (75%). Flaxseed Oil contains 73% PUFA, 18% MUFA, 9% SFA (ALA 55% of total fatty acids) and the richest source of phytoestrogen called lignans which are probably used for prevention of breast cancer (Conners, 2000).

Sesame is a flowering plant and is widely cultivated in tropical regions around the world for its edible seeds, which grow in pods. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago. It was a major summer crop in the Middle East for thousands of years, as attested by the discovery of many ancient presses for sesame oil in the region. Sesame is highly tolerant to drought like conditions, making it suitable to grow where other crops may fail. Sesame has one of the highest oil contents of any seed.

Oil bearing seeds are much higher in proteins than are the cereals seeds (Malik , et al., 2012). Sesame seed showed a high content of oil (52%), protein (24%) and ash (5%) (Mahabadi , Khodayari, Hassani Bafrani , Nikzad , & Taherian, 2013). Sesame oil comprises approximately 50% of the seed weight good type of monounsaturated and polyunsaturated fatty acids having large amounts of natural antioxidants (Choi , et al., 2004). Considering the importance of flax seed and sesame seed oils as energy and essential fatty acid source, studies were designed with following objectives;

- To study the growth performance of fish with and without plant growth promoters in diet.
- To determine the effects of above growth promoters on hematology of fish.
- To determine the effect of these additives on body composition of fish.

Materials and Methods

Experimental Site:

Studies were conducted in Fish Seed Hatchery, Department of Fisheries and Aquaculture, C-Block, Ravi Campus Pattoki. Trial was continued for 90 days. Fingerlings of grass carp, *Ctenopharyngodon idella*

Experimental design:

Studies were designed following completely randomized statistical design (CRD). There were three treatments and a control. Control group was totally free of any supplement. Detail of dietary treatments has been given in table 1 below.

Table .1: Dietary treatments

Treatments	Detail of feeds
Control	Inert/ Basal feed only(32% C.P)
Treatment # 1	Basal feed 98% + 2% *Linum usitatissimum* (linseed(flax seed)) fat soluble extract
Treatment # 2	Basal feed 98% + 2% *Sesamum indicum* (sesame seed) fat soluble extract
Treatment # 3	Basal feed 96% + 2% *Linum usitatissimum* linseed fat soluble extract + 2% *Sesamum indicum* sesame seed fat soluble extract

Fingerlings were randomly stocked in 8 rectangular fiber glass tanks with 30 fingerlings in each tank weighing 7.26 g on the average. Tanks were well cleaned before stocking and disinfected with $KMnO_4$. Tanks were randomly allotted to all the four dietary groups two per group. Each dietary group was replicated. Four rectangular cemented tanks were disinfected for fish stocking. Prepared feeds were then offered to fish to the respective dietary group. Detail of ingredients along with their composition has been given in table 2.

Feed Formulation and preparation

Feed was prepared by combining calculated amount of feed ingredients to form a mixture to meet that was made accurate to meet the specific goals of rapid growth & production. Four diets were prepared viz. Control with 32% C.P, Treatment 1 basal diet supplemented with 2% *Linum usitatissimum* (linseed) extract, Treatment 2 basal feed with 2% *Sesamum indicum* (sesame) seed extract, and Treatment 3 basal feed with 2% *Linum usitatissimum* (linseed) extract + 2% *Sesamum indicum* (sesame) seed extract. Pelleted feed was sun dried for 4 hours and then in oven for 48 hours at 100 ^{0}C. Feed formulation was done by using the "Pearson Square Method".

Table No.2: Proximate analysis of feed ingredients used in feeds

Sr. No.	Ingredients	% used in feed	Fat%	Moisture%	Protein%	Fiber%	Ash%	P %	CP contribution
1	Guar meal	40	1.85 ±0.6	5.80± 1.1	26.56± 1.5	10.94± 1.1	13.12± 6	0.68 ±0.2	10.62
2	Cotton seed meal	35	3.47 ±0.9	7.64± 19	41.31± 2.7	15.86± 0.9	12.59± 1.1	0.37 ±0.4	14.46
3	Rice polished	2.5	3.43 ±0.2	1.88± 0.4	12.04± 1.2	21.02± 3.0	07.14± 0.3	0.27 ±0.0	0.3
4	Maize gluten meal	20	6.3± 0.5	10.94 ±1.1	30.01± 3.5	16.86± 1.1	15.36± 0.9	0.56 ±0.1	6.0
5	Wheat bran	2.5	2.94 ±0.8	5.60± 0.6	14.89± 3.8	20.35± 1.6	15.02± 1.6	0.64 ±0.2	0.37
	100								31.75

Water Quality Parameters:

Water quality parameters viz., dissolved oxygen, pH, electrical conductivity and water temperature were monitored daily by using DO meter (YSI 55 Incorporated, Yellow Springs, Ohio, 4387, USA), pH meter (LT-Lutron pH-207, Taiwan) and electrical conductivity meter (Condi 330i WTW 82362 Weilheim Germany), respectively.

Statistics

Values were expressed as means ± standard error of means (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to assess statistically significant differences among different treatments. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using PASW Statistics 18.0 software (IBM SPSS Statistics, IBM, Chicago, USA).

Results

Water quality parameters

Detail of water quality parameters observed has been given in table 3 below. All the water quality parameters remained within an acceptable range. Non of them can be considered the cause of any unusual change in outcome of the research work.

Table 3: Treatment wise detail of water quality parameters observed during the course of an experiment.

Sr. No.	Parameters	Normal Values	Control	Treatment 1	Treatment 2	Treatment 3
1	DO	5-12 ppm (mg/L)	8.61± 0.02	7.84± 0.56	8.23± 0.07	8.18± 0.03
2	pH	6.5-8.5	8.04± 0.01	7.87± 0.02	8.01± 0.01	7.94± 0.06
3	Temperature	24-32 C°	30.17± 0.07	30.10± 0.10	31.19± 0.10	30.74± 0.05
4	T.D.S	50-250 mg/L	231± 6.06	211± 5.10	224± 15.07	195± 11.05
5	Salinity	< 1.00 ppt	0.88± 0.01	0.78± 0.04	0.81± 0.03	0.87± 0.08
6	E.C	0-800 µS /cm	218± 10.06	211± 4.02	219± 8.04	207± 15.01

Growth and Survival

All the fingerlings were captured on fortnight basis from each tank by nylon hand net. Fingerlings survival and morphometric characteristics viz., wet body weight gain and total length were measured and recorded individually to observe their growth under different treatments. Fish were then released back in respective tank for further rearing. Weight gain was higher in fish in T_1 which were fed with diet containing 2% *Linum usitatissimum* (linseed) fat soluble extract as growth promoter. Weight gain by fish in Treatment 2 fed on sesame extract and 3 fed with *Linum usitatissimum* +*Sesamum indicum* extract were same but slightly higher than Control (Basal feed) (Table 4).

Table 4: Treatment wise detail of weight gain observed during the course of an experiment.

Sr.#	Treatments	Initial weight (g)	Final weight (g)	Difference (g)	weight gain%
1	Control	7.33±0.2	15.98±1.1	8.65±0.3	118.00±5%[a]
2	Treatment 1	7.14±0.4	17.38±1.2	10.24±0.3	143.41±6.1%[b]
3	Treatment 2	7.27±0.3	16.49±1.0	9.22±0.3	126.82±3.9%[c]
4	Treatment 3	7.30±0.3	16.86±1.1	9.56±0.4	130.95±4.2%[c]

F.C.R & FCE (Feed Conversion Ratio, Feed Conversion Estimation)

Best FCR and FCE was observed in Treatment 1 with 2% linseed extract which was the higehst in control group. Treatment 3 seconded it which also has 2% linseed extract with 2% sesame oil extract. Control group and Treatment 2 with 2% sesame seed extract did poorly with the highest values of FCR and the lowest FCE(Table 5).

Table 5: Treatment wise detail of F.C.R. and F.C.E observed during the course of an experiment.

Sr.No.	Treatments	F.C.R	F.C.E %
1	Control	1.51±0.0[a]	66.18±0.03[b]
2	Treatment 1	1.28±0.0[c]	78.34±0.03[a]
3	Treatment 2	1.42±0.03[ab]	70.51±0.03[c]
4	Treatment 3	1.36±0.07[bc]	73.14±0.2[c]

Length Studies

Length of fish followed the same trend as was observed in weight gain and FCR values, highest in Treatment 1 and the lowest in control group without any additive (Table 6).

Table 6: Treatment wise detail of length observed during the course of an experiment.

Obs.	Treatments	Initial length (cm)	Final length (cm)	Difference (cm)
1	Control	7.56 cm	10.69 cm	3.13 cm±0.2
2	Treatment 1	8.11 cm	12.35 cm	4.24 cm±0.2
3	Treatment 2	7.24 cm	10.90 cm	3.66 cm±0.3
4	Treatment 3	7.70 cm	11.59 cm	3.89 cm±0.1

Proximate Analysis of Fish (Carcass of Fingerling *Ctenopharyngodon idella*)

Fish (Carcass) sample was proximately analyzed following (AOAC, 2003) (Table 7). Highest protein percentage and comparatively lower fat contents were observed in treatment 1 when compared to its counter parts. Other parameters fluctuated in correspondence with these values but performance of fish in treatment 1 remained superior irrespective of the parameter tested.

Table 7: Proximate Analysis of fish in different treatments.

Sr.No.	Parameters	Control	Treatment 1	Treatment 2	Treatment 3
1	Fat %	4.13 ± 04^{d}	6.17 ± 03^{d}	7.53 ± 01^{c}	10.16 ± 02^{a}
2	C.P. %	47.25 ± 06^{c}	52.50 ± 04^{a}	48.57 ± 03^{bc}	51.61 ± 03^{b}
3	Moisture%	79.36 ± 11^{b}	76.19 ± 05^{d}	78.39 ± 04^{c}	80.42 ± 03^{a}
4	D.M %	20.21 ± 02^{c}	23.54 ± 07^{a}	21.26 ± 11^{b}	19.15 ± 05^{d}
5	Fiber %	1.55 ± 01^{c}	3.84 ± 04^{a}	2.71 ± 03^{b}	2.29 ± 01^{bc}
6	Ash %	14.61 ± 06^{a}	9.39 ± 01^{d}	13.21 ± 03^{b}	12.39 ± 01^{c}

Blood Sampling

At the end of trial blood was drawn puncturing heart and gills containing EDTA to avoid blood clotting. Blood was transported to Laboratory for hematology studies.

Table 8: Hematological parameters observed among various treatments.

Sr. No.	Parameters	Control	Treatment 1	Treatment 2	Treatment 3
1	HB.	8.83± 0.52^{b}	**11.43**± 0.50^{a}	7.38± 0.25^{c}	8.98± 0.72^{b}
2	TLC	7800.00± 100.00^{c}	**10800**.00± 100.00^{a}	7689.33± 95.00^{c}	9800.00± 100.00^{b}
3	Neutrophils	20.00± 1.00^{d}	22.33± 0.57^{c}	**33.**00± 1.00^{a}	28.00± 1.00^{b}
4	Lymphocytes	**84.**00± 1.00^{a}	66.00± 1.00^{d}	71.00± 1.00^{c}	73.00± 1.00^{b}
5	Monocytes	2.33± 0.57^{b}	3.00± 1.00^{b}	**6.00**± 1.00^{a}	4.33± 1.00^{b}
6	Eosinophils	3.00± 1.00^{b}	**4.21**.00± 1.00^{a}	1.33± 0.57^{c}	3.35± 0.57^{b}
7	Platelets	198333± 577.35^{d}	234000.00± 1000.0^{b}	185333.33 ±577.4^{c}	**267000**.00 ±1000.0^{a}
8	RBCs	2.67± 0.20^{c}	3.24± 0.15^{b}	3.19± 0.20^{b}	**4.52**± 0.05^{a}
9	HCT	28.89± 0.67^{c}	**43.73±** 0.50^{a}	29.35± 0.58^{c}	34.80± 0.57^{b}
10	MCV	**103.53**± 0.57^{a}	97.03± 0.68^{b}	78.81± 0.59^{c}	80.19± 1.00^{c}
11	MCH	23.55± 0.51^{c}	**30.70**± 0.72^{a}	24.20± 0.57^{c}	25.47± 0.60^{b}
12	MCHC	26.18± 1.00^{c}	29.89± 1.00^{b}	**34.48**± 0.60^{a}	27.26± 1.10^{c}

Keywords; Hb ; Hemoglobin, TLC; Total leukocytes count, RBCs; Red blood cells (Erythrocytes), HCT or PCV; Packed cell volume or hematocrit, MCV; Mean cell volume, MCH; Mean cell hemoglobin, MCHC; Mean cell hemoglobin concentration, etc. Hb, TLC, Eosinophils, HCT, MCH were the highest in treatment

1 while other parameters fluctuated selectively among the treatments, lower in some while higher in others.

Discussion

Higher growth rates, lower FCRs, higher SGR, better protein values and lower fats were observed in fish fed on linseed oil included diets. The optimum level of dietary nutrients normally enhances growth and feed efficiency (Shiau , 1997) and so the decreased weight gain and the specific growth rate in previous studies may be due to higher energy content in the form of high carbohydrate and low lipid containing diets (Page & Andrews , 1973). Addition of lipids in present studies did improve growth and other biochemical parameters (Table 4, 5, 7 and 8). An inverse relationship between growth and dietary lipid/ energy was however, reported by (Daniels & Robinson , 1986) in juvenile red drum, *Sciaenops ocellatus*.

Knowledge of the optimal level of protein and protein-sparing effects of non-protein nutrients such as lipids and carbohydrate can be used effectively in reducing feed costs (AOAC, 2003) and improve growth which was quite obvious in current studies because lipids and specifically linseed oil did significantly ($P<0.05$) better in diets where it was included 2% of the total diet (Table 4, 5, 7 and 8). Lin (Lin, Cui, Hung, & Shiau, 1997) reported that better SGR in Magur grow-out feeding in their studies may be due to better carbohydrate and lipid utilization. Fattier nature of Magur further indicates that they may be able to better utilize lipids for growth. Levels of lipid utilization can also vary with the source of lipids utilized (Austreng, Risa , Edwards, & Hvidsten , 1977). Current studies witnessed this in the form of better growth, FCR, FCE which were observed in diet containing linseed oil compared to sesame oil or combination of both(Table 5).

Increase in weight in T_1 resulted in improvements of all the growth related parameters (Table 5). Highest FCE and lowest FCR value of 1.28 was quite astonishing which not only significantly improved growth but also cut down the feed cost to half with improvement in water quality and rise in production. Our SGR values are comparable with those of (De Silva, Gunasekera, & Atapattu, 1989). Although the carcass protein, carbohydrate, and lipid contents increased after feeding the test diet, there was no appreciable change in body composition of the following treatments. Unlike previous studies with increase in growth, there were drastic changes in protein levels. The highest protein level was observed in fish fed on linseed oil while the lowest in control group. Fats were the lowest in control and the second lowest in T_1 (Table 7).

Lipids are a concentrated and highly digestible source of energy (Habib , Hasan , & Akand , 1994) and are favored over carbohydrate as an energy source (Wee & Ng, 1986). The control diet was devoid of lipids which could not spare protein for growth and so the proteins might have been used for energy production and not for growth which might have depressed growth in previous as well as in current studies. Deposition of high lipid contents in the fishes fed higher amounts of lipid may be due to the availability of sufficient energy in those diets (Shimeno , Hosakawa, & Takeda , 1979) which was also very clear in current studies too because with the rise in lipid concentration there was proportionate rise in lipid deposition in fish. Fatty carcasses of fishes at higher dietary lipid and carbohydrate levels were also reported by Wee and Ng (1986).

The requirements of dietary lipid vary among different species according to their mode and habits of feeding. Lin (1997) reported that the capacity to utilize different lipid sources varies among fish species. Tilapia (Anderson , Jackson , Matty , & Copper , 1984), yellowtail (Furuichi & Yone, 1980) and channel catfish (Wilson & Poe , 1987) grew better when fed a lipid with enriched carbohydrate diet. On the other hand, there was no significant difference in net weight gain between lipid and starch fed white sturgeon (Hung , Fynn-Aikins, Lutes , & Xu , 1989). According to the researchers the air breathing fishes did not intake the purified diets (Qin , Arlow , Fas, Denfuda , & Weidenbach , 1997).

Martino (2002) reported in Surubim, a carnivorous freshwater fish in Brazil, that fish weight increased with dietary lipid although many species like salmonids, sea bass or rainbow trout, where a protein sparing effect of lipids has been well demonstrated (Lee & Putnam , 1973) an increase in dietary lipid level from 40 to 120 g per kg does not appear to improve protein utilization in grass carp with no clear protein sparing effect of dietary lipid. Our results were not only quite different but considerably better than previous studies, wherever, linseed oil was added to feed (Dias, Alvarez , Diez , Arzel , & Corraze , 1998). Addition of sesame oil not remained much behind but tried to approach linseed oil (Table 3, 4, 7 and 8).

Peres & Oliva-Teles (1999) believed this lack of protein sparing effect by dietary lipid may be related to the high protein level of the diet and according to Dias *et al*. (1998), the beneficial effects of an increase in lipid level from 100 to 180 g per kg in sea bass diets were significant only with a low protein diet, but not with a high protein diet. Our studies are in close agreement with those of previous studies and further affirm that even in high protein diets, the protein sparing effect of lipid is possible within a low upper limit. This was further proved by the lowest protein retention in the lipid-free diet group.

The significant decreased lipid retention with the increased dietary lipid levels, suggest an increased proportion of lipid used for energy. This agrees with Cho and Watanabe (1985) who observed in rainbow trout, that the highest lipid diet did not promote the highest lipid retention. Peres & Oliva-Teles (1999) also reported decreasing lipid retention when dietary lipid increased from 120 to 300 g per kg. The relationship of body lipid content with protein and moisture contents is a common phenomenon in fishes, and our results are comparable to those of Peres & Oliva-Teles (1999). Köse and Yildiz (2013) replaced fish oil with sesame oil in combination with other vegetable oils in diets with regard to growth performance, feed utilization, desaturation and elongation, whole fish and liver fatty acid profiles of juvenile rainbow trout.

Sesame oil (SO) used in the feeds was a mixture of linseed (LO), sunflower (SFO) and fish oil (FO), whereas the control diet contained only FO. Duplicate groups of 60 rainbow trout (~7 g) held under similar culture conditions were fed 2% of their body weight per day for 75 days. At the end of feeding trials, there was no difference in feed utilization efficiency or growth performance between the control group and the groups with added sesame oil ($P > 0.05$). However, viscero-somatic and hepato-somatic index values were significantly higher ($P < 0.05$) in fish fed with FO30/SO35/SFO35 diets.

Results showed that total body lipid levels of fish fed diets containing sunflower oil were higher than in the other experimental groups ($P < 0.05$). However, crude lipid levels were similar in fish fed the control diet and the diet with sesame oil (FO30/LO35/SO35), which is sunflower oil-free. Crude lipid levels of fish livers were not influenced by the diets ($P > 0.05$). Diets with sesame oil increased desaturation and elongation of 18: 3n-3 towards n-3 HUFA. They reported that addition of sesame oil in combination with other vegetable oils increased the nutritional quality of the whole fish and liver of juvenile rainbow trout, in particular the docosahexaenoic acid (DHA) level indicating usefulness of sesame oil in aquaculture diets.

Similarly Castro, et al., (2015) weighed up the impact of partial (70%) and complete (100%) dietary replacement of fish oil (FO) by linseed oil (LO) on sensory and quality attributes during the edible shelf life of gilthead sea bream (*Sparus aurata*). Physico-chemical parameters (pH, torrymeter, total volatile basic nitrogen, thiobarbituric acid reactive substances and texture), and sensory analysis, both in cooked and raw fish were carried out during 17 days of ice storage. Throughout ice storage, feeding with LO diets, TBARS values remained lower on muscle than those found when feeding FO control diet. On freshly caught fish (day 0 of ice storage), statistically significant dietary texture variations were recorded on cooked fillet fed FO diet. No sensory differences on Quality Index Method, sensory profile or Torry scheme were found with partial or total LO replacement diets.

The feed efficiency in terms of Feed Conversion Ratio (FCR) recorded as 2.46 to 3.22 among all the feeding trials. It could be concluded, based on the results of this trial, that a diet formulated with a gross energy of 19.55 kJ/g is sufficient to promote better feed efficiency and growth performance in *C. batrachus* grow-out however, the best growth was recorded in linseed oil (LINOL) followed by beef tallow(BETAL) and mix oil(MIXOL). Though sensory parameters were not taken into consideration but better body composition witnesses its better sensory qualities which was apparent in current results (Table 4, 5 and 7).

Similarly FCR values were significantly higher than observed in previous studies and were really quite unexpected like 1.28 one of the best value in fish feed with 2% linseed oil (Table 5). Begum and Navaraj (2012) used the mixture of two plant extracts (*Solanum trilobatum* and *Ocimum sanctum*), extracted with 70% ethanol and screened for their antimicrobial activities against *Aeromonas hydrophila*, a bacterial pathogen. Using disk diffusion assays, equal proportions of plant mixture extract (mixture of *Solanum trilobatum, Ocimum sanctum* in 1:1 proportion) were mixed thoroughly with the artificial feeds at concentrations of 0.0 (A), 3 (B), 30(C), 300(D) mg kg^{-1} of dry diet. The prepared diets were fed to healthy *Mystus keletius* for 60 days and then challenged with *A. hydrophila*.

The hematological, biochemical and immunological parameters of fish were investigated at 20, 40 and 60 days of feeding and also on 10th day post challenge in order to evaluate the immune response and resistance against *A. hydrophila* infection of fish. Results indicated that respiratory burst activity, serum bactericidal activity, lysozyme activity, serum protein, albumin, globulin, WBC, RBC and haemoglobin content were enhanced ($p<0.05$) in fish fed herbal diets compared to the control group. On 10 days post-challenge, the total survival rates were 38.09% in control group (A) and 72.15%, 80.95%, 85.71% in groups B, C, D respectively. Among different groups, D generally showed the best performance in the experiment.

Further research is needed to isolate and characterize the active compounds from these plants. Effects of linseed oil are very much obvious though we did not attempt the challenge tests but we did analyze the hematological parameters which also remained superior in linseed oil than sesame oil or combination of both (Table 8). It appears that linseed oil also contribute significantly to the immunological capabilities of the animal but sesame oil do has antagonistic effects when combined with linseed oil which demand further studies.

References

Anderson , J., Jackson , A., Matty , A., & Copper , B. (1984). Effects of dietary carbohydrate and fiber on the tilapia *Oreochromis niloticus* (Linn.). *Aquaculture Journal, 37*(4), 303-314.

AOAC. (2003). Association of Official Analytical Chemists. In K. Helrich, *Official Methods of Analysis of the Association of Official Analytical Chemists* (15 ed.). Arlington, Virginia,.

Austreng, E., Risa , S., Edwards, D., & Hvidsten , H. (1977). Carbohydrate in rainbow trout diets. II. Influence of carbohydrate levels on chemical composition and feed utilization of fish from different families. *Aquaculture Journal, 11*(2), 39-50.

Begum, S., & Navaraj, P. (2012). Synergistic effect of Plant Extracts Supplemented Diets on Immunity and Resistance to *Aeromonas hydrophila* in *Mystus keletius*. *Journal of Pharmacy and Biological Sciences, 2*(4), 30-36.

Castro,Castro, PL., Caballero, MJ. Gines, R., Pene, Castro, P., Caballero, M., Gines, R., & Penedo, J. (2015). Linseed oil inclusion in sea bream diets: effect on muscle quality and shelf life. *Aquaculture Research, 46*(2), 75–85.

Cho , C., & Watanabe , T. (1985). Dietary energy and lipid requirements of rainbowtrout (*Salmo gairdneri*) at different water temperature. *Energy Metabolismof Farm Animals, 32*(2), 206–209.

Choi , S., Wang , X., Park, G., Lim, S., Kim, K., Bai, S., & Shin, I. (2004). Dietary dehulled soybean meal as are placement for fish meal in fingerling and growing olive flounder Paralichthys olivaceus (*Temminck et Schlegel)*. *Aquaculture Research, 35*(3), 410-418.

Conners, W. (2000). Importance of n-3 fatty acids in health and disease. *The American Journal of Clinical Nutrition, 71*(1), 171-175.

Daniels, W., & Robinson , E. (1986). Protein and energy requirements of juvenilered drum (*Sciaenops ocellatus*). *Aquaculture journal , 53*(2), 243-252.

De Silva, S., Gunasekera, R., & Atapattu, D. (1989). The dietary protein requirements of young tilapia and an evaluation of the least cost dietary protein levels. *Aquaculture Journal , 80*(2), 271- 284.

Dias, J., Alvarez , M., Diez , A., Arzel , J., & Corraze , G. (1998). Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European seabass (*Dicentrarchus labrax*). *Aquaculture Journal, 161*(4), 169–186.

Furuichi, M., & Yone, Y. (1980). Effect of dietary dextrin levels on the growth and feed efficiency, the chemical composition of liver and dorsal muscle, and the absorption of dietary protein and dextrin in fishes. *Bulletin of the Japanese Society for the Science of Fish, 46*(2), 225-229.

Habib , M., Hasan , M., & Akand , A. (1994). Dietary carbohydrate utilization by silver barb *Puntius gonionotus*. In D. S. Silva, *Asian Dishes Soc Spec Publ.Asian Fisheries Society.* (pp. 57-62). Manila, Philippines.

Hasan, M., Moniruzzaman, M., & Omar Farooque, A. (1990). Evaluation of leucaena and water hyacinth leaf meal as dietary protein sources for the fry of Indian major carp, *Labeo rohita* (Hamilton). In R. Hirano, & I. Hanyu, *The Second Asian Fisheries Forum* (pp. 275-278). Manila: Asian Fisheries Society.

Hung , S., Fynn-Aikins, K., Lutes , P., & Xu , R. (1989). Ability of juvenile white sturgeon (*Acipenser transmontanus*) to utilize different carbohydrate sources. *Journal of Nutrition, 119*(2), 727-733.

Jena , J., Aravidakshan, P., Suresh, C., Muduli, H., Ayyappan, S., & Chandra, S. (1998). Comparative evaluation of growth and survival of Indian major carps and exotic carps in raising fingerlings. *J. Aquacult. Tropics, 13*(2), 143-149.

Köse, I., & Yildiz, M. (2013). Effect of diets containing sesame oil on growth and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology, 29*(6), 1318-1324.

Lee , D., & Putnam , G. (1973). The response of rainbow trout of varying protein energy ratios in a test diet. . *Journal of Nutrition, 103*(2), 916–922.

Lin, J., Cui, Y., Hung, S., & Shiau, S. (1997). Effect of feeding strategy and carbohydrate source on cabohydrate utilization by white sturgeon (*Acipenser transmontanus*) and hybrid tilapia (*Oreochromis niloticus x O. aureus*). *Aquaculture Journal , 148*(2), 201-211.

Mahabadi , A., Khodayari, M., Hassani Bafrani , H., Nikzad , H., & Taherian, A. (2013). Effect of Diet Contains Sesame Seed on Epididymal Histology of Adult Rat. *The International Journal of Morphology*, *31*(1), 264-270.

Malik , K., Pervaiz , K., Khansa , L., Rashid , F., Nisa, F., & Naz, S. (2012). Effect of feeding rapeseed meal on the liver weight and hepato-somatic index (HIS) content of liver of Japanese quail. *African Journal of Microbiology Research, 6*(9), 19-28.

Martino , R., Cyrino , J., Portz , L., & Trugo , L. (2002). Effect of dietary lipid level on nutritional performance of the surubim, *Pseudoplatystoma coruscans*. *Aquaculture Journal, 209*(3), 209–218.

Mokolensang , J., Yamasaki , S., & Onoue , Y. (2003). Utilization of Shochu distillery by-products for culturing the common carp *Cyprinus carpio L. rohita*.*Journal of Biological Sciences, 3*(5), 502-507.

Page, J., & Andrews , J. (1973). Interactions of dietary levels of protein and energy on channel catfishes (*Ictalurus punctatus*). *Journal of Nutrition, 103*(3), 1339-1346.

Peres , H., & Oliva-Teles , A. (1999). Effect of dietary lipid level on growth performanceand feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture Journal , 179*(3), 325–334.

Qin , J., Arlow , W., Fas, Denfuda , D., & Weidenbach , R. (1997). Growth and survival of larval Magur (*Clarias batrachus*) fed different diets. *Aquaculture Journal , 198*(3), 105-113.

Shiau , S. (1997). Utilization of carbohydrates in warm water fish-with particular reference to tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture journal, 151*(4), 79-96.

Shimeno , S., Hosakawa, H., & Takeda , M. (1979). The importance of carbohydrate in the diet of a carnivorous fishes. In J. Halver , & K. Tiews, *World Symposium on Finfish Nutrition and Fish food Technology* (Vol. 1, pp. 127-143). Hamburg, Heeneman, Berlin.

Wee , K., & Tacon , A. (1982). A preliminary study on the dietary protein requirement of juvenile Magur. *Bulletin of the Japanese Society for the Science of Fish., 48*(2), 1463-1468.

Wee, K., & Ng, L. (1986). Use of cassava as an energy source in a pelleted feed for the tilapia, *Oreochromis niloticus*. *Aquaculture Research*, *17*(2), 129-138.

Wilson , R., & Poe , W. (1987). Apparent inability of channel catfish to utilize dietary mono- and disaccharides as energy sources. *Journal of Nutrition*, *117*(5), 280-285.