Effect of Hemp Seed Oil and Hemp Seed on Proximate Composition, Growth Performance, and Growth Gene of Freshwater Fish *Cirrhinus mrigala*

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Abstract

The present study is conducted to analyze the nutritional effect of hemp seed oil and hemp seed supplemented in feed on proximate composition, growth performance and Insulin-like growth Factor-I of Mrigal (Cirrhinus mrigala) for fifty days. For this purpose, fingerlings of Mrigal are maintained on hemp supplemented in diet of hemp seed oil (HO) at three concentration (1, 2 and 3%), and hemp seed (HS) at three concentration (5, 10 and 15%) for fifty days. At end of experimental trail moisture contents are significantly higher in control, ash contents are in hemp seed (HS15 %,) crude protein are in hemp seed (HS10%) and hemp seed (HS15%), ether extract in hemp seed (HS15%) and energy content in (HO3%), and (HS15%) in comparison to control and others hemp feeding groups. Similarly, hemp supplementation also showed positive effect on final body weight (FBW), percentage weight gain (PWG), specific growth rate (SGR) and feed conversion efficiency (FCR), on fingerlings of Mrigal. Insulin-like Growth factor-I (IGF-I) in liver and muscle tissues of fingerlings of Mrigal resulted significantly higher in all hemp treated groups in comparison to control. Hemp seed showed better effect in comparison to hemp seed oil, however, percent inclusion of hemp seed (HS15%) had shown a better result in comparison to control and other percentage inclusion of hemp seed and hemp seed oil. Hempseed products could be recommended as a feed ingredient for fish for enhancing the nutritional values of fish, which in turn can have a good impact on consumer health.

Keywords*:* Hempseed; Oil; Proximate Composition; Growth Performance; Growth Gene; Mrigal.

Introduction

The nutritional requirement of human beings is increasing with increasing population. The proper utilization of aquaculture can satisfy nutritional demands of human beings. Aquaculture provide us white meat which are an important source of protein, vitamins, essential fatty acids,

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and minerals (FAO, 2010), and being rich in omega-3 fatty acids, lowers the risk of heart diseases thus increases life expectancy (Abbas et al*.,* 2010). In aquaculture, diet plays a significant role in growth and development of fish. In Aquaculture good quality of feed are being considered in fish nutrition aiming to improve fish growth, improve feed efficiency, maintain health status, stress tolerance, and resistance to diseases (Oliva-Teles, 2012). However, inappropriate diets not only decrease feed efficiency but exposing fish to certain diseases, and also bring several defects and deficiency symptoms such as pathological alterations, vitamin scarcities, behavioral changes, and produce harmful interactions amongst feed nutrients (Puangkaew et al., 2004).

Feed plays a substantial role in increasing the survival and growth rate by increasing the feed conversion efficiency of fish (Hassan et al., 1996; Hassan et al., 2009). Generally, formulated feed have more ingredients by adding them essential nutrients such as essential amino acids, fatty acids, minerals, and vitamins, which can stimulate the immune system, raise growth proportion, and help to prevent diseases (Landolt, 1989). Percentile of crude protein and other ingredients are considered most important for the preparation of artificial feeds (Galtin, 2002; Bhosale et al., 2010). Diets fortified with specific nutrients (amino acids, essential fatty acids, vitamins, and minerals) at required levels may improve health condition and disease resistance (Alagawany et al., 2021).

In a natural environment, a vast variety of food is available for survival and growth of fish, but the problem occurs when the fish is reared under intensive culturing system where fish is reared on prepared feeds (Tacon and De Silva, 1997). The prepared and formulated feeds play a central role in increasing growth, and survival rate by improving the feed conversion efficiency (FCE %) of fish and also enhancing fish meat quality by affecting its chemical composition. The nutritional quality is optimized by using prepared feeds (Hassan, 1996; Jena et al., 1998).

Marijuana or Indian hemp (*Cannabis sativa* L) is considered as a nutritional and medicinal plant for thousands of years (Callaway, 2004). Hemp seed possessing appropriate amount of protein, carbohydrates and oil, 20−30%, 25−35%, 10−15% respectively. Hemp seed contains a rich set of minerals such as P, K, Mg, Ca, Fe, Na, Mn, Zn and Cu (Borhade, 2013; Isahq et al., 2015), and has elevated levels of vitamins A, C, E, β-carotene, Thiamine (B1), and Riboflavin (B2) (Orhan et al., 2000).

Insulin-like growth factor-I (*IGF-I*) is a polypeptide hormone that shows a similar molecular structure to insulin and plays a significant role in the growth and development of vertebrates (Wood et al., 2005; Patel et al*.,* 2005). IGF-1 plays a significant role in the complex system of the body by regulating several important functions such as growth, reproduction,

inhibits apoptosis, and selectively promotes mitogenesis and differentiation (Reinecke and Collet, 1998). Remarkably keeping in view different properties of hemp like pharmacological activities (Callaway, 2004; Whiting et al., 2015), therapeutic role and ameliorative effect of hemp (Afridi et al., 2024; Afridi et al., 2024), antibacterial (Ali et al., 2012), antifungal, and anti-leishmanial activity (Radwan et al., 2009). Thus, the present study is formulated to analyze the beneficial effect of hemp seeds oil and hemp seeds supplemented diet on proximate composition, growth performance, and growth gene of fingerlings of Mrigal *(Cirrhinus mrigala)* under controlled conditions.

Materials and Methods

Preparation of Hemp Supplemented Feed

Test Animals

Fingerlings of Mrigal are obtained by conducting induced breeding experiments at the Fisheries and Aquaculture research station of Quaid-i-Azam University Islamabad. After yolk sac absorption, swim-up fry of Mrigal are shifted to earthen nursery ponds, then to rearing ponds for rearing up to fingerling stage. Mrigal fingerlings having average weight 40.64 ± 2.39 g, and average length of 19.46 ± 0.68 cm are selected and initially stocked separately in well-aerated circular fiberglass tanks (8000 L) to acclimatize in laboratory condition for a week.

Feeding Experiments

A completely randomized experiments in a replicate of three are conducted under laboratory condition. First uniform sized fingerlings of *Cirrhinus mrigala* are transferred from fiberglass tank to 21 glass aquaria (120 × 60 × 60 cm), well equipped with water heaters for temperature regulation and air stones connected to aerators. They are stocked at a stocking density of 2 g/L (15fingerlings/aquarium) following protocol of (Afridi et al., 2019). The water temperature is set at 26.5°C throughout the experiment and fish are acclimatized again for 3 days in settled aquaria. During acclimatization and experimental period, they are fed control diet twice a day (at 9:00 hrs and 16:00 hrs). They are offered their respective feed of hemp seed oil and hemp seed for 50 days at the rate of 5% body weight. After every 48 hrs, uneaten feed and fecal matter from each aquarium is removed through siphoning, water quality parameters; are checked regularly before and during the bioassay tests**.** Before the end of the experiment, the fish are starved for 24 hrs. Fish are captured from each aquarium, anesthetized with buffered MS222 (10 mg/L), decapitated, and

two tissues of muscle and liver are collected for analysis of proximate composition and Insulin-like growth Factor-I and stored at -80°C.

Proximate Composition

The moisture, dry matter, ash content, crude protein, crude fats, and gross energy are determined in control and hemp supplemented feeding groups using 20 gm muscle tissues of Mrigal fingerling per the methodology of AOAC (2000) from Veterinary Research Institute (VRI) Peshawar. The moisture content is determined by weighing the initial wet weight of a fresh sample then dried it in the oven at 45 ºC for 72 hrs until gaining a constant weight. Crude protein is determined by nitrogen determination using the Kjeldahl micro method (Sutharshiny and Sivashanthini, 2011), and conversion of nitrogen to protein by multiplying with the factor 6.25. The lipid content is determined with the help of the soxhlet apparatus using the non-polar organic solvent hexane. For ash contents, a prewashed crucible is placed in a muffle furnace at 100ºC for an hour. After cooling and weighing, 2 g sample is placed in the crucible and again placed in a muffle furnace at 550 ºC for 5 hrs. Later on, it is again placed in a desiccator, and is weighed quickly after cooling to prevent moisture absorption. The gross energy is measured through bomb calorimeter in (cal/g).

Growth Parameters

At the end of feeding trial, fish are weighed individually again for final body weight to assess their growth performance. The Percentage weight gain (% WG), Specific growth rate (SGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE) is calculated by the following methods described by Wu et al., (2012). The growth performances of fish are assessed by using the following formulas.

$$\text{Percentage weight gain} = \frac{W_f - W_i}{W_i} \times 100$$

Where, W_f = Final weight of fish and W_i = Initial weight of fish.

$$\text{Specific growth rate} = \frac{(\text{In Final weight of fish}) - (\text{In Initial weight of fish})}{\text{Total experimental days}} \times 100$$

$$\text{Food conversion ratio} = \frac{\text{Total diet consumed (g)}}{\text{Total wet weight gain (g)}}$$

$$\text{Feed conversion efficiency} = \frac{1}{\text{FCR}} \times 100$$

Gene Expression

Tissues Collection and Preparation

The desired tissues (muscle and liver) are Preserved in 2 ml RNA free eppendorf tubes or cryo tubes using RNA later solution in such quantity to completely sink the whole tissues sample and preserved these samples at -80Cº. Samples are transported to Marine Toxicology and Fish Physiology Laboratory at the Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami Florida, the United States of America through FedEx courier. The growth gene (insulin-like growth Factor-I) (IGF-I) in the liver and muscle tissues of Mrigal are analyzed with help of qPCR.

RNA Extraction and qRT-PCR Analysis

RNA is extracted from liver and muscle tissues following the manufacturer's protocol. Tissue homogenization is performed in a safety cabinet using RNA stat, chloroform, and isopropanol under controlled conditions. After precipitation and washing with 75% ethanol, RNA is dissolved in nuclease-free water. RNA purity and concentration are measured using a Nanodrop spectrophotometer.

For purification, 10 ng RNA is treated with TURBO DNase, followed by inactivation and centrifugation to isolate RNA. RNA concentrations are re-quantified and stored at -20°C or -80°C. Purified RNA (200 ng/µL) is used for cDNA synthesis with the High Capacity cDNA Reverse Transcription Kit. Gene expression is quantified using Power SYBR® Green Master Mix on a Bio-Rad iCycler-MyIQ platform. qPCR reactions (12 µl total) included 6.0 µl of master mix, 360 nM primers, 50 ng cDNA, and molecular-grade water. Relative transcript levels are calculated using the comparative Ct method, normalized to actin gene expression, and analyzed using LightCycler 4.1 software. Four technical replicates per sample are performed, and data are expressed as mean fold change ± SEM.

Statistical Analysis

All data are expressed as mean ± SEM. Significant differences in proximate composition, and growth performance of Mrigal fingerlings between control and hemp experimental fed groups are identified by using one-way ANOVA. Once significant differences are identified, then the comparison among the means is evaluated by tukey HSD post hoc test using SPSS. For gene expression, qRT-PCR outcomes are expressed as mean fold change ± SEM. The P-values less than 0.05 are considered to be statistically significant.

Results

The results of present studies demonstrate the nutritional effects of graded levels of hempseed oil and hempseed supplemented in diet for fifty days on proximate composition are shown in (Table, 1), growth performance in (Table, 2), and Insulin-like Growth Factor-I (IGF-I) in the liver in (Graph, 1) and muscle in (Graph, 2) of fingerlings of *Cirrhinus mrigala.*

Table 1: Showing effect of hemp seed oil (HO) and hemp seed (HS) on proximate composition of Mrigal (Cirrhinus mrigala) fingerling after feeding hemp supplemented diet for fifty days.

Proximate	Control	Hemp seed oil (%)			Hemp seed (%)		
		HO 1%	HO 2%	HO 3%	HS 5%	HS 10%	HS 15%
Moisture	76.33 ±2.66a	75.0 ±4.35ab	72.66 ±2.90ab	72.66 ±3.84ab	74.00 ±3.60ab	68.33 ±2.02b	70.33 ±2.02ab
Ash	13.42 ±0.38ab	11.45 ±0.98bc	13.87 ±0.50ab	14.53 ±0.85ab	11.09 ±0.98bc	11.87 ±1.05bc	16.70 ±0.48a
Crude protein	58.17 ±0.90ab	55.09 ±1.57bc	56.02 ±1.20ab	57.99 ±0.99ab	58.30 ±1.78ab	60.12 ±0.93a	62.35 ±1.05a
Ether extract	9.72 ±1.64c	8.71 ±1.26c	12.76 ±0.56ab	7.61 ±0.60c	9.90 ±0.84c	11.49 ±0.67bc	13.71 ±0.58a
Energy	4846.50 ±5.15ab	4763.2 ±12.89b	4845.74 ±6.77ab	4895.61 ±2.52a	4762.5 ±17.14b	4855.59 ±5.59ab	4906.63 ±6.70a

Data presented as Mean ± S.E. Means with different superscripted letters are significantly different at $P < 0.05$ (ANOVA followed by HSD post hoc test). Control= basal diet; HO1%= 1% hemp seed oil, HO2%= 2% hemp seed oil, HO3%= 3% hemp seed oil: HS5%= 5% hemp seed, HS10%= 10% hemp seed, HS15%= 15% hemp seed.

Proximate Composition

The positive effect of hemp supplemented diet in graded level on proximate composition of Mrigal fingerlings are shown in Table 1. In Mrigal, the moisture contents are significantly higher in control, ash contents in hemp seed HS15%, crude protein in hemp seed (HS10%) and hemp seed (HS15%) and ether extract in hemp seed (HS15%) and energy content in (HO3%) and (HS15%), in comparison to control and other hemp treated group. Analysis of one-way ANOVA followed by Tukey's HSD post hoc test, showing positive effects of graded levels of hemp-supplemented feed on proximate composition of Mrigal fingerling. Hemp seed showed better effect in comparison to hemp seed oil, however, percent inclusion of hemp seed (15%) had shown a better result in comparison to other hemp seed and hemp seed oil groups.

Table 2: Showing effect of hemp seed oil (HO) and hemp seed (HS) on growth performance of Mrigal (Cirrhinus mrigala) after 50 days hemp feeding in graded level.

Parameter	Control	Hemp seed oil (%)			Hemp seed (%)		
		HO 1%	HO 2%	HO 3%	HS 5%	HS 10%	HS 15%
W_i (g)	41.24 ±2.69[a]	41.98 ±2.09[a]	42.23 ±2.85[a]	42.03 ±4.51[a]	40.78 ±2.28[a]	41.01 ±3.28[a]	42.60 ±3.17[a]
W_f (g)	45.42 ±1.17[c]	47.61 ±1.45 [b]	49.78 ±0.80 [a]	48.87 ±1.74 [a]	47.19 ±1.57[b]	49.92 ±1.51 [a]	50.69 ±0.52[a]
%WG	15.56 ±1.89[b]	16.91 ±1.52[ab]	18.67 ±2.41 [a]	19.83 ±0.67 [a]	20.25 ±1.45[ab]	21.82 ±21.75 [a]	21.73 ±0.93[a]
SGR (%)	12.22 ±2.12c	13.15 ±1.08ab	15.32 ±2.11 [a]	13.88 ±.43 [ab]	13.16 ±1.05b	15.02 ±.033 [a]	18.00 ±0.51[a]
FCR	3.99 ±1.14a	3.67 ±0.58[b]	3.87 ±0.84[bc]	2.98 ±0.61[bc]	3.66 ±0.47[ab]	2.89 ±0.68[b]	2.34 ±0.12[c]
FCE (%)	26.1 ±0.38[c]	28.15 ±0.99[ab]	31.67 ±0.52[a]	31.87 ±0.32[a]	30.32 ±0.62[ab]	33.18 ±0.31[ab]	35.02 ±0.78[a]

Data presented as Mean ± S.E. Means with different superscripted letters are significantly different at $P < 0.05$ (ANOVA followed by HSD post hoc test). Control= basal diet; HO1%= 1% Hemp seed oil, HO2%= 2% Hemp seed oil, HO3%= 3% Hemp seed oil: HS5%= 5% Hemp seed, HS10%= 10% Hemp seed, HS15%= 15% Hemp seed.

Growth Performance

The beneficial effect of hemp supplemented in feed i.e., hemp seed oil (HO: 1 %, 2%, 3%) and hemp seed (HS: 5%, 10%, 15%) on growth performance of Mrigal fingerlings are shown in Table 2. Results validates positive and significantly ($P < 0.05$) effect of hemp supplemented in diets on growth performance in comparison to control group of Mrigal fingerlings for fifty. No mortality of *C. mrigala* is observed during the feeding trial. Fish fed with the experimental diets exhibited higher growth parameters including %WG; SGR (%); FCE (%) of *C. mrigala* after 50 days hemp feeding in graded level. At the end of feeding trial, significantly higher ($P < 0.05$) final body weight (50.69±0.52 g) and %weight gain (21.73±0.93 %) is observed in group of fish fed with hemp seed (HS 15%) group as compared to control given basal diet (W_f 45.42±1.17 g; %WG, 15.56±1.89 %). The final body weight and % weight gain of Mrigal is significantly higher at end of feeding experiment. The final body weight ($W_{f)}$ (g) and %WG values showed a similar trend and significantly higher ($P < 0.05$) of group of fish fed with hemp seed oil and hemp seed in comparison to control group. The trend of SGR (%) is observed significantly higher ($P < 0.05$) in hemp seed oil (HO2%) and hemp seed (HS10%; HS15%) in comparison to control group of basal diet. Feed conversion factor is significantly higher ($P < 0.05$) in control group of basal diets in comparison to all other hemp feeding group. Feed conversion efficiency (FCE %) showed similar trend to final body weight ($W_{f)}$ (g) and %WG values. However, FCE % significantly lowest is

observed in control group of basal diet in comparison to all hemp feeding group. Significantly similar variation are observed in hemp seed oil (HO2%; HO3%) hemp seed oil (HS15%), while two group hemp seed oil (HO1%) and hemp seed (HS 10%), showed similar significant variation in comparison to control. Following one-way ANOVA followed by Tukey's HSD post hoc test, reveal significant and positive effect of hemp supplemented feed on growth performance of Mrigal after fifty days hemp supplementation. Hemp seed showed better effect in comparison to hemp seed oil.

Gene Expression

The nutritional effect of hemp supplemented in diets on Insulin-like growth factor-I (IGF-I) in the liver and muscle in of fingerlings of Mrigal as shown in Figures 1 and 2. Insulin-like Growth Factor-I (IGF-I) resulted significantly changed ($P < 0.05$) in the liver of all hemp treated group in comparison to control group of Mrigal fingerling. The Insulin-like Growth Factor-I (IGF-I) significantly highest ($P < 0.05$) in hemp seed (HS15%), and lowest is recorded in control group in the liver of Mrigal.

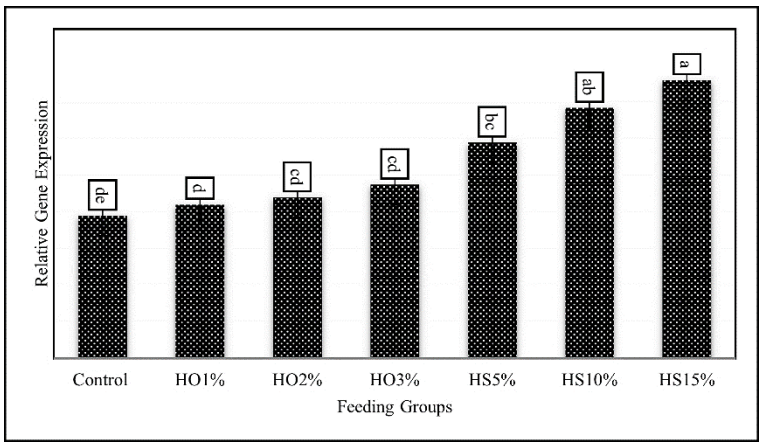

Figure 1: Relative gene expression of Insulin-like growth Factor-I (IGF-I) in control and hemp treated groups in liver of Mrigal fingerling after fifty days hemp feeding in graded level.

Insulin-like Growth Factor-I (IGF-I) resulted significantly changed ($P < 0.05$) in muscle in all hemp treated group in comparison to control of Mrigal fingerling. Similarly, in muscle tissues, growth gene is significantly ($P < 0.05$) up regulated in hemp seed (HS15%) in comparison to control group of fingerlings of Mrigal. However, hemp seed 15% (HS15%) resulted significantly ($P < 0.05$) up regulated in comparison to

control and all hemp treated groups. Analysis of one-way ANOVA followed by Tukey's HSD post hoc test, growth gene is up regulated in hemp feeding group in comparison to control, while 15% inclusion shown better effect as compared to other hemp feeding of hemp seed oil in liver and muscles tissues.

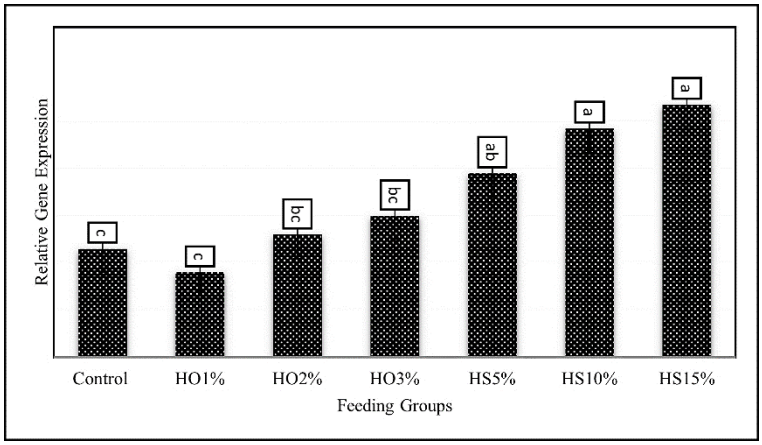

Figure 2: Showing relative Gene expression of Insulin-like growth Factor-I (IGF-I) in control and hemp treated groups in muscle of Mrigal fingerling after fifty days hemp feeding in graded level.

Discussion

World are facing hunger, malnutrition, and nutrient deficiency due to global population expansion. Aquaculture has the potential to significantly contribute good quality of food by providing freshwater and marine fish. (Pradeepkiran, 2019; FAO, 2010). Fish contains better dietary requirements of amino acids as compared to meat, milk and egg, and being rich in polyunsaturated fatty acids, particularly omega-3 (eicosapentaenoic and docosahexanoic acids) (Olomu, 1995), which lowers the risk of heart diseases thus increases the life expectancy (Abbas et al., 2010).

In this study fingerlings of Mrigal are maintained on two types of hemp (*Cannabis sativa*) supplemented feed, either on graded levels of hemp seed oil (HO) and hemp seed (HS) for 50 days. This study report for first time the beneficial impacts of hemp supplemented in diets on proximate composition, growth performance and growth gene of a freshwater fish Mrigal. Results showing that hemp feeding has significantly increased ($P < 0.05$) contents of ash, crude protein and ether extract in muscle tissues of Mrigal fingerlings after 50 days hemp feeding. We can suggest that hemp feeding enhanced the quality of fish meat by affecting its chemical composition. To determine proximate composition

of under test organism its feed analysis is crucial. Analysis of proximate composition reflects the feed composition given to test organism. Qiu et al. (2017) reported that fatty acid profile of yellow croaker (*Larmichthys crocea*) muscles reflect the fatty acid composition of the diets after using different sources of lipids. In this study hemp feeding trail are conducted under same water temperature till to end. According to Love (1970) chemical composition varies significantly according to species, as well as its ecological, physiological and environmental factor. Many researchers have reported the influence of rearing environment such as salinity (Jana et al., 2006; Partridge and Jenkins, 2002), intrinsic factor like species, age and sex (Bandarra et al., 1997; Gruger, 1967), age and body size (Jonsson and Jonsson, 2003), migration, sex, temperature, season and starvation (Ali et al., 2006). The female fish contained more moisture and lipids than those of male fish (female > male) while protein and carbohydrates are more in male fish (male > female) (Islam and Joadder, 2005).

For increased demand of fish meat, more fish feed is needed. To meet this demand of feed, supplementary or prepared feed is supplied throughout the world (Jhingran, 1991). In aquaculture fish nutrition have improved significantly with regard to growth and feed efficiency, therefore mass production of several species of cultured fish have been well established for at least two decades (De Verdal et al., 2018). In aquaculture practices fish farmers usually prefer those supplementary/artificial feed which are nutritive, local, cheap and easily available in market. In this regard, hemp satisfies all these requirements that is ideally exist in best feed of Pakistan. Adding hemp seed in fish feed enhances the essential nutrients such as fatty acids, vitamins, minerals, that raises the growth proportion, stimulate immune-system, and help to prevent diseases (Landolt, 1989).

There is lack of knowledge of feed formulation for a specific fish species. Generally researchers considered type of species and age of prime importance for feed formulation (Jhingran, 1991). Specific feed formulation is suggested for specific fish species for monoculture. In this studies hemp supplementation showed positive effect on final body weight (FBW), percentage weight gain (PWG), specific growth rate (SGR) and feed conversion efficiency (FCR), on fingerlings of Mrigal. Available literature indicates that formulated feed having more ingredients showed more advantageous by improving the feed conversion efficiency of fish resulting in higher growth and survival rate (Jena et al., 1998). In contrast, with inappropriate diets fish is easily exposed to diseases, and resulting in a retard growth rate with ultimately decreases in feed conversion efficiency. Keeping in view its nutritional importance, hemp can be introduced in fish feed industries for obtaining best growth and survival

rate in aquaculture sector of Pakistan (Afridi et al., 2019; Afridi et al., 2024).

Cannabis sativa L. (Famous as marijuana or Indian hemp) is a native and wild plant of Pakistan and nowadays mostly cultivated in the Federally Administered Tribal Area (Ex-FATA). Supplemented diet are being evaluated for their antioxidant potential, because fish are potentially at risk of peroxidative attack due its large quantities of highly unsaturated fatty acids in its tissues and diets. Since long hempseed, has been recognized as a food and therapeutic agent for both animals and humans (Callaway, 2004). Besides protein and fibers, hemp seeds are good source of essential amino acids, polyunsaturated fatty acids (PUFA), vitamins, minerals and antioxidants. Hemp seed oil is over 80% polyunsaturated fatty acids (Callaway, 2004) with abundant amounts of essential fatty acids and tocopherols (Vitamin E) which act as good antioxidant (Kriese et al, 2004).

In essential fatty it contain three omega such as Linoleic Acid (18:2 ω6), Alpha Linolenic Acid (ALA) (18:3 ω3), Oleic Acid (18:1 ω9), and Gamma Linolenic Acid (GLA) (18:3 ω6) in abundant amounts (Kriese, et al, 2004). It is a complete balance diet having omega 6 and 3 (n6/n3) ratio of 2.5 which is greater than fish oil. The "omega-6" linoleic acid (18:2n6, LA) component is present at about 55% and "*omega*- 3" alpha-linolenic acid (18:3n3, ALA) occur at about 20% (Kriese et al., 2004). On the basis of available literature, it is considered a super food and can be used as an alternative of fish oil due to high omegas ratio that found in hemp oil (Afridi et al., 2019)

Hemp seed and hemp seed oil are plant derived products that contain all essential nutrients that required for optimum fish growth. Feed that contain animal protein are more expensive as compared to plants source of protein. It is therefore imperative that feed of animal protein should be substituted with plant protein ingredients such as hemp seed and soybean to reduce feed cost (Mohsen and Lovell, 1990). The feed plays an important role that ensures optimal growth for different fish species reared under different conditions (Thorarinsdottir, 2011). Feed is important for economically productive aquaculture systems that largely depend on low cost and nutrient rich feed (Akiyama, 1992). A balance feed formulation can lessen the burden on captured fisheries by increasing its survival rate and thus boosting the production (Wan, 2015). In this period, enormous amounts of supplemented feed is needed for intensive culturing of fish. Intensive culturing system relay on external source of feed such as compounds feed. In intensive culturing system fish are stocked at higher densities that cannot be supported by natural food sources. Compound feed usually contain higher percentages of protein supplements. The essential

ingredients are more in hemp seed in comparison to soybean. Hemp seed can be added in compound feed instead of animal protein and even plant source of protein such as soybean meal, peanut meal, and cottonseed meal (Potin and Saurel, 2020).

To get insight knowledge of hemp feeding the up and down regulation of insulin-like growth factor (IGF-I) is studied in fingerlings of Mrigal. The growth gene (insulin-like growth factors IGF-1) is the important promoter of growth in vertebrates (Fuentes et al., 2013) and primary mediator of many responses regulated, and plays an essential role in growth hormone throughout the body (Butler and Le Roith, 2001). In this study hemp seed significantly up regulated IGF-1 in comparison to hemp seed oil. Hemp seed (HS) at (5%, 10%, 15%) have showed better effect in comparison to hemp seed oil (HO) at concentration (1%, 2%, 3%) on expression of growth gene in liver of Mrigal. IGF-1 is more expressed in liver tissues in comparison to muscle of Mrigal as it evident from fold change of Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). Insulin-like growth factors (IGF-I and -II) along with their binding proteins and receptors are known to play a dominant role in regulation of growth both in livestock and fish (Buyse and Decuypere, 1999). This study favors the beneficial effect of hemp seed oil and hemp seed on proximate composition, growth performance and Insulin-like growth Factor-I of Mrigal (*Cirrhinus mrigala*) for 50 days. Hemp seed showed better results on all under studied parameter in comparison to hemp seed oil. Hemp seed and its oil is strongly recommended for fish, and poultry feed on graded level for enhancing all essential amino acids, fatty acids, mineral and vitamins not only in fish bodies but the human which consume them.

Conclusions

The present studies, for first time examine the nutritional effect of hemp seed oil and hemp seeds supplemented fed on growth performance, Proximate composition, and growth gene (Insulin-like Growth Factor-I) of Mrigal (*Cirrhinus mrigala*) after 50 days hemp feeding in graded level. Hemp seed oil (HO) at (1 %, 2%, 3%) and hempseed (HS) at (5%, 10%, 15%) concentration showed significant and positive effect but concentration specific effects on growth performance, proximate composition, amino acid profile and growth gene (Insulin-like Growth Factor-I) of Mrigal fingerling in comparison to control after 50 days hemp feeding. Hemp seed showed better effect in comparison to hemp seed oil, however, percent inclusion of hemp seed (15%) had shown a better result in comparison to other hemp seed and hemp seed oil. Hempseed products could be recommended as a feed ingredient in fish and poultry for

enhancing the nutritional values which in turn can have a good impact on consumer health.

Ethics statement and biosafety

The research is conducted by following compliance with ethical standards provided by society for the prevention of cruelty to animals (SPCA) of Pakistan. The ethical approval is obtained from the "Bioethical Committee of the Faculty of Biological Sciences on the use of animals for Scientific Research", and the ethical approval number granted for this study is BEC-FBS-QAU2017-67.

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