# Nutritional Status Assessment of Wild and Cultured Stock of Major Carps in Central Punjab, Pakistan

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#### ABSTRACT

This study aimed to compare the nutritional status of wild and cultured carps viz, Catla catla, Cirrhinus mrigala, and Labeo rohita, in terms of vitamin analysis, omega-3, and chemical composition of fish meat. One hundred and fifty (150) samples of each of three species were collected from wild stock i.e., Tremmu Headworks at district Jhang and cultured stock from Fisheries Research Farms University of Agriculture, Faisalabad. After following the standard analysis, results showed that wild carps had a higher percentage of omega-3 compared to cultured carps. The higher levels of omega-3 (21.69±0.51%) was observed in wild C. mrigala and lower level of omega-3 (10.85±1.01 %) was found in farmed L. rohita. Significant differences were observed in protein, ash, crude fat, and carbohydrates contents between wild and farmed carps. The maximum and minimum proteins were estimated in wild and farmed C. catla, respectively. The highest ash contents (2.57±0.06 %) were in wild C. catla and minimum (1.68±0.12 %) in farmed reared L. rohita while maximum carbohydrates (2.73 ±0.10 %) were recorded in wild L. rohita and minimum (1.70±0.1 %) in wild C. catla. Fat contents were significantly higher in cultured L. rohita (2.96±0.1%) compared to its counterpart, which has 1.35±0.1% crude fat. There was non- significant difference in vitamin C and E content of wild and cultured groups. The study concluded that differences in the chemical and omega-3 fatty acid composition between cultured and riverine carps may be attributed to the versatility of nutritive sintake.

# EINTRODUCTION

The production of aquaculture has reached to 82.1 million tons. It is expected to increase by 32% with a total yield of 108.5 million tons in 2030, making it the dominant protein source from fish and shellfish for consumption

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by human beings (FAO, 2020). Indian major carps are economically important fish species native to south Asian countries (Hossain *et al.*, 2022). Indian major carps are the major source of food in the Aquaculture industry and 75% of the aquaculture economy is contributed by Indian major carps. *Labeo rohita* is an Indian major carp that ranks amongst the top eleven finfish species cultured in world aquaculture (FAO, 2020).

Fish nutrition can influence production costs and water quality in fish farming. Nutrition is crucial to the long-term development of aquaculture. The most important component in aquaculture viability in all raising methods is appropriate nutrition. Currently, there has been a lot of concern are there due the fish fatty acid profile and nutritional conditions. Fish has demonstrated exceptional effects of necessary fatty acids in human nutrition (Zhang



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#### Key words

Wild and farmed major carps, Nutritional status, Omega-3 fatty acid, Chemical composition, Amino acid profile, Meat quality

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*et al.*, 2014). Fish fatty acids play a dynamic part in the inhibition of rheumatoid arthritis, high blood pressure, diabetes, heart disease and stroke (Hussain *et al.*, 2011; Afonso *et al.*, 2016; Mohanty *et al.*, 2016). The DHA (docosahexanoic acid) and EPA (Eicosapentaenoic acid) and are important long chain n-3 poly unsaturated fats (PUFA) present in lipid of fish. PUFA in fish differs from other fats or oils as they can freely flow through blood vessels (Mesías *et al.*, 2015). PUFA consumption has been well-thought-out vital for human disease control fitness and nutrition (Sujatha and Joice, 2013). The consumption of high levels of fatty acids (n-3 PUFA) from fish are connected with reduced inflammatory markers and increased high-density lipoprotein (Hustad *et al.*, 2021; Asher *et al.*, 2021).

Moisture, lipids, ash and protein are the key component of fish which make up 96%–98% of the fish body conformation (Begum *et al.*, 2012). The proximate composition of fish includes (moisture, lipids, protein and ash) are evaluated as chemical composition (Rani *et al.*, 2016). The chemical composition of fish flesh ideally predicted by flesh superiority, biological state, dietary value, and environment (Ravichandran *et al.*, 2011). An important role in catabolic processes is played by the amount of percentage of moisture in an animal body (Ch *et al.*, 2013). Size of various fish species is directly proportional to their ash content (Ahmed, 2011). Similar fish individuals chemical structure may vary in feeding and surrounding conditions, water quality and depth (Drazen, 2007; Ahmed, 2011).

Fish fatty acids and chemical composition (Moisture, protein, fat and ash) is influenced by different factors like the fish is either from wild environment or cultured their diet status like its origin and composition and some physiological factors which include age, sex, reproductive cycle, nutrition, seasonal change, geographical location (Vsetickova *et al.*, 2020). The carp farming at three different geographical sites in Austrian ponds resulted in great variety in the carp meat composition and quality. The fat content of carp varied greatly in all three farms ranging from 2.7 to 6.9% (Bauer and Schlott, 2009). Fish cultured in fresh water farms have reduced fat (n-3 fatty acid) levels as compared to their natural counterparts (Sales, 2010).

Vitamins are compounds (organic) essential for growth, reproduction, survival and nutrition and is required in very small amounts to maintain their health (Parisi and Guerriero, 2019; Kong *et al.*, 2021; Ghafarifarsani *et al.*, 2022). Vitamin nutrition plays a critical role in the sustainable aquaculture sector by improving aquatic animal health while decreasing feed and food waste. When the fish are fed with low fishmeal and high lipid fed diet the, nutritional disorders may occur that can impair the water quality. These nutritional disorders can be prevented by appropriately administering vitamins (Krogdahl *et al.*, 2020). Under perfect cultured conditions Vitamins act as coenzymes and maintain the metabolic reaction (Berntssen *et al.*, 2016; Soto-Dávila *et al.*, 2020).

Hydrophilic vital vitamins include vitamin C is which act as an important immune defensive and antioxidant componenet for fish. Fish require vitamin C to maintain their normal healthy body state. Various studies have confirmed that vitamin C enhances the aquatic animal growth performances (Shahkar *et al.*, 2015) and is vital for maintaining the physiological body function of aquatic animals (Ren *et al.*, 2007).

Vitamin E is very important for fish growth and development. The presence of rich polyunsaturated fatty acid, in fish diet and also in its body making them more vulnerable to peroxidation. This peroxidation can be prohibited by suitable supplementation of vit E in fish diet (El-Sayed and Izquierdo, 2022). Vitamins C and E are very important for fish growth, so it is very important to determine the appropriate supplementation level of vitamin C and E in fish diet to improve the nutritional status of cultured fish. The proposed investigation intended to iteract the analysis of the omega-3 fatty acid composition and vitamin (C and E) of riverine and cultured Indian major carps.

#### **MATERIALS AND METHODS**

Indviduals of different species of Indian major carps included 3 species (*Catla catla, Labeo rohita, Cirrhinus mrigala*) were collected from University of Agriculture, Faisalabad (Fisheries Research Farms) Tremmu headwork's, River Chenab at district Jhang. A total of one hundred and fifty (150) individuals with weight ranging from (200 to 250) g and length ranging (18 to 25) cm were collected. Total trial duration was 8 months and experiment was started from September 2022 to May 2023.

#### *Proximate analysis*

After the trial, speceimens were collected from different experimental groups. The fish were starved for 24 h and then anesthetized by immersing in 3000mg/L clove oil for 40-60 sec. All the fish were killed with sharp blow on head and samples were frozen at -20 °C. To measure the proximate components i.e. crude lipids, proteins, and ash, standard procedures AOAC (2016) were followed.

#### Fatty acid study

Accelerated solvent extraction (ASE) was used for total lipids removed from fish muscle. For fatty acids determination, the fat extract was used. Trans-esterification technique was used for fatty acid methyl esters (FAME) and with help of gas chromatography (GC) (Araki and Sako, 1987). Lipid profile (n-3 fatty acids) was determined by Trbović *et al.* (2013) method.

#### Vitamins and amino acid analyses

Standard protocols were used for the vitamin analysis (Ozogul *et al.*, 2011). Rapid HPLC method was used to determine vitamin E and method of Begum *et al.* (2012) was used to estimate the contents of Vit C in fish sample.

Aracus Amino Acid Analyzer Membrane Pure (Germany) was used to determine the amino acid profiles of 3 experimental species of fish.

#### Statistical analysis

The fatty acid and proximate body composition was calculated using variance analysis (ANOVA) and Tukey's test was applied at 5% level of significance to compare the variable between means of sample.

#### Physico-chemical parameters of water

Various physicochemical parameters of water were determined every fortnight from samples taken from two different locations: pH, DO (mg/L), alkalinity (mg/L), hardness (mg/L), TSS (mg/L) and TDS (mg/L). HANNA HI-9146), was used for determination of dissolved oxygen HANNA HI-98107 was used for pH, YL-TDS2-A for temperature YL-TDS2-A for TDS. Alkalnity and hardness was calculated by titration method and TSS was calculated by evaporation method.

# RESULTS

Table I shows proximates composition of there major carps.

There is no significant difference in the moisture

control of cultures and wild fish. The wild *Catla catla* however had ignificantly lower moisture content compared to other wild fishes.

When moisture content was compared among carps of wild and cultured sources, there was a pointed variance except *Catla catla*. The maximum moisture (74.77±0.48 %) in farmed *Cirrhinus mrigala* and minimum moisture (73.95±0.37 %) was observed in wild *C. catla*. Farmed captured fish species showed less percentage of moisture (74.38±0.30 %) in *L. rohita* followed by *C. catla*. and *C. mrigala* While wild-captured fish species showed maximum moisture (74.33%±0.30 %) in meat of *L. rohita* followed by *C. mrigala* and *C. catla* (Table I). The results showed that moisture percentage fluctuated nonsignificantly within and between both groups.

The protein contents were significantly different in wild and farmed fishes. The wild *C. catla* has  $19.45\pm0.13\%$  protein as against  $18.20\pm0.20\%$  in farmed *C. catla* (Table I). A substantial difference in fat content was detected among the species of both groups. Farmed carps had  $2.96\pm0.10\%$  fat contents compared to  $2.33\pm0.09\%$  wild fish (Table I).

A significant difference in farmed and wild stock species was also recorded in ash contents. Wild species showed highest ash content compared to farmed specimens. However, it was observed that meat of wild-captured *C. catla* showed a highest percentage ( $2.57\pm0.06$  %) of ash (Table I) followed by *L. rohita* and *C. mrigala*. While, the maximum ash percentage ( $1.98\pm0.04$  %) was recorded in farmed *C. mrigala*.

Carbohydrates are difficult to quantify and are frequently overlooked in chemical composition studies, these are taken into account by comparing all body composition metrics.

Species	Proximate composition parameters					
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)	
Cultured fish						
Labeo rohita	74.38±0.49a	18.37±0.10bc	2.96±0.10a	1.68±0.12c	2.72±0.10a	
Cirrhinus mrigala	74.77±0.48a	18.48±0.19b	2.85±0.12a	1.98±0.04bc	1.83±0.08c	
Catla catla	74.50±0.35a	18.20±0.18c	2.94±0.10a	1.82±0.05c	2.50±0.09bc	
Wild fish						
Labeo rohita	74.33±0.30a	19.30±0.26a	1.35±0.16c	2.34±0.09ab	2.73±0.10a	
Cirrhinus mrigala	74.30±0.46a	18.95±0.20ab	2.15±0.10b	2.32±0.12ab	2.28±0.08b	
Catla catla	73.95±0.37b	19.45±0.13a	2.33±0.09b	2.57±0.06a	1.70±0.10c	

Table I. Comparative proximate composition among wild and cultured major carps. Values are given as Mean±SE of three fish species.

Extreme carbohydrates  $(2.73\pm0.10 \%)$  were recorded in river-captured *L. rohita* followed by *C. mrigala* and *C. catla*. In contrast, the maximum  $(2.72\pm0.10\%)$  amount of these carbohydrates in *L. rohita* followed by *C. catla* and *C. mrigala* was observed in farmed captured fish (Table I).

A substantial difference was found at p<0.05 when the percentage of omega-3 fatty acids in carps of both sources was examined. The percentage of omega-3 in wild rohu 17.80 $\pm$ 0.57 %, cultured 10.85 $\pm$ 1.01 %, wild mori: 21.69 $\pm$ 0.51%, cultured individual: 11.39 $\pm$ 0.45 % and wild thaila 18.08 $\pm$ 0.71 %, cultured individual: 13.54 $\pm$ 0.35 % were observed. The wild mori has the highest omega-3 content, followed by thaila and rohu. The interference further showed that omega-3 differs significantly in both groups and three species (Table II).

Table II. Levels of omega 3 (%) fatty acid, vit C (mg/100g) and Vit E (IU) in wild captured and farm-raised major carps. Values are given as Mean±SE of three fish species.

Species	Cultured	Wild
Omega 3 level (%)		
Labeo rohita	10.85±1.01c	17.80±0.57b
Cirrhinus mrigala	11.39±0.45c	21.69±0.51a
Catla catla	13.54±0.35c	18.08±0.71b
Mean	11.93±0.47B	19.19±0.57A
Vitamin C level (mg/100g)		2
Labeo rohita	0.63±0.02c	0.61±0.009c
Cirrhinus mrigala	0.74±0.02b	0.79±0.01b
Catla catla	0.99±0.01a	0.96±0.009a
Mean	0.78±0.03A	0.79±0.03A
Vitamin E level (IU)		
Labeo rohita	0.74±0.01c	0.69±0.01c
Cirrhinus mrigala	0.69±0.01b	$0.64{\pm}0.01b$
Catla catla	0.54±0.02a	0.54±0.02a
Mean	0.66±0.02A	0.63±0.02A

Averages with parallel letters in a column and row are statistically insignificant (P>0.05). Capital letters imply the overall mean, whereas small letters represent the interaction between means.

#### Vitamin C and E content

Three fish species from both wild and farmed sources of vitamin C and E exhibited highly significant differences, but differences between wild and farmed groups were not statistically significant. In particular mean values of vitamin C ( $0.78\pm0.03$  and  $0.79\pm0.03$ ) were statistically insignificant (Table II). The mean values of vitamin E ( $0.66\pm0.02$  and  $0.63\pm0.02$ ) in farmed and wild species of carps were also statistically non-significant (Table II).

#### Water quality parameters

Water quality parameters were checked from both sites for all selected species. The values of water quality parameters are given into the Table III.

Table III. Fortnightly observations of physiocochemical parameters (pH, temperature, dissolved oxygen, alkalinity, hardness, total suspended solids and total dissolved solids) for selected species of wild and farmed culture.

Parameters	Farmed	Wild	
pH	8.35±0.02	7.16±0.01	
Т	27.3±0.55	26.1±0.57	
DO	7.65±0.03	$8.42 \pm 0.02$	
Alkalinity	205.31±1.32	$185.30 \pm 1.29$	
Hardness	234.31±1.82	202.65±1.64	
TSS	163.25±1.76	$145.20 \pm 1.73$	
TDS	834.10±1.73	771.00±1.67	

T, temperature; DO, dissolved oxygen; TSS, total suspended solids; TDS, total dissolved solids.

#### Amino acid profile of wild and cultured species

Figure 1 shows the results of amnio acid profile of wild and farmes species of major carps. Colour variation shown higher and lower values of amino acids. In this observations Nine esessntial and eight non-essential amnio acids recorded into the wild nad farmed cultured species of carps. Values of Gly, Ser, Pro, His, Ala, Tyr, Met, Phe, Arg, Thr, Ilu and leu were similar in farmed and wild carps. Significantly higher values of Glu Asp, Cys, Lys, valine, were found in farmed and wild *C. catla* than other wild and farmed carps fish. Over all there was no significant differences among wild and farmed species of major carps.



Fig. 1. Polar heatmap of essential and non essential amino acids of wild and farmed species of major carps. W, wild fish; F, farmed fish; EAA, essential amino acids; NEAA, non essential amino acids.

#### DISCUSSION

Fish is a highly nutritious and healthy food source containing balanced protein, lipid and minerals. Humans can easily digest fish meat compared to other animal meat sources (Miao et al., 2020). Fish is a nutritionally balanced food source and a variety of essential nutrients in fish make it an important food source easily available worldwide. Fish has anti-inflammation, anti-oxidation, wound healing, cardioprotection, and neuroprotection hepatoprotection properties, proving that fish consumption has numerous health benefits (Chen et al., 2022). Fish constitute 17% of animal protein sources, 6% of entire protein consumption of humans and global annual per capita fish consumption has reached to 20.5 kg (Avigliano et al., 2019). Wild fish normally feed on natural food while farmed fish are fed nutritionally well-adjusted diet. The deviation in feed sources causes a significant variation in the proximate composition of these fish species. Malnourishment is a terrific danger for the modest nations. Sufficient food is attained from farmed fishes, so they have better growth rates and different nutritionary levels than their wild counterparts (Khan et al., 2014).

The current investigations compared the nutritional value of meat from domesticated and wild carps. Our investigations of the n-3 fatty acid content of flesh from farmed and wild major carps exposed that the wild major carps have a much greater concentration of n-3 fatty acid (19.190.57%) than the farmed major carps (11.930.47%). Afterwards, high quantities of this vital fatty acid and its precursors being widely accessible in diet may be the cause of wild carps' high n-3 fatty acid content. On the other hand, lower concentrations of n-3 fatty acids in big carp raised in farms may be justified by the fatty acid composition of their food. These outcomes are consistent with those reported by Sharma et al. (2010) in Rohu. They concluded that DHA and EPA were principal polyunsaturated fatty acid that were displayed in extreme amounts 18.98±0.21 % in wild Rohu as compared to farmed-reared Rohu that contain 12.63±0.43 % and in sea bass (Alasalvar et al., 2002; Mnari et al., 2007) and in sea bream (Grigorakis et al., 2002a), while Ackman (2008) observed significant altitudes of essential n-3 fatty acid profile in tropical freshwater fish than marine stock. Tarricone et al. (2022) studied the fatty acid composition of wild and farmed sea bass. They found no significant difference in n-3 polyunsaturated fatty acid of wild and farmed seabass fillets.

In the current findings, non-significant variations  $(p \ge 0.05)$  were recorded for moisture in fish muscles between farm and riverine captured specimens of major carps. Yeannes and Almandos (2003) also described

parallel explanations in Paralabrax clathratus, Islam and Joadder (2005) in Glossogobius giuris, and Hussain *et al.* (2011) in Thala (*C. catla*). The protein contents of both groups of major carps differed significantly. The highest protein (19.45±0.13 %) in wild *C. catlaa* and minimum protein (18.20±0.20 %) in cultured *C. catlaa* was also observed during current study. While contradictory results were also detected in other fish species (Mahboob *et al.*, 2003; Dempson *et al.*, 2004; Osman *et al.*, 2007).

Ahmed *et al.* (2015) conducted a study to evaluate the meat quality of wild and farm-cultured *C. mrigala*. They found that the protein content of cultured farm *C. mrigala* was substantially higher than that of wild-captured fish. Additionally, comparable results were made by Omoniyi *et al.* (2013), who claimed that wild *Clarias gariepinus* has a greater moisture content than its counterpart. According to Ahmed *et al.* (2015), wild *C. mrigala* had a higher moisture content than farm-cultured ones.

Both groups recorded a notable variance in fat contents among the species of carps. Major carps caught on farms had the top in contrast percentage of fat content compared to fish caught in the wild. Fat deposition among farmed collected fish and wild stock may due to differences in feeding habits. Pond-reared fish needs less energy to obtain diet and has less space, which increases fat percentage in the body of fish as parallel to wild individuals who need more space and energy. Yesilayer and Genc (2013) also observed farmed rainbow trout having significant lipid contents. Adeosun et al. (2014) also concluded that pond cultured Clarias gariepinus have more fat deposition than wild stock. Comparable conclusions were also noted in sea bass (Alasalvar et al., 2002) and in Sparus aurata (Grigorakis et al., 2002). The results of our study are according to Mehboob et al. (2003) who indicated out significantly higher lipid substances in cultivated L. rohita than in its wild stock. Ahmed et al. (2015a) reported similar results: Fat content of farm cultured indian major carp (C. mrigala) was significantly higher than wild captured fish.

Hadyait *et al.* (2018) found different results to our findings that fat content of fish was notably higher in wild fish than farmed counterparts.

In the current study a significant difference in ash contents in both farmed and wild stock species was recorded major carp species from the wild had the highest levels of ash in comparison to those from farms. According to similar observations by Omoniyi *et al.* (2013), wild *C. gariepinus* has a higher ash concentration than its cultivated counterpart. Hadyait *et al.* (2018) studied the proximate composition of wild and farmed Indian carps (*L. rohita* and *C. mrigala*). They found that ash content of farmed *L. rohita* and *C. mrigala* was greater than that of

wild counterpart.

Carbohydrates are generally ignored in proximate analysis while they are an valuable segment of human nutrition. During current investigation significant content of carbohydrates were recorded in river captured (*L. rohita* followed by *C. mrigala* and *C. catla.*) than farmed captured fish specimens. While Ch *et al.* (2013) reported higher content of carbohydrate in the meat of reared carp *C. mrigala.* 

Vitamins are very important for fish as they perform the variety of function such as improve the flesh attributes (Wu *et al.*, 2020) stimulates immunity (Soto-Dávila *et al.*, 2020) support the bone development (Mazurais *et al.*, 2008) and contribute detoxification (Berntssen *et al.*, 2016). Aquatic organisms including the fish have restricted ability to synthesize vitamin C due to the absence of L-gulonolactone oxidase involved in ascorbic acid biosynthesis (Ai *et al.*, 2006). Harsij *et al.* (2020) observed that when fish was fed with supplemented vitamin C and vitamin E, fish showed enhancement in growth, antioxidant capacity and immune response.

In our study, a non-significant difference was found in the vitamin C and E content of wild farmed major carps. Vitamin E and C mean values were 0.656±0.023 IU, 0.784±0.031 mg/100g in farmed stock and 0.626±0.019 IU, 0.786±0.029 mg/100g in wild stock. The level of vitamin E in major carp meat was within the range found in C. mrigala, C. catla, and L. rohita (Mohanty et al., 2016). Our results are in line with study of Johnston et al. (2006) who reported that Vitamin E content was found non- significant in wild and farmed salmon. Rigos et al. (2012) founded that vitamin C content was slightly lower in wild fish liver as compared to the farmed fish. However, significant differences were found in muscle vitamin C of farmed and wild fish. Vitamin E concentration was substantially higher in liver and muscle of farmed fish as compared to their wild conspecifics. In our study the mean value of vitamin E was slightly lower in wild fish as compared to farmed one and these results were not parallel to Kaba et al. (2009) who discovered that wild fish contain more vitamins E and B2 than cultured fish. In present study vitamin C and E content differ significantly among indian major carps. Different results were found in the study conducted by Paul et al. (2016) who reported no significant difference in vitamin E content among three indian major carps. Maximum vitamin C content was found in farmed Catla catla (0.991±0.013) and minimum in wild (0.610±0.009) L. rohita. Maximum value for vitamin E was found in farmed L. rohita (0.741±0.012) and minimum in farmed C. catla (0.542±0.017).

Amino acids (AAs) are the primary constituents utilized to measure the nutritional value of fish (Hussain et al., 2018; Mohammed and Alim, 2012). There were a total of 17 AAs found in dried meat samples from the wild and farm-raised C. catla, L. rohita, and C. mrigala species. The intensity of essential amino acids (EAA) in C. catla, L. rohita and C. mrigala was assessed as 9 g/g of dry meat and 8 for non EAA, respectively in fishes collected from the farmedand wild from the river. When the AA profiles of major carps from two different habitats were evaluated, farmed fish showed superior AA balance and much greater quantities than wild counterparts. It was observed that when all the species of both sites were comparison the catla calta fish gives better amino aicd profile than other fishes of wild and farmed. It was also noted that the amount of amino acids were higher in farmed. The results were similar with Mehmood and Mateen (2020). When the AA profiles of grass and silver carp in two different environments were evaluated, farmed fish showed superior AA balance and much greater amounts than their wild counterparts. EAA supplementation had a significant impact on animal growth, according to Yamamoto et al. (2005) and Yang et al. (2010) reports. This was because of the altered nutritional status. Additionally Ch et al. (2013) noted that compared to both wild and silver carp, farmed grass carp have better flesh quality for human consumption.

## CONCLUSIONS AND RECOMMENDATIONS

Based on current examination, it is presumed that chemical and omega-3 fatty acid composition of river and cultured stock of carps may be attributed mainly to the feed utilization of fish. Data is deficient regarding the comparison of vitamins in wild and major carps. More research needs to be conducted to assess the required level of micronutrients in cultured Indian major carps. The required quantities of omega-3 fatty acids, amino acids and vitamins (C and E) can be incorporated into fish feed to improve the flesh quality of major carps raised in farms.

# DECLARATIONS

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Ethical statement

The work has been approved by the Institutional Biosafety and Bioethics Committee (IBC) of University of Agriculture, Faisalabad. The work has followed all the limitations for the fish trial.

Statement of conflict of interest

The authors have declared no conflict of interest.

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