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Role of Taurine supplemented LSM Based Diet on ADC of Nutrient and Mineral Retention in *Cyprinus carpio* Juveniles

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ABSTRACT

Taurine (Tau) is vital compound that is limited to oilseed based diets and its inclusion is essential in aquafeeds particularly when low fishmeal levels are used. Fish feed with addition of organic acids lowers the pH of fish gut that ultimately improves the hydrolysis of phytate, improves minerals absorption, upsurges the pace of gastric activities and augment digestion. The present study was designed to judge the effectiveness of Tau supplemented LMS (linseed meal) based diet in enhancing mineral absorption and nutrient digestibility in selected fish fingerlings. Graded levels of the prepared feed containing (0, 2, 4, 6, 8, and 10 g/kg) of Tau were fed to *C. carpio* fingerlings for 60 days. Test diets were prepared using Linseed meal and other ingredients. Highest digestibility of gross energy (72%), crude fat (66%) and crude proteins (73%) was observed in fish at 4 g/kg level of Tau in LSM based diet. Likewise, better absorption of the majority minerals occurred on said level. Our results showed that Tau could be used as a feed additive in linseed meal based diet to confer improved health status and higher growth in common carp at a 4 g/kg level in a plant-based diet.

Keywords: Aquaculture, *Cyprinus carpio*, LSM, Mineral absorption, Nutrient digestibility, Tau

INTRODUCTION

Currently aquaculture represents, 47% of total global fish production, and till 2050 it has been projected to increase its share about 52% (Sofia, 2018). The rapidly increasing human population has created nutrition-related issues, particularly concerns related to food security, all over the world. To maintain an uninterrupted supply of nutritious fish and ensure food security, the production of highly proteinaceous and economical feed needs to be increased. In recent decades, there has been an acute decline in the fish harvesting from wild water sources was noted, which moved the attention to the aquaculture sector. The annually fish consumption all over the world, is increasing since the population is increasing at exponential rate. The said factor presenting that, in future, this sector will play a very important role to meet the goals of FAO i.e. “A World without Hunger and Malnutrition” (FAO, 2018).

There is an urgent need to look for more efficient aqua-feeds as trends in aquaculture have shifted towards intensive culturing (Tacon *et al*., 2012). Aqua feeds mainly represents about 50 to 60% of total expenditure in culture of fish (Essa *et al*., 2010). Food fish reared in captivity gain their major protein fractions from fish meal (FM) which is an important constituent of aqua feeds and harbors essential amino acids and other indispensable biomolecules/nutrients such as fatty acids, plus the attractant fractions ensure that fish readily accept the feed (Shahzad *et al*., 2020; Shahzad *et al*., 2021a). The most viable and financially sound alternative in this regard are plant proteins which can facilitate more economically efficient fish rearing for both the developing and the developed world (Hassaan *et al*., 2018). Recently, it has become very necessary to replace fishmeal with locally available and low cost protein sources (Hassaan *et al*., 2018; Shahzad *et al*., 2021b). Amidst all plant protein-based ingredients, Linseed also called Flaxseed is one of the most significant oilseed meals for industrial as well as for the feed and food industry (Singh *et al*., 2011). Tau is a non-protein amino acid and is an

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important participant in many biotic processes involving salt regulation, bile acid conjugation, antioxidant functioning and early neural, visual and muscular systems development (Salze *et al*., 2012; Salze & Davis, 2015).

Common carp is among the most commonly cultivated freshwater fish species globally, demonstrating 80% of fresh water fish formulation (Dawood & Koshio, 2016). It is an economical and important food fish, which plays a vital role in sustaining Pakistan's food economy (Khan *et al*., 2016). Available literature has revealed that various oil plant sources are being considered as possible substitutes for FM in the diet of various fish species, however, limited amount of literature is available on the LSM (Linseed meal) based diet for Chinese carp i.e., *C. carpio*. Tau is a critical constituent in fish nutrition, specifically when supplying with plant protein-based diets. The most suitable protein source in fish feeds is undoubtedly fishmeal. Nevertheless, plant protein-based diets have been utilized in industry, but there are some constraints in nutritional content. Fragmentary replacement of fishmeal with Tau can lower feed expense as well as boost growth performance in fish (Zhang *et al*., 2019). The current study has been designed to calculate the optimum level of alternative plant protein sources such as linseed meal commonly called flax seed for commercially important species, such as *C. carpio,* to both spike up the production of cultured fish (this species in particular), all the while avoiding the economic restraints placed by high-priced fishmeal.

MATERIALS AND METHODS

Fish Fingerlings with Trial Conditions

Study was conducted in the Fish Nutrition Lab, Zoology Department, University of Education, Township Campus, Lahore, Pakistan. *C. carpio* fingerlings were purchased from head balloki, about 70 km from Lahore. For two whole weeks, or 14 days, the fingerlings were gently introduced to and adjusted with the experimental conditions. Specifically designed 70 liters V-shaped aquaculture tanks were used for the stock of experimental fingerlings and were fed two times a day on a basal diet (Allan & Rowland, 1992). For the supply of air by the capillary system, an air pump was used during the whole experimental period. Water quality parameters (pH, dissolved oxygen, and temperature etc.) were monitored and maintained in normal range.

Experimental Design

This experiment was centered on linseed as the basis of our custom-designed experimental feed for carp fingerlings. The ingredients were mixed in gradually increasing proportions with 0, 1, 2, 3, 4, and 5 % of Tau, a sulfonated amino acid. Of the six diets, one was the control, and the other five were increasing grades of the Tau-supplemented LSM experimental diets which were fed at 4% of the fish's body to the six fish groups: one control, and five experiment groups, for 60 days (Table 1). For each treatment, triplicate tanks were employed, and each housing 15 fingerlings. Each linseed-based diet with addition of Tau was compared with control and other test diets to check the ADC% of nutrients and minerals absorption in fish by using Completely Randomized Design (CRD).

Feed Pellet Formation

All ingredients for the preparation of the experimental diet were brought from a local market in Lahore (Table 1). Standard methods, as used by AOAC (1995), were employed to test the chemical constituents of the feed prior to the creation of the experimental diet. 1% chromic oxide was also added, it served as an inert marker. The ingredients for the diet were thoroughly mixed for 20-25 minutes in an electric mixer. After this, fish oil was added slowly to make suitable dough. Meanwhile, up to 20% distilled water was added to prepare the required texture and feed pellets were prepared using a feed pelleting machine. The feed pellets were dried in a shady place. All diets were supplemented with set percentages of Tau except the control diet (without Tau addition) and stored at 4^0C until use.

Feeding Procedure and Sample Collection

All six groups of *C. carpio* fingerlings were given their corresponding diets twice each day during the experimental period in both the morning and evening for 2 hours. Following this feeding period, the leftovers were siphoned out of the tank water via specially designed valves. Plus, the fecal matter was gathered carefully, being extra careful not to break the feces which would've led to leaching of its constituents in the water. Following the collection, the feces were air-dried inside an oven at 70ºC, and after this, they were stored for analysis of chemical constituents.

Analysis of Prepared Feed and Collected Feces

Homogenized samples of the feed and the feces were subjected to oven-drying at 105ºC for 12 hours, following which, the moisture content was calculated for both. To calculate the levels of crude protein (CP) constituents (N x 6.25), the Micro Kjeldahl Apparatus was put to service, for crude fat (EE) evaluation, the Soxhlet system was employed. Contents of crude fiber were calculated as loss on ignition of dried lipid-free residues after digestion, whereas ash was estimated via electric furnace (Naberthern B170) to invariable weight. For the evaluation of the total energy level of the samples, an oxygen bomb calorimeter was employed. For the total carbohydrate content calculation, the given formula was used:

Total carbohydrate (%) =100- (Moisture% + EE% + CP% + Ash% + CF %)

Assessment of Minerals

For the assessment of minerals (Ca, P, Cu, and Mg) in prepared samples of feces and diets, the samples were subjected to atomic absorption as per the protocol dictated by AOAC (1995). Flame photometry (Jenway PFP-7, UK) was put to use for the quantification of potassium and sodium fractions in the sample. The chromic oxide fractions of the samples, which were used as markers, were oxidized using a molybdate reagent, and following this, the fractions were evaluated (based on their absorbance) via UV-VIS 2001 Spectrophotometer at 370nm wavelength. A spectrophotometer was employed once again to estimate the phosphorus (P) contents in the samples by observing their absorbance at 720 nm wavelength.

Calculation of ADC%

Apparent digestibility coefficient % of nutrients in all feeds was calculated by the standard formula National Research Council (1993).

$$\text{ADC\%} = 100 - 100 \times \frac{\text{\%nutrient /mineral in feces} \times \text{\% marker in diet}}{\text{\%marker in feces} \times \text{\% nutrient /mineral in feed}}$$

Statistical Analysis

Following this, the ADC% statistics (for gross energy, EE, and CP levels), degree of absorption of minerals (Mg, Ca, K, Cu, Na, and P), and other parameters for quantifying growth were then treated with inferential statistical treatment, i.e. one-way analysis of variance. After comparing the parameters via Tukey's Honesty Significant Difference at $p<0.05$, a statistically significant difference was determined (Snedecor & Cochran, 1991). For statistical analysis, the CoStat Computer Package was employed.

RESULTS AND DISCUSSION

Nutrients Digestibility Parametric

Analyzed nutrients i.e., crude proteins, crude fat, and gross energy contents in diet, feces, and digestibility values are demonstrated in Table I, Table II, and Fig 1, respectively.

During the period of 70 days, trial fingerlings were fed on isoenergetic and isocaloric diets, maximum apparent digestibility coefficient of all nutrients were observed i.e. crude proteins (73%), crude fat (66%), and gross energy (72%) were observed at 4gkg^{-1} Tau level, followed by CP (67%), EE (63%) and GE (64%) at 6gkg^{-1} LSM based diet as shown in Fig 1. The results have shown that minimum loss of nutrients in feces occurred in group III which was fed on the optimal level of Tau at the rate of 4gkg^{-1}. Nonetheless, the lowest value of CP digestibility (48%) was found in fingerlings fed with a control diet while the lowest EE (44%) and GE (40%) were analyzed in fish fed with test diet VI having 10gkg^{-1} LSM based diet (Fig 1).

Fingerlings fed on a Tau supplemented diet indicated altogether improved digestibility in contrast to those in the control treatment. These results provided much evidence that Tau supplemented LSM based diet improved the absorption of nutrients in *C. carpio* fingerlings as compared to control treatment (without Tau addition). Present findings indicated that absorption of all nutrients in *C. carpio* fingerlings begin to increase from a 2gkg^{-1} level-based diet and to its peak value at 4gkg^{-1}. It was also noticed that further inclusion of Tau (higher levels 6gkg^{-1} and 10gkg^{-1}) supplementation diminished the nutrient absorption. As a consequence of better efficacy of digestive enzymes which then led to better absorption via the digestive system, the protein levels in the fish body elevated significantly.

Absorption of minerals (Cu, P, Mg, Na, K, and Ca)

Mineral constitution in LSM based diets and feces are shown in Table III and Table IV, respectively. It was noticed that all experimental diets had nearly the same mineral composition, whereas mineral contents of feces were different when analyzed, in all fish feeding groups. Lowest discharge of majority of minerals (Ca; 0.41%, Na; 0.0045%, K; 0.33%, P; 0.61, Cu; 0.0025) in feces occurred at 4 gkg^{-1} level based diet while for Mg, lowest discharge (0.0041 %) was noted at 6gkg^{-1} level based diet. Low discharge of minerals in water is necessary to prevent algal bloom or eutrophication. Whereas, the maximum amount of mineral (Ca, Na, K, and P) was discharged through feces when fingerlings were fed on the control diet.

Table I: Ingredients and chemical composition (%) of feed ingredients and diets (Dry based)

Ingredients	TDs	DM (%)	CP (%)	CF (%)	Ash (%)	EE (%)	CHO	GE (kcal/g)
Linseed meal	33	89.91	32.64	12.93	11.74	3.73	38.96	3.99
FM	16.5	91.54	49.31	1.29	24.66	6.99	17.75	2.23
Corn gluten (60%)	13.5	90.37	59.34	1.41	1.63	4.79	32.83	4.38
Wheat flour	17.5	92.73	9.43	2.88	2.06	2.41	83.22	3.09
Rice polish	8.5	91.86	13.02	13.06	11.17	12.76	49.99	3.03
Fish oil	7	-						
Vitamin premix	1	-						
Mineral premix	1	-						
Ascorbic acid	1	-						
Chromic oxide	1	-						
Tau level (gkg^{-1})*	0.0-10	-						

*Tau was incorporated with partial replacement of Wheat flour. TDs: Test diets, DM: Dry Matter, CP: Crude Protein, CF: Crude fiber: EE: Ether Extract, CHO: Carbohydrate, GE: Gross Energy

Proximate composition (%) of diets with varying amounts of Tau

Treatments	Tau gkg^{-1}	Protein in diet (%)	Fat in diet (%)	Gross energy in diets (kcal^{-g})
TD-I	0.0	32.47±0.36^{a}	5.33±0.28^{a}	3.06±0.08^{a}
TD-II	02	32.48±0.37^{a}	5.34±0.34^{a}	3.06±0.11^{a}
TD-III	04	32.48±0.24^{a}	5.34±0.24^{a}	3.07±0.06^{a}
TD-IV	06	32.47±0.26^{a}	5.34±0.23^{a}	3.06±0.09^{a}
TD-V	08	32.49±0.21^{a}	5.33±0.22^{a}	3.05±0.06^{a}
TD-VI	10	32.49±0.13^{a}	5.35±0.12^{a}	3.07±0.09^{a}

Table II: Effect of dietary Tau on CP, EE, and GE in feces of *C. carpio* fed on LSM based diets supplemented with dietary Tau.

Treatments	Tau gkg^{-1}	CP (%)	EE (%)	GE (kcal^{-g})
TD-I	0	18.99±0.68^{a}	3.31±0.26^{a}	2.04±0.08^{ab}
TD-II	02	12.81±0.40^{cd}	2.39±0.22^{bc}	1.26±0.09^{b}
TD-III	04	9.64±0.43^{e}	2.00±0.12^{c}	0.97±0.04^{d}
TD-IV	06	11.67±0.80^{d}	2.16±0.14^{c}	1.23±0.07^{c}
TD-V	08	13.43±0.50^{c}	2.76±0.16^{b}	1.85±0.06^{b}
TD-VI	10	15.64±0.24^{b}	3.36±0.12^{a}	2.06±0.09^{a}

EE = Crude fat, CP = Crude protein, GE = Gross energy (a-e). The mean within columns with dissimilar superscripts are quite different at *p<0.05.* Data are mean of three replicates with 15 fish fingerlings in each.

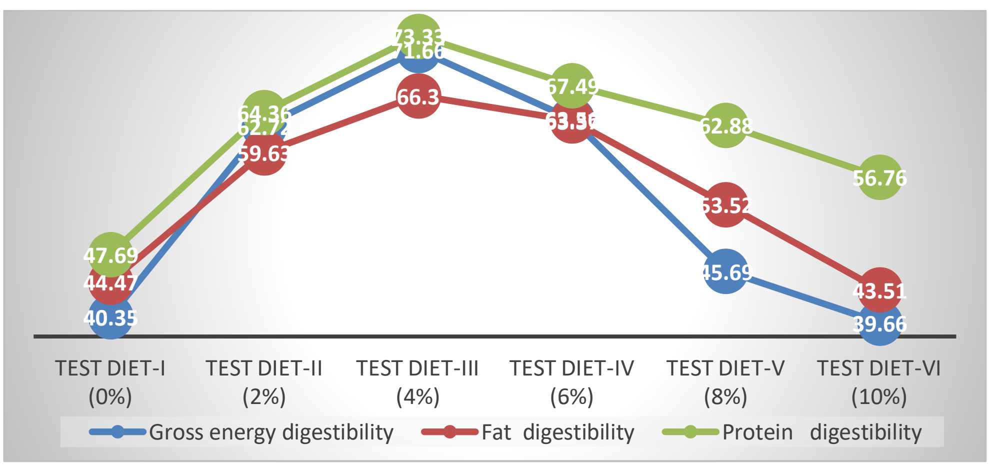

Fig. 1: Graphical representation of nutrient digestibility values.

Table III: Mineral composition (%) of linseed meal-based diets supplemented with Tau.

Treatments	Tau g/kg	Ca	Na	K	P	Cu	Mg
TD-I	0.0	1.10±0.04	0.0137±0.0021	1.17±0.06	2.32±0.09	0.0068±0.0005	0.0117±0.0006
TD-II	02	1.10±0.03	0.0147±0.0035	1.17±0.08	2.31±0.11	0.0067±0.0007	0.0119±0.0007
TD-III	04	1.09±0.05	0.0143±0.0025	1.19±0.06	2.32±0.11	0.0067±0.0005	0.0118±0.0010
TD-IV	06	1.10±0.03	0.0140±0.0030	1.18±0.09	2.33±0.08	0.0067±0.0005	0.0118±0.0006
TD-V	08	1.11±0.03	0.0133±0.0021	1.17±0.04	2.32±0.09	0.0068±0.0005	0.0118±0.0008
TD-VI	10	1.11±0.03	0.0150±0.0036	1.17±0.09	2.34±0.05	0.0067±0.0006	0.0118±0.0009

Data are mean of 3 replicates with 15 fish fingerlings in each

Table IV: Mineral composition in feces of *C. carpio* fed with Tau supplemented LSM based diet.

Treatments	Tau g/kg	Ca	Na	K	P	Cu	Mg
TD-I	0.0	0.59±0.03^{b}	0.0068±0.0011^{ab}	0.71±0.06^{ab}	1.07±0.04^{b}	0.0038±0.0003^{a}	0.0056±0.0003^{a}
TD-II	02	0.47±0.03^{c}	0.0065±0.0016^{ab}	0.55±0.04^{cd}	0.86±0.06^{c}	0.0032±0.0003^{abc}	0.0052±0.0001^{ab}
TD-III	04	0.41±0.03^{c}	0.0045±0.0009^{b}	0.33±0.02^{e}	0.61±0.03^{d}	0.0025±0.0003^{c}	0.0044±0.0004^{bc}
TD-IV	06	0.48±0.04^{c}	0.0066±0.0011^{ab}	0.50±0.04^{d}	0.88±0.10^{c}	0.0029±0.0004^{bc}	0.0041±0.0001^{c}
TD-V	08	0.58±0.03^{b}	0.0072±0.0013^{ab}	0.63±0.03^{bc}	1.12±0.05^{b}	0.0034±0.0002^{ab}	0.0051±0.0005^{ab}
TD-VI	10	0.72±0.02^{a}	0.0095±0.0023^{a}	0.75±0.04^{a}	1.37±0.05^{a}	0.0037±0.0002^{a}	0.0055±0.0004^{a}

(a-e) The mean within columns having different superscripts are quite different at *p<0.05.* Data are mean of 3 replicates with 15 fish fingerlings in each.

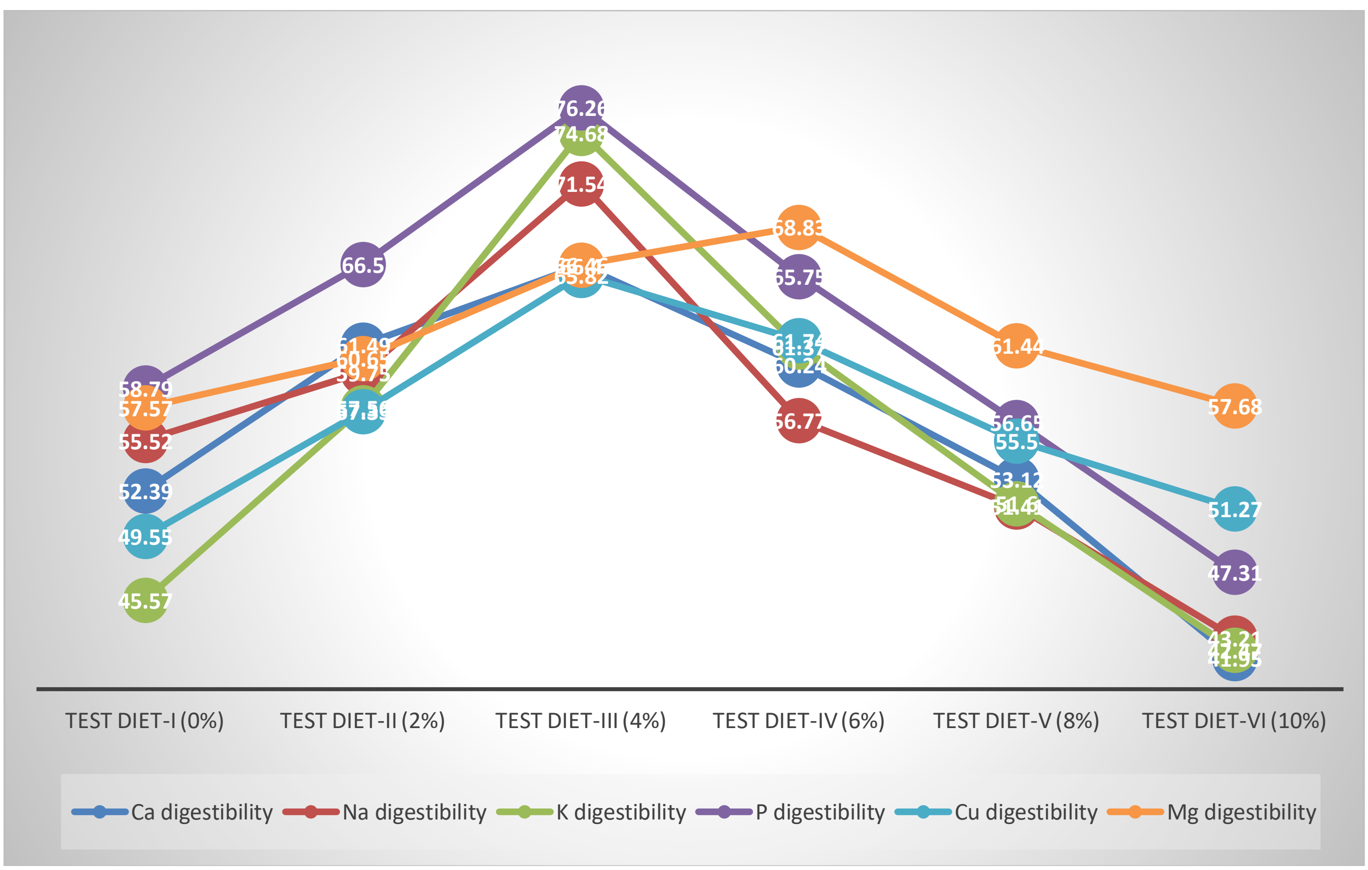

Fig. 2: Graphical representation of minerals absorption.

The absorption of various minerals including Ca, Na, K, P, Cu, and Mg improved significantly with the addition of Tau in LSM based diet. The highest absorption values of the majority of minerals i.e. Ca (66%), K (75%), Cu (66%), Na (72%), and P (76%) were observed at 4gkg^{-1} Tau supplemented LSM based diet followed by K (61%) and Cu (62%) in group IV when fish were fed on 6gkg^{-1} level based diet, while second-highest absorption values for Ca (62%), Na (60%) and P (67%) were found in group II, fed on a 2gkg^{-1} level-based diet. For Mg, improved absorption (69%) was recorded in group IV at a 6gkg^{-1} level-based diet, followed by 67% in group III which were fed on a 4gkg^{-1} LSM based diet. The lowest absorption of minerals i.e., Ca (42%), Na (43%), K (42%), and P (47%) were recorded in fish fed on a 10 gkg^{-1} Tau-supplemented LSM based diet (Figure 2). But some of the minerals such as Cu (50%) and Mg (58%) were found lowest in fish that were fed with the control diet. Results indicated that there were highly significant differences (P<0.05) in the absorption of minerals of the control group and other Tau-supplemented treatments. The results showed that Tau supplemented LSM based diet augmented ingestion of minerals in *C. carpio* fingerlings as compared to control treatment (without Tau addition). The current outcomes pointed out that absorption of the majority of minerals in *C. carpio* fingerlings began to increase from 2gkg^{-1} and rose to its maximum value at 4gkg^{-1} other than the Mg for which maximum retention occurred at 6gkg^{-1}. It was noticed that further inclusion of Tau (higher levels 8gkg^{-1} and 10gkg^{-1}) resulted in a decreased absorption of minerals.

These results suggested that utilization of LSM with Tau supplementation can be beneficial in the retention of nutrients and minerals in the fish body thereby reducing leaching of nutrients and minerals in the water. Less excretion of nutrients in water is crucial in reducing the problems related to aquatic pollution. In the current research work, improvement in nutrient and mineral absorption was observed in *C. carpio* fingerlings fed on LSM with simultaneous supplementation of Tau at an optimal level of 4 gkg^{-1} level-based diet.

DISCUSSION

The present study has clearly indicated that nutrient digestibility, as well as mineral retention in *C. carpio* fingerlings, is maximally increased when fed on an LSM based diet supplemented with Tau at an optimum level of 4gkg^{-1}. It was found that the 4gkg^{-1}level of Tau was sufficient to hydrolyze phytic acid and to release the bound minerals and nutrients of a plant-based diet (LSM). Higher enzyme secretion, causing the better nutrient digestibility, may be the reason for improved growth when Tau was used as supplement in comparison of control group. Adding Tau to the diet of the fish proved to be beneficial for the secretion of intestinal enzymes, most prominently, proteases, lipases, and amylase. This increased secretion led to better digestion of proteins, lipids, and carbohydrates (most notably starch), which made available more biomolecule monomers for absorption in blood via the intestines. Previous studies have already proven the ability of this sulfonated amino acid to improve the nutrient digestion in nektonic beings. In other studies, with carps (Liu *et al*., 2006; Luo *et al*., 2006), it was discovered that adding Tau to fish feed had a direct relation with enhanced growth, better digestibility of nutrients, and improved biomolecule absorption. Similar results were absorbed with common dentex (*Dentex dentex*) when it was treated with the experimental feed at 2.0 gkg^{-1} concentration (Chatzifotis *et al*.,2008), cobia (*Rachycentroncanadum*) at 5.0 gkg^{-1} diet (Lunger *et al*., 2007), rainbow trout at 8.5 gkg^{-1} diet (Gaylord *et al*.,2007), white sea bass (*Atractoscion nobilis*) at 9.9 gkg^{-1} diet (Jirsa *et al*., 2014), *O. niloticus* at 10 gkg^{-1} diet (Al-Feky *et al*., 2016), *Pelteobagrus fulvidraco* at 10.9 gkg^{-1} diet, turbot (*Psetta maxima*) at 11.5 gkg^{-1} diet (Qi *et al*., 2012), Japanese flounder (*Paralichthys olivaceous*) at 16.6 gkg^{-1} diet (Kim *et al*., 2005).

The Tau requisites of fish have a diverse range (from 2.0 to 16.6 gkg^{-1} diet) which is most likely influenced by dietary sources of protein and levels, experimental conditions, assimilation rate, fish species, and sizes (Salze & Davis, 2015). They delineated that several fishes could biosynthesize Tau starting with cysteine and methionine. Diets used in present study were lacking in cysteine and methionine when compared with their need by common carp. This recommends that *C. carpio* fed with low-FM diets can't biosynthesize sufficient amount of Tau and will need an extraneous supply. The literature review indicated that there was no study conducted on the role of Tau in mineral absorption in freshwater fishes, particularly common carp, which is an important food fish and has huge economic potential in Pakistan.

According to the results of the present study, it was noted that ADC% of nutrients i.e., CP (73%), EE (66%), and GE (72%) was significantly improved (P<0.05) in *C. carpio* fingerlings fed on Tau (4gkg^{-1}) supplemented LSM based diet. According to Al-Feky *et al*. (2016), using Tau (10gkg^{-1}) supplemented soybean meal-based diet for tilapia showed improvement in larval growth rates and feed utilization efficiency i.e., EE 58.24% and CP 169%. In another feeding trial by Qi *et al*. (2012), positive results were found in terms of SGR (2.0) final body weight 25g when juvenile turbot were fed on using a casein-based diet supplemented with 1.5 % Tau level.

In contrast to our results, a recent study by Abdel-Tawwab and Monier, 2018 recorded total lipids (3.2%) in the proximate chemical composition of common carp fed on SBM supplemented with a 5gkg^{-1} level-based diet.

The addition of Tau in the fish diet herein reinforced the activities of intestinal amylase, protease and lipase, of common carp, which promoted the decomposition of dietary carbohydrates, lipids, and protein resulting in better growth and feed utilization. In former studies, Tau has been stated to play a crucial role in enhancing nutrient digestion and absorption in underwater animals (Nguyen *et al*., 2015; Richard *et al*., 2017; Salze *et al*., 2012). Improving digestive enzyme activities would enable improved nutrient availability (Hoseinifar *et al*., 2017) hence, appropriately explaining the better growth observed in fish-fed Tau-supplemented feed. Similarly, lipase activity estimated in the intestinal digestion of yellowtail (*Seriola quinqueradiata*) fed several SBM-based formulations, was increased on supplementing the diets with Tau (Nguyen *et al*., 2015).

From the current research work, it was noted that optimal inclusion (4 gkg^{-1} level) of Tau in LSM based diet significantly ($P<0.05$) reduces the excretion of essential minerals and nutrients in water through feces. The literature review indicated that no study had been conducted on the role of Tau in mineral absorption in freshwater fishes, particularly common carp. So, the current result indicates that Tau supplementation at optimum level (4 gkg^{-1} level) leads to better absorption of minerals since it acts as a chelating agent which helps in the retention of various essential minerals.

CONCLUSION

Based on present results and available data, it was concluded that dietary Tau supplementation in plant meal-based diet imparted beneficial effects on the wellbeing of Common carp by improving the digestibility of essential nutrients and mineral absorption, and ultimately, growth in fingerlings when fed on the level of 4gkg^{-1} of Tau.

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