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Alterations in biochemical parameters of fish species under choline administration directly into the pond water in a semi-intensive fish farming system: A comparative study

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Abstract
The present study deals with the impact of choline chloride supplementation, applied directly into the pond water in a semi-intensive farm culture system for 90 d on two Indian Major Carps (IMCs) (*Catla catla*, *Labeo rohita*) and two air-breathing teleostean fishes (*Anabas testudineus*, *Clarias batracus*).The experiment was conducted in two seasons (breeding and dry) under choline (treated) and non-choline (control) exposure. Biochemical parameters, *viz*., PRO (total protein) content gained maximum significantly ($p< 0.01$) in treated-breeding with the lowest in control-dry in intestine, liver, muscle, brain. IMCs showed significant ($p< 0.01$) increased amylase activity in the liver and intestine in treated-dry and treatment-breeding conditions, while liver amylase activity in both the seasons increased manifolds significantly ($p< 0.01$) in air-breathing fishes, showing a sharp decline only in the intestine. Protease and lipase activity in the intestine and liver of choline-fed fishes disclosed declining trend in the breeding season significantly ($p< 0.01$), but treated (choline-fed) IMCs under dry revealed the significant ($p< 0.01$) increasing and decreasing trend of protease and lipase activity respectively; the reverse trend at dry season was found in air-breathing fishes under choline-fed condition. ALP (alkaline phosphatase) in intestine indicated significant ($p< 0.01$) lowest activity in treatment-breeding, highest in treatment-dry. This study explored the higher activity towards total protein generation, especially muscle; higher carbohydrate digestion; elevated lipid digestion in choline-fed fishes. So, the fishes from this farm-based pisciculture system are healthy and its yield can be prescribed as a rich source of quality fish food for human health.

Keywords: Choline, Indian major carps (IMCs), air-breathing fishes, biochemical parameters

Introduction
Availability of choline or its metabolites maintains the structural integrity as well as signalling functions of the cellular membrane in animals. Actually, in the diets, methyl constituents (*viz*., betaine causes methylation of homocysteine to make methionine) are derived from choline and its metabolites. Moreover, it also performs some important cellular functions like cholinergic neurotransmission, transmembrane signalling and lipid transport or metabolism [1]. It can enhance the neural tube closure and hippocampal development in case of human and some cellular activities *e.g*., signalling in neurons; in case of liver cells, transport of lipoproteins from hepatic cells are also being triggered by the presence of this lipolytic factor and thus preventing hepatic carcinogenesis in the animal body [2]. Properties of metabolites of choline are detrimental, such as betaine acts as methyl group donor and also induce renal osmolytic activity, while, acetylcholine plays an important role in neurotransmission. Further, phosphatidylcholine and sphingomyelin are necessary and mandatory items to act as building blocks of bio-membranes, whereas, glycerophosphocholine and phosphocholine act as intra-cellular storage polls of choline [3, 4]. The physiological functions of fish is maintained by protein [5], and choline supplementation in diet caused an enhancement of crude protein content in different organs of juvenile Jian carp *Cyprinus carpio* [6]; in *Oncorhynchus mykiss* choline with soya lecithin [7]. Activity of different digestive enzymes like amylase, protease and lipase due to different feed supplementation was recorded by different authors, such as reduced amylase activity in juvenile *Totoaba macdonaldi*, fed with soybean meal with

Taurine [8, 9], elevated level of intestinal amylase activity with the increased dose of choline content in the diet in blunt snout bream [10], in juvenile Jian Carp [6], and in eel [11]; while, decreased lipase activity in liver and intestine of juvenile Jian Carp was observed due to elevation of choline in the diet [6], in eel [11], and in Caspian Sea brown trout (*Salmo trutta*) [12]. But in case of supplementation of metabolites of choline, *viz*., dietary phosphatidylcholine decreased the protease activity in intestine and liver in Caspian Sea brown trout [12]. Again, supplementation of betaine and lecithin during recovery of the endosulfan exposed fish, the *Labeo rohita* fingerlings showed normal activity of ALP (alkaline phosphatase) due to easy liberation of phosphate ions to combat stress [13].

So, the primary aim of this study is to analyse and compare the biochemical alternations, especially digestive enzymes, total protein content (PRO) and alkaline phosphatase activity in the different tissues of two Indian Major Carps (IMCs), *i.e*., *Catla catla* and *Labeo rohita* along with two air-breathing fishes *e.g.*, *Clarias batracus* and *Anabas testudineus* under a semi-intensive farming system by the administration of choline directly into the pond water in addition to usual farm-made-aqua-feed during dry (Nov. to Jan.) and breeding (June to Aug.) seasons.

2. Materials and Methods

2.1. Preparation of culture ponds

Experimental ponds are located at Khano village, Galsi-II Block, Purba Bardhaman, West Bengal, India under the Aqua-farm of Chandimata Self Help Group and each having effective water area (EWA) 0.50 bigha (or 0.20 acre). Two treatment ponds (P1 and P2) and one control pond (C) are geographically located at latitude and longitude N $23^0$19′872″, E $87^0$43′702″ and N $23^0$19′834″, E $87^0$43′751″ and N $23^0$19′924″, E $87^0$43′877″ respectively. Preparation of ponds for acclimatization as well as experimentation was done by following the usual protocol beforehand (manured and fertilised as per fishery protocol) [14-18].

2.2. Procurement of fish species and culture

Fish specimens were collected from Khano village and were acclimatized in an already prepared single pond (called acclimatization pond) for two weeks before liberation into the experimental ponds and fed regularly with farm-made-aqua-feed (Table 1) at the rate of (3-4) % of total biomass perday. Now, after acclimatization, in the dry season (Nov. to Jan.), two Indian Major Carps (IMCs) *Catla catla* (catla), *Labeo rohita* (rahu) and two air-breathing carnivorous fishes *e.g*., *Clarias batracus* (magur), *Anabas testudineus* (koi) of both the sexes were released into the pre-prepared experimental field ponds. The test fishes were also fed with farm-made-qua-feed at the rate of (3-4) % of total biomass in a pond per day. Out of these ponds, two with farm-made-aqua-feed plus choline supplemented (T: treatment), *i.e.*, in P1 and P2, and one with normal farm-made-aqua-feed fed (control, *i.e*., without choline supplementation) (C). A total number of 900 fishes at the rate of Catla: Rahu: Magur: Koi = 2:5:1:1 was released in each experimental pond from the acclimatization pond after 15 days of acclimatization. So, a total of 2700 fishes were taken into consideration for this present experiment for this season.

During breeding season in our present experiment (June to Aug.), the same stoking density and culture ratio were maintained [a total number of 900 fishes (both the sexes) per experimental pond at the rate of Catla: Rahu: Magur: Koi = 2:5:1:1]. The experimental design was identical with the dry season and the feeding of farm-made-aqua-feed was maintained in the three experimental ponds (P1& P2: treatment and C: control) as per the protocol of dry season. Here also a total of 2700 fishes were chosen from the acclimatization pond for experimenting with these test fishes in the breeding season.

Choline of commercial synthetic formulation of Meden Pharma Pvt. Ltd., Boisar-401506, Maharashtra was procured and administrated at the rate of 350 g $bigha^{-1}$ $15d^{-1}$ directly into the P1 and P2 ponds during the time of experimentation in both the season.Results were recorded as TD, TB, CD and CB for representing the treatment-dry, treatment-breeding, control-dry and control-breeding conditions in culture practices.The water quality of the experimental ponds was monitored and analysed **[19]** for both the seasons in every fortnight gap (Table 2) and finally expressed in mean value.The avg. value was considered in case of treatment (T: avg. of P1 & P2) ponds during the time of experimentation in both the seasons.

2.3. Sampling

After completion of the experiment of 90-d, the fishes (species wise) were harvested randomly both from treatment (n=5; 3 sample from P1 and 2 sample from P2) and control (n=5, from C pond) ponds in both the seasons and anesthetized with tricaine methanesulphonate (MS 222) for collection of desired tissues like intestine, liver, brain, and muscle and kept at -80^0C for biochemical analysis, *viz*., total protein content (PRO), alkaline phosphatase (ALP) activity, amylase, protease and lipase activity.

2.4. Biochemical analysis

Amylase activity was measured by Bernfeld *et al*. (1955) [20] and lipase activity by the method of Cherry and Crandall (1932) [21]; protease activity by Snell and Snell (1959) [22] and total protein content was measured by the Folin-Phenol reaction method of Lowry *et al*., 1951) [23]. Finally, alkaline phosphatase (ALP) activity was measured by Bergmeyer *et al*. (1976) [24] by using MERCK kit (Merck cat. #1730PDLFT.0045)

2.5. Statistical analysis

Analysis of variance (One-way ANOVA) followed by Tukey's test at the significance level of 0.05 according to Zar (2010) [25] using SPSS Ver19 **[26]** was followed for statistical analysis of the enzyme activity.

3. Results and Observations

Results of biochemical analysis were recorded as CD, TD, CB and TB in Tables 3a and 3b.

3.1. Total protein (PRO): Protein content was observed maximum in intestine, liver, muscle and brain at TB in all the cultured species under choline-exposure (Table 3a). In *C. catla* and *C. batracus*, the trend was in the tune of liver>intestine>muscle>brain, whereas, in *L. rohita* and *A. testudineus* it was intestine>liver>muscle>brain. The minimum of protein content was found in CD in all the cultured fishes, where, the decreasing trend was presented as brain<muscle<intestine<liver in *C. catla* and *C. batracus*, whereas, *L. rohita* and *A. testudineus* showed the trend of brain<muscle<liver<intestine.

3.2. Amylase: Present study depicted the maximum amylase activity in the intestine and liver of *C. catla* and *A. testudineus* respectively at TD, whereas, the minimum amylase activity was found in the intestine of *C. catla* at CB and liver of *C. batracus* respectively. Moreover, it is to note that the activity of amylase to the choline-exposed fishes was increased manifolds both in the intestine and liver in both in the seasons except a straight decline was noticed in the intestine of air-breathing fishes under choline-exposure in both the seasons compared to their control groups. (Table 3b).

3.3. Protease: Present study revealed that the protease activity was dropped down in both the seasons in the intestine and liver of choline exposed IMCs as well as the air-breathing fishes, except a slight elevation was observed both in intestine and liver of choline-fed IMCs under TD condition in comparison to their non-choline-exposed fishes. Interestingly, the maximum hike in protease activity in intestine and liver was noticed in *C. catla* and *L. rohita* respectively under TD condition, while, least value in intestine and liver was accorded by *C. catla* in CD and *L. rohita* in TB respectively among all the experimented fishes (Table 3b).

3.4. Lipase: In the present study, the lipase activity declined maximally in both the seasons to the intestine and liver of treated IMCs as well as air-breathing teleosts except in TD of air-breathing fishes depicted an elevating trend both in intestine as liver under choline exposure compared to their control fishes. However, highest elevation of lipase activity both in intestine and liver was depicted in *C. batracus* under TD condition, while, minimum value was accorded both in intestine and liver of *C. batracus* under TB condition (Table 3b).

3.5. Alkaline phosphatase (ALP): ALP is a zinc-containing metallo-enzyme. It has an important role in phosphorus metabolism. The present study presented the maximum hike of ALP activity in intestine was occurred in TD of all the experimented fishes under both the seasons, whereas, TB showed the minimum value. The ALP activity was reduced in the TB compared to CB, whereas, the activity was increased in TD compared to CD. However, the maximum gain of ALP activity in intestine was noticed in *C. batracus* under TD condition, while *C. batracus* under TB condition depicted the lowest value (Table 3b).

4. Discussion

The digestive physiology of aquatic organisms is maintained by different enzymatic activities and these are essential for digestion processes and physiological functions of the fish **[27]**.

4.1. Total protein (PRO): Protein attributes the general metabolism and good health of fish; so, enhancement of protein (PRO) in different tissues of animals is clearly determined by the process of balancing between synthesis of protein and its gradual degradation [28]. Total protein content (mgg^{-1}) in different tissues under two different experimental sets, such as control, treatment in breeding and dry season (CB & CD and TB & TD) varied significantly in the present study with the higher elevation in TB than in TD compared to CB & CD, specifically, it showed highest value in TB and lowest in CD in intestine, liver, muscle and brain of *C. catla*, *L. rohita*, *A. testudineus* and *C. batracus*. The higher rate of metabolism in breeding season forces the fishes for higher intake of foods; so, sometime it causes the breakdown of tissue protein due to scarcity of foods, and thus, releasing the protein to the plasma to maintain the equilibrium of the concentration of protein in the plasma during protein deficiency [29]. Application of choline into the pond water resulted into enhanced body crude protein content in different organs is also reported in *Cyprinus carpio*, when supplemented with the dietary choline chloride [6], and secretion of hepatic lipoprotein in juvenile cobia, *Rachycentron canadum* and *Micropterus salmoides* [30,31]. Besides, it was also indicated that the enhanced protein content and metabolic rate was recorded in *Oncorhynchus mykiss* with choline supplemented diets containing soy lecithin, and autoclaved isolated soy protein [7]. On the other hand, carbofuran treated *Clarias batrachus* showed decreased total protein content and subsequently it was recovered by choline [32].

4.2. Amylase, Protease and Lipase: These enzymes are responsible for breaking down the complex food items of vertebrates and bony fishes, *viz.*, proteins, carbohydrates or lipids into smaller molecules, like amino acids, simple sugars and fatty acids respectively[33], like incorporation of rubber seed meal (260 g kg^{-1}) in the diets of juvenile tilapia (*Oreochromis niloticus* × *O. aureus*) noticeably showed declining trend of hepatic protease, lipase and amylase activities, and thereby reduced the dry matter and crude protein digestibilities [34]. In an another note, it was revealed that the protease and lipase activities were found to be higher in small sized fish while amylase activity was observed maximum in the large sized fish. The protease and lipase activities appeared to be higher in the intestine while amylase activity was increased in the liver [35]. On the other hand, it was also ascribed higher amylase activity in fish when fed with diets containing plant-based ingredients [36].

Present study showed the increased amylase activity in liver and intestine of IMCs and air-breathing fishes in both the seasons except the decreasing trend in intestine of air-breathing fishes. Amylase analysis indicated the higher rate of carbohydrate digestion, because carbohydrate gives the instant energy source during the stress condition as well as during higher metabolism in intestine of juvenile *Totoaba macdonaldi*, fed with soybean meal diets [8,9]. But with constant supplemented taurine resulted into reduction in amylase activity and reduced amylase activity reflected less carbohydrate digestion in the lumen of the intestine as found in the intestine of air-breathing fishes in our present experiment. On the other hand, significant decrease of amylase activity in intestine was noticed with the increase of lipid level in the diet of blunt snout bream, but intestinal amylase increased significantly with the hike of dietary choline level when dietary lipid level was limited up to 150g kg^{-1} [10], as occurred in the case of IMCs under both the seasons in our present study, may be due to sufficient utilization of choline. In an another experiment, with the increase of dietary choline levels in the diet of juvenile Jian Carp, the activity of amylase in the intestine increased manifolds [6] and the same trend also found in eel [11], in the intestine of juvenile Jian carp, fed with xylanase up to a certain level in the plant-protein enriched diet [37]. Moreover, *Artemia* fed striped bass fish (*Morone saxatilis*), Caspian Sea brown trout fish (*Salmo trutta*), fed with dietary phosphatidylcholine indicated higher amylase activity [38,12], as occurred in our present experiment.

In our present study, it revealed that the protease activity was reduced in most of the cases due to less reactivity towards protein digestion [39]. However, it was observed that the protease activity was hiked in intestine of *Clarias batrachus* (Linn.) after application of dietary protein alike our present experiment in treated IMCs in dry season, but to report as to lower physiological as well as metabolic activities to mitigate stress [40]. Moreover decreased protease activity was also been reported in intestine and liver tissue by the supplementation of dietary phosphatidylcholine in Caspian Sea brown trout [12], in *Channa punctatus*, after the application of *Nerium indicum* leaf extracts, treated against the piscicidal activities [41]. Nevertheless, inclusion of tannin (extracted from *Accacia* sp., 6.25 to 200 µg) in the diet of *L. rohita* fingerlings showed the reduced protease activity from 24.07 to 90.21% [42]. Similarly, *in vitro*, protease activity of *L. rohita* fingerlings reported to be inhibited by incorporation of tannin extracted from *Pistia* sp [43].

IMCs under choline exposure indicated declining trend of lypase activity in both the seasons, while during dry season in case of air-breathing fishes under treated condition showed increasing trend of lipase activity in intestine and liver alike increased lipase activity was observed in the intestine and liver with higher levels of soybean meal in the diets of juvenile *Totoaba macdonaldi*, but in later study, soybean meal with low taurine content decreased the production of bile salts and therefore, limited the lipid digestion to produce inadequate liver function [9].But it was also reported that the inverse relationship was found in case of silvery-black porgy [44]. Interestingly, in similar to our results with the IMCs, decreased lipase activity was noticed in liver hepatopancreas and intestine of juvenile Jian Carp with the increasing levels of dietary choline levels because of the presence of phosphatidylcholine in lipid digestion [6], but the inverse relationship in eel was noticed when the diet contained increased amount of choline [11]. Moreover, on similar to our result, lipase activity was significantly reduced by the supplementation of dietary phosphatidylcholine in Caspian Sea brown trout (*Salmo trutta*) [12], in the viscera of freshwater fish during fermentation [45].

4.3. Alkaline phosphatase (ALP): ALP is considered as a general marker of nutrient absorption in the absorptive process in fish [46]. Findings in the present study reflected a declining trend of ALP activity in intestine of treated fishes in breeding season appeared to be normal to liberate phosphate ions to combat stress due to higher metabolic activity and mobility in *L. rohita* fingerlings, exposed to endosulfan and subsequent recovery from stress by the application of betaine and lecithin [13]. But in an another study it showed the increased ALP activity in *L. rohita* in the diet containing *Microcystis* due to breakdown of reserved energy (glycogen), required for growth and survivability of the fishes [47], whereas, activation of ALP activity was alike during treated dry condition indicating inactivation of phosphorylase enzymes, resulting into glycogen synthesis in the tissue, while, similar inhibition of ALP activity in treated breeding condition, resulted into breakdown of glycogen to meet up the energy demand under stress or otherwise depicting lowered transphosphorylation and uncoupling of oxidative phosphorylation [48]. On the other hand, an elevated trend of ALP in intestine of all fishes in field condition was observed in dry season under choline exposure, where, the metabolic activity, movement of fishes and protein synthesis were less, resulted into synthesis of glycogen in liver as found in *C. punctatus* [49], in *C. carpio* [50] and in Nile tilapia exposed to deltamethrin [51]. In another study it was noticed that proximal intestine showed highest ALP activity in juvenile Jian Carp under choline-fed diet, whereas, distal intestine reflected the lowest activity [6].

Table 1: Ingredients (g kg^{-1}) for formulation of farm-made-aqua feed and proximate composition of the basal experimental diet

Ingredients	g kg^{-1}	Nutritional content (g Kg^{-1})		
Fish meal *	195	Dry matter	:	982
Soya meal*	130	Crude protein	:	350
Ground nut oil cake *	45	Crude fat	:	48.85
Yellow corn (maize) *	120	Crude Ash	:	53.53
DO_RB * (De-oiled rice bran)	230	NFE	:	547.62
Broken rice *	145			
Silky bran *	45			
Vitamin premix ** [6]	40			
Mineral premix ** [6]	40			
Sodium chloride *	10			
Crude protein, crude fat, crude ash and moisture content were measured value [52]				
Nitrogen free extract; NFE (%) = 100- (% crude protein + % total fat + % ash)				
* Local market (Khano, Galsi, Galsi-II Block, Purba Bardhaman, West Bengal, India) **Matsya Chas Sahayata Kendra, Tinkonia, Gurudwara, near Burdwan Municipality, Purba Bardhaman, West Bengal, India				

Table 2: Analysis of physicochemical parameters of pond water under CD, TD, CB and TB conditions

Sl. No	Parameter	Unit	CD	TD	CB	TB	p Value
01	Transparency	cm	27.43±0.44	24.93±0.58	20.33±0.55	19.01±0.53	<0.01**
02	Temperature	^0C	18.00±0.49	18.83±0.59	29.88±0.42	31.35±0.56	<0.01**
03	Electrical conductivity	µS/cm	532.00±2.79	538.00±2.99	671.83±3.44	679.83±4.09	<0.01**
04	pH		7.35±0.26	7.85±0.16	8.02±0.40	8.35±0.72	<0.05*
05	Carbon di-oxide	mg/l	5.37±0.31	4.53±0.26	5.28±0.33	4.70±0.21	<0.01**
06	Dissolved oxygen	mg/l	5.48±0.16	5.87±0.39	6.70±0.45	7.12±0.20	<0.01**
07	Total alkalinity	mg/l	279.67±3.14	285.67±1.55	317.33±1.72	338.33±3.74	<0.01**
08	Phosphate (as PO_4^{3-})	mg/l	0.43±0.03	0.55±0.01	0.85±0.05	1.30±0.15	<0.01**
09	Total-hardness	mg/l	144.67±1.80	159.17±0.82	190.33±1.32	185.33±1.10	<0.01**
10	Chloride (Cl^-)	mg/l	87.83±0.82	96.33±0.66	52.00±0.43	61.50±0.65	<0.01**

11	Ammonical nitrogen (NH_4^+-N)	mg/l	0.86±0.03	1.28±0.11	0.87±0.15	1.47±0.21	<0.01**
12	Nitrate nitrogen (NO_3^-N)	mg/l	0.38±0.01	0.61±0.05	0.42±0.07	0.76±0.05	<0.01**
13	Sodium (Na^+)	mg/l	67.17±1.36	67.83±1.08	70.83±1.43	83.33±0.80	<0.01**
14	Potassium (K^+)	mg/l	16.50±0.43	17.00±0.52	17.50±0.45	20.33±0.89	<0.01**

Data are represented as mean ± SD (n-6). One-way ANOVA conducted, significance '**' when $p< 0.01$ and '*' when $p< 0.05$.

Table 3a: Responses of total protein content of fish tissues in *L. rohita, C. catla, C. batracus and A. testudineus* under CD, TD, CB and TB conditions

Total protein (PRO) (mgg^{-1})										
Species	**Organ**	**CD (control-dry)**	**TD (treatment-dry)**	**% of inc. /dec. (col D vs C)**	**CB (control-breeding)**	**TB (treatment-breeding)**	**% of inc. /dec. (col G vs F)**	**p value (among col. C, D, F, G)**	**MAX**	**MIN**
A	B	C	D	E	F	G	H	I	J	K
C.catla	Intestine	23.81±1.68 ^{a,b}	26.93±0.96 ^{c}	13.07	24.64±0.94 ^{b,c}	32.77±1.58 ^{d}	32.96	< 0.01	32.77(TB)	23.81(CD)
L. rohita		61.13±2.81 ^{a}	73.26±3.33 ^{b}	19.85	80.19±1.52 ^{c}	95.01±4.16 ^{d}	18.48	< 0.01	95.01(TB)	61.13(CD)
A. testudineus		88.57±1.95 ^{a,b}	96.95±3.35 ^{c}	9.46	92.55±5.40 ^{b,c}	122.44±2.64 ^{d}	32.30	< 0.01	122.44(TB)	88.57(CD)
C. batracus		33.71±0.77 ^{a}	43.72±1.72 ^{c}	29.70	40.85±0.79 ^{b}	60.31±1.95 ^{d}	47.64	< 0.01	60.31(TB)	33.71(CD)
C.catla	Liver	45.13±3.79 ^{a}	53.97±2.19 ^{b,c}	19.59	55.06±1.99 ^{c}	63.07±1.36 ^{d}	14.54	< 0.01	63.07(TB)	45.13(CD)
L. rohita		34.56±1.64 ^{a,b,c,d}	37.61±1.87 ^{c}	8.84	34.62±1.73 ^{b}	44.25±2.49 ^{d}	27.80	< 0.01	44.25(TB)	34.56(CD)
A. testudineus		42.44±2.82 ^{a,b,c}	47.69±2.20 ^{c}	12.35	45.48±3.00 ^{b,c}	56.21±3.69 ^{d}	23.60	< 0.01	56.21(TB)	42.44(CD)
C. batracus		47.51±4.53 ^{a,c}	59.51±1.72 ^{c}	25.25	54.35±5.62 ^{b}	68.12±0.63 ^{d}	25.33	< 0.01	68.12(TB)	47.51(CD)
C.catla	Muscle	17.69±1.18 ^{a,b}	20.84±0.62 ^{c}	17.78	18.12±0.91 ^{b}	23.30±1.55 ^{d}	28.61	< 0.01	23.30(TB)	17.69(CD)
L. rohita		26.24±1.37 ^{a}	28.90±0.46 ^{b,c}	10.11	30.52±1.27 ^{c}	37.34±0.36 ^{d}	22.35	< 0.01	37.34(TB)	26.24(CD)
A. testudineus		21.39±0.62 ^{a}	30.78±0.93 ^{b}	43.90	39.07±0.43 ^{c}	48.41±0.85 ^{d}	23.90	< 0.01	48.41(TB)	21.39(CD)
C. batracus		22.82±0.78 ^{a}	33.52±0.56 ^{c}	46.90	28.53±0.62 ^{b}	48.89±1.77 ^{d}	71.33	< 0.01	48.89(TB)	22.82(CD)
C.catla	Brain	7.51±0.02 ^{a}	8.79±0.04 ^{c,d}	17.07	8.71±0.58 ^{b,c}	9.60±0.78 ^{d}	10.23	< 0.01	9.60(TB)	7.51(CD)
L. rohita		5.91±0.08 ^{a}	6.90±0.34 ^{b}	16.75	8.00±0.62 c	10.36±0.63 ^{d}	29.38	< 0.01	10.36(TB)	5.91(CD)
A. testudineus		5.05±0.67 ^{a}	7.19±0.58 ^{c}	42.27	7.03±0.57 ^{b,c}	9.50±0.40 ^{d}	35.14	< 0.01	9.50(TB)	5.05(CD)
C. batracus		8.56±0.44 ^{a,b,c}	8.95±0.55 ^{c}	4.49	8.92±0.59 ^{b,c}	10.93±1.64 ^{d}	22.58	< 0.01	10.93(TB)	8.56(CD)

Data are reported as Mean ± SD (n=5). One-way ANOVA followed by Tukey's test conducted. Values with same superscripts in the same row are not significantly different ($p< 0.05$)

Table 3b: Responses of amylase, protease, lypase and ALP activity of fish tissues in *L. rohita, C. catla, C. batracus and A. testudineus* under CD, TD, CB and TB conditions

Amylase (mg maltose liberated min^{-1} mg^{-1} protein)										
Species	**Organ**	**CD (control-dry)**	**TD (treatment-dry)**	**% of inc. /dec. (col D vs C)**	**CB (control-breeding)**	**TB (treatment-breeding)**	**% of inc. /dec. (col G vs F)**	**p value (among col. C,D,F,G)**	**MAX**	**MIN**
A	B	C	D	E	F	G	H	I	J	K
C.catla	Intestine	31.34±0.43 ^{b}	69.23±1.27 ^{d}	120.90	29.76±1.57 ^{a,b}	58.82±0.81^{c}	97.65	< 0.01	69.23 (TD)	29.76 (CB)
L. rohita		25.00±0.22 ^{a}	28.19±0.27 ^{b,c}	12.76	28.44±0.54 ^{c}	36.52±0.61 ^{d}	28.41	< 0.01	36.52 (TB)	25.00 (CD)
A. testudineus		29.97±0.43 ^{d}	15.61±0.47 ^{b}	-47.91	20.11±0.48 ^{c}	8.50±0.14 ^{a}	-57.73	< 0.01	29.97 (CD)	8.50 (TB)
C. batracus		67.06±1.00 ^{d}	39.04±0.76 ^{b}	-41.78	51.58±4.20 ^{c}	22.30±1.60 ^{a}	-56.77	< 0.01	67.06 (CD)	22.30 (TB)
C.catla	Liver	21.82±0.52 ^{c}	37.93±1.27 ^{d}	73.83	13.82±0.62 ^{a}	15.61±0.59 ^{b}	12.95	< 0.01	37.93 (TD)	13.82(CB)
L. rohita		15.33±0.46 ^{b}	40.62±1.36 ^{d}	164.97	14.97±0.66 ^{a,b}	40.55±1.30 ^{c,d}	170.88	< 0.01	40.62 (TD)	14.97 (CB)
A. testudineus		17.32±0.10 ^{b}	42.87±0.50 ^{d}	147.52	15.64±0.08 ^{a}	37.35±0.83 ^{c}	138.81	< 0.01	42.87 (TD)	15.64 (CB)
C. batracus		32.40±1.11 ^{c}	35.57±1.17 ^{d}	9.78	26.24±0.75 ^{a,b}	26.75±0.41 ^{b}	1.94	< 0.01	35.57 (TD)	26.24 (CB)
Protease (µg tyrosine liberated h^{-1} mg^{-1} protein)										
C.catla	Intestine	8.33±0.04 ^{a,b,c}	18.38 ±1.51 ^{d}	120.65	9.60±0.12 ^{c}	8.80±0.38 ^{b,c}	-8.33	< 0.01	18.38 (TD)	8.33(CD)
L. rohita		3.94±0.07 ^{c,d}	3.98± 0.73 ^{d}	1.02	2.78±0.01 ^{b}	2.23±0.05 ^{a,b}	-19.83	< 0.01	3.98 (TD)	2.23(TB)
A. testudineus		1.56±0.02 ^{c,d}	0.92±0.01 ^{a}	-41.03	1.57±0.04 ^{d}	1.29±0.06 ^{b}	-17.88	< 0.01	1.57 (CB)	0.92 (TD)
C. batracus		10.09±0.05 ^{d}	6.03±0.01 ^{b}	-40.24	7.76±0.05 ^{c}	4.09±0.06 ^{a}	-47.33	< 0.01	10.09 (CD)	4.09 (TB)
C.catla	Liver	6.97±0.65 ^{c,d}	7.31±0.97 ^{d}	4.88	6.20±0.55 ^{b,c,d}	5.50±0.73 ^{a,b}	-11.22	< 0.01	7.31 (TD)	5.50 (TB)
L. rohita		8.40±0.54 ^{c,d}	8.80±0.50 ^{d}	4.76	7.85±0.44 ^{b,c}	6.56±0.15 ^{a}	-16.38	< 0.01	8.80 (TD)	6.56 (TB)
A. testudineus		5.39±0.86 ^{d}	5.30±0.30 ^{c,d}	-1.67	4.65±0.20 ^{b,c,d}	3.02±0.17 ^{a}	-34.99	< 0.01	5.39 (CD)	3.02 (TB)
C. batracus		5.96±1.54 ^{d}	4.34±0.27 ^{b,c}	-27.18	5.27±0.16 ^{c,d}	0.97±0.02 ^{a}	-81.50	< 0.01	5.96 (CD)	0.97(TB)
Lypase (µmol of free fatty acid liberated hr^{-1} mg^{-1} protein)										
C.catla	Intestine	15.51±0.64 ^{d}	8.17±0.63^{a}	-47.31	12.04±0.54 ^{c}	9.51±0.66 ^{b}	-21.01	< 0.01	15.51(CD)	8.17(TD)
L. rohita		13.29±0.19 ^{d}	10.90±0.05^{c}	-18.03	10.19±0.04 ^{b}	7.93±0.06 ^{a}	-22.19	< 0.01	13.29(CD)	7.93(TB)
A. testudineus		12.19±0.39 ^{c}	14.53±0.32^{d}	19.22	8.34±0.58 ^{b}	5.55±0.63 ^{a}	-33.44	< 0.01	14.53(TD)	5.55(TB)
C. batracus		14.18±0.09 ^{c}	16.60±0.26^{d}	17.07	11.12± 0.52 ^{b}	10.31±0.11 ^{a}	-7.30	< 0.01	16.60(TD)	10.31(TB)
C.catla	Liver	13.85±0.56 ^{d}	10.53±0.49^{c}	-23.97	9.91± 0.08 ^{b,c}	8.72±0.03 ^{a}	-12.01	< 0.01	13.85(CD)	8.72(TB)
L. rohita		15.29±0.28 ^{d}	11.81±0.38 ^{b,c}	-22.79	12.04± 0.25 ^{c}	9.51±0.32 ^{a}	-21.01	< 0.01	15.29(CD)	9.51(TB)
A. testudineus		9.97±0.05 ^{c}	15.44±0.25 ^{d}	54.82	9.45± 0.28 ^{b}	7.13±0.08 ^{a}	-24.50	< 0.01	15.44(TD)	7.13(TB)
C. batracus		14.40± 0.09 ^{c}	16.16±0.22 ^{d}	12.20	11.86± 0.12 ^{b}	11.10±0.20 ^{a}	-6.42	< 0.01	16.16(TD)	11.10(TB)
ALP (nanomole p-nitrophenol released min^{-1} mg^{-1} protein)										
C.catla	Intestine	12.26±0.11 ^{b}	18.27±0.09^{d}	48.94	16.86± 0.03 ^{c}	9.87±0.21 ^{a}	-41.45	< 0.01	18.27(TD)	9.87(TB)
L. rohita		13.79±0.40 ^{b}	22.51±0.23^{d}	63.17	20.26± 0.29 ^{c}	9.10±0.42 ^{a}	-55.09	< 0.01	22.51(TD)	9.10(TB)
A. testudineus		16.84±0.32 ^{b}	32.06±0.83^{d}	90.34	24.76± 0.15 ^{c}	9.64±0.15 ^{a}	-61.07	< 0.01	32.06(TD)	9.64(TB)
C. batracus		26.94±0.37 ^{b}	39.97±1.34^{d}	48.38	33.57± 0.08 ^{c}	20.25±1.22 ^{a}	-39.69	< 0.01	39.97(TD)	20.25(TB)

Data are reported as Mean ± SD (n=5). One-way ANOVA followed by Tukey's test conducted. Values with same superscripts in the same row are not significantly different ($p< 0.05$), ALP- Alkaline phosphatase

5. Conclusion

The present work has been able to disclose the influences of direct choline administration in the water body of farm-fish culture for production of enriched fish food, having high protein content in liver, intestine and especially in muscle tissue. Increased amylase activity and decreased lipase and protease activity were observed in maximum, especially, in muscles, which indicate the higher carbohydrate digestion, maximum utilization of feed due to higher metabolic rate, enhanced lipid digestion, under choline exposure. From analysis of digestive enzymes, it can be concluded that the metabolism in liver was enhanced in IMCs and air-breathing fishes during breeding season compared to dry showing a distinct utilization of protein to combat stress as well as to achieve good health, especially, in breeding season under choline exposure. So, here choline supplementation enhanced the digestive and absorptive functions of the fishes resulting into a quality fish for consumption of human beings with conducive aquatic body for sustainable fish culture.

6. Conflict of Interest

The authors announce no conflict of interest with the contents of this article

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