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

Subhas Das, Atanu Patra, Arghya Mandal, Niladri Sekhar Mondal, Sukhendu Dey, SK Mirjan and Apurba Ratan Ghosh

DOI: [https://doi.org/10.22271/fish.2020.v8.i6a.2352](https://doi.org/10.22271/fish.2020.v8.i6a.2352)

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 batrachus*). 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-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 constitutents (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 intracellular storage pols 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 *Oncorhynus 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 Labo 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° 19' 872", E 87° 43' 702" and N 23° 19' 834", E 87° 43' 751" and N 23° 19' 924", E 87° 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 per day. 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-aqua-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 = 2.5:1:1.1 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 administered at the rate of 350 g bigha1 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°C 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. batrachus* 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 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. batrachus* under TD condition, while, minimum value was accorded both in intestine and liver of *C. batrachus* 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. batrachus* under TD condition, while *C. batrachus* 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 [29].

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. batrachus*. 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 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 reported in intestine (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 lipase 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 *Tutobia macdonaldi*, but in later study, soybean meal with low taurine content decreased the production of bile salts liver with higher levels of soybean meal in the diets of *Channa punctatus* [50] and in Nile tilapia exposed to *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 phospholipase 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 cholone 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          |
| DO₃B * (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 | °C    | 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±3.49 | 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  | 5.82±0.33   | 4.72±0.21  | <0.01** |
| 06     | Dissolved oxygen | mg/l | 5.48±0.16  | 5.87±0.39  | 7.60±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 PO₄³⁻) | 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** |
Table 3a: Responses of total protein content of fish tissues in L. rohita, C. catla, C. batrachus and A. testudineus under CD, TB, CB and TD conditions.

| Species   | Organ   | Total protein (PRO) (mg/g) |
|-----------|---------|---------------------------|
|           |         | CD (control-dry) | TD (treatment-dry) | % of inc. / dec. (col D vs C) | CB (control-breading) | TB (treatment-breading) | % of inc. / dec. (p value among col. C, D, F, G) | MAX | MIN |
| A. testudineus | Intestine | 3.31±1.94 a,b | 3.74±1.94 a,b | 0.42±0.10 c | 13.05±0.63 b | 3.49±0.63 c | 0.64±0.03 c | 0.01 | 0.05 |
| L. rohita   | Liver   | 43.5±8.54 a,b | 47.5±8.54 a,b | 8.56±0.44 c | 21.82±0.52 c | 21.82±0.52 c | 8.56±0.44 c | 0.01 | 0.05 |
| C. catla    | Brain   | 75.1±0.2 a,b | 78.5±0.2 a,b | 4.34±0.27 c | 15.51±0.64 c | 15.51±0.64 c | 4.34±0.27 c | 0.01 | 0.05 |
| C. batrachus| Muscle  | 8.6±0.4 a,b | 8.9±0.4 a,b | 0.79±0.04 c | 8.9±0.4 a,b | 7.13±0.08 a,b | 0.79±0.04 c | 0.01 | 0.05 |

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

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

| Species   | Organ   | Amylase (mg maltose liberated min⁻¹ mg⁻¹ protein) |
|-----------|---------|-----------------------------------------------|
|           |         | CD (control-dry) | TD (treatment-dry) | % of inc. / dec. (col D vs C) | CB (Control-breading) | TB (Treatment-breading) | % of inc. / dec. (p value among col. C, D, F, G) | MAX | MIN |
| A. testudineus | Intestine | 3.31±1.94 a,b | 3.74±1.94 a,b | 0.42±0.10 c | 13.05±0.63 b | 3.49±0.63 c | 0.64±0.03 c | 0.01 | 0.05 |
| L. rohita   | Liver   | 43.5±8.54 a,b | 47.5±8.54 a,b | 8.56±0.44 c | 21.82±0.52 c | 21.82±0.52 c | 8.56±0.44 c | 0.01 | 0.05 |
| C. catla    | Brain   | 75.1±0.2 a,b | 78.5±0.2 a,b | 4.34±0.27 c | 15.51±0.64 c | 15.51±0.64 c | 4.34±0.27 c | 0.01 | 0.05 |
| C. batrachus| Muscle  | 8.6±0.4 a,b | 8.9±0.4 a,b | 0.79±0.04 c | 8.9±0.4 a,b | 7.13±0.08 a,b | 0.79±0.04 c | 0.01 | 0.05 |

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

Table 3c: Responses of lipase and ALP activity of fish tissues in L. rohita, C. catla, C. batrachus and A. testudineus under CD, TB, CB and TD conditions.

| Species   | Organ   | Lipase (µg protein) | ALP (µg protein) |
|-----------|---------|---------------------|------------------|
|           |         | CB (Control-breading) | TB (Treatment-breading) | p value (among col. C, D, F, G) | MAX | MIN |
| A. testudineus | Intestine | 3.31±1.94 a,b | 3.74±1.94 a,b | 0.42±0.10 c | 13.05±0.63 b | 3.49±0.63 c | 0.64±0.03 c | 0.01 | 0.05 |
| L. rohita   | Liver   | 43.5±8.54 a,b | 47.5±8.54 a,b | 8.56±0.44 c | 21.82±0.52 c | 21.82±0.52 c | 8.56±0.44 c | 0.01 | 0.05 |
| C. catla    | Brain   | 75.1±0.2 a,b | 78.5±0.2 a,b | 4.34±0.27 c | 15.51±0.64 c | 15.51±0.64 c | 4.34±0.27 c | 0.01 | 0.05 |
| C. batrachus| Muscle  | 8.6±0.4 a,b | 8.9±0.4 a,b | 0.79±0.04 c | 8.9±0.4 a,b | 7.13±0.08 a,b | 0.79±0.04 c | 0.01 | 0.05 |

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

7. Acknowledgements
The authors are thankful to Deptt. Of Fisheries, Govt. of West Bengal and Chandimata Fish Farm, Khano, Purba Bardhaman, WB, India for rendering constant support and kindness in our research work at field level. We are also obliged to DST-FIST sponsored Deptt. Of Environmental Science, The University of Burdwan, for providing laboratory facilities for performing the analytical part of the research work.

8. Funding source
This research has no such involvement of any funding source(s) or sponsor(s) (Central/State Govt. / Public/ Undertakings etc.) to conduct the research program.

9. References
1. Zeisel, SH, Blusztajn K. Choline and Human Nutrition. Annual Rev. of Nutrition 1994;14(1):269-296. doi:10.1146/annurev.nu.14.070194.001413
2. Zeisel SH. Choline: an essential nutrient for humans. Nutrition 2000;16(7,8):669-671. doi:10.1016/s0899-9007(00)00349-x
3. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of Choline-Containing Compounds and Betaine in Common Foods. The Journal of Nutrition 2003;133(5):1302-1307. doi:10.1093/jn/133.5.1302
4. Zeisel SH, Niculescu MD. Perinatal Choline Influences Brain Structure and Function. Nutrition Reviews 2006;64(4):197-203. doi:10.1301/nr.2006.janr.197-203
5. David M, Mushiger SB, Shivakumar R, Philip GH. Response of *Cyprinus carpio* (Linn.) to sublethal concentration of cypermethrin: alterations in protein metabolic profiles. Chemosphere 2004;56(4):347-352. doi: 10.1016/j.chemosphere.2004.02.024
6. Wu P, Feng L, Kuang SY, Liu Y, Jiang J, Hu K et al. Effect of dietary choline on growth, intestinal enzyme activities and relative expressions of target of rapamycin and elf4e-binding protein2 gene in muscle, hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 2011;317(1-4):107-116. doi:10.1016/j.aquaculture.2011.03.042
7. Poston HA. Response of rainbow trout to soy lecithin, choline and autoclaved isolated soy protein. The Progressive Fish-Culturist. 1991;53(2):85-90. doi: 10.1577/1548-8640(1991)053<0085:RRTTTS>2.3.CO;2
8. Haard NF, Dimes LE, Arndt RE, Dong FM. Estimation of protein digestibility-IV. Digestive proteinases from the pyloric caeca of coho salmon (*Oncorhyncus kisutch*) fed diets containing soybean meal. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 1996;115(4):533-540. doi:10.1016/s0305-0491(96)00189-7
9. Fuentes-Quesada JP, Viana MT, Rombonzo AN, Guerrero-Rentería Y, Nomura-Solis M, Gomez-Calle V et al. Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. Aquaculture 2018;495:78-89. doi:10.1016/j.aquaculture.2018.05.025
10. Li JY, Li XF, Xu WN, Zhang CN, Liu WB. Effects of dietary choline supplementation on growth performance, lipid deposition and intestinal enzyme activities of blunt snout bream *Megalobrama amblycephala* fed high-lipid diet. Aquaculture Nutrition 2015;22(1):181-190. doi:10.1111/anu.12231
11. Yang DQ, Chen F, Ruan GL. Effect of dietary choline on the growth, tissue lipid content and activities of digestive enzymes of *Monopterus albus*. Journal of Fisheries of China 2006;30(5):676-682.
12. Kenari AA, Sotoudeh E, Rezaei MH. Dietary soybean phosphatidylcholine affects growth performance and lipolytic enzyme activity in Caspian brown trout (*Salmo trutta Caspius*) alevin. Aquaculture Research 2011;42(5):655-663. doi:10.1111/j.1365-2109.2010.02587.x
13. Kumar N, Jadhao SB, Chandan NK, Kumar K, Jha AK, Bhushan S et al. Dietary choline, betaine and lecithin mitigates endosulfan induced stress in *Laboe rohita* fingerlings. Fish physiology and biochemistry 2012;38(4):989-1000. doi:10.1007/s10695-011-9584-y
14. Alikunhi KH. Studies on Composite Fish Culture production by compatible combinations of Indian and Chinese Carps. J. Ind. Fish. Assoc 1972;1(1):26-27.
15. Chaudhuri H, Chakrabarty RD, Sen PR, Rao NGS, Jena S. A new high in fish production in India with record yields by composite fish culture in freshwater ponds. Aquaculture 1975;6(4):343-355. doi:10.1016/0044-8466(75)90113-1
16. Jhingran VG. Fish and Fisheries of India. Hindustan Publishing Corporation (India), Delhi. Hindustan Pub. Corp. (India) 1982; (DLC) 92900656 (OCoLC) 2530965.
17. Ayyappan S, Jena JK. Grow-Out Production of Carps in India. Journal of Applied Aquaculture. 2003;13(3,4):251-282. doi:10.1300/j02813n03_04
18. Ayyappan S, Ahmad-Ali S. Analysis of feeds and fertilizers for sustainable aquaculture development in India. In M.R. Hasan, T. Hecht, S.S. De Silva and A.G.J. Tacon (eds). Study and analysis of feeds and fertilizers for sustainable aquaculture development. FAO Fisheries Technical Paper 2007,497. Rome, FAO: 191–219.
19. APHA. In: Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. (Eds.). Standard Methods for the Examination of Water and Wastewater. 22th ed.
American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA 2012.

20. Bernfeld in, Colowick SP, Kaplan NO. Methods in Enzymology. Academy Press, New York. (Eds.); 1955:149-541.

21. Cherry IS, Crandall LA. The Specificity of pancreatic lipase: Its appearance in the blood after pancreatic injury. American Journal of Physiology-Legacy Content. 1932;100(2):266-273. doi:10.1152/ajplegacy.1932.100.2.266

22. Snell FD, Snell CT. Colorimetric methods of analysis. D. van Nostrand 1959.

23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry 1951;193:265-275.

24. Bergmeyer HU, Bowers-Jr GN, Horder M, Moss DW. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes Part 2. IFCC method for aspartate aminotransferase. Clinica Chimica Acta 1976;70(2):19-42. doi:10.1016/0009-8917(76)90437-x

25. Zar JH. Biostatistical Analysis, 5th ed. Pearson Education Singapore Pte. Ltd., New Delhi; (Indian Branch) 2010

26. Kinnear PR, Gray CD. SPSS 17 Made Simple. Psychology Press, East Sussex, UK 2009.

27. Fernandez I, Moyano FJ, Diaz M, Martinez T. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J. Exp. Mar. Biol. Ecol. 2001;262(1):1-12. doi:10.1016/s0022-0981(01)00228-3

28. Gabriel UU, Obomanu FG, Edori OS. Biochemical Changes in Hybrid Catfish (Heterobranchus bispinor x Clarias gariepinus) Treated with Nauracon. Chinese J. Appl. Environ. Biol 2010;16(3):353-357. doi:10.3724/SP.J.1145.2010.00353

29. Shamnugam A. Fundamentals of Biochemistry for Medical Students, College of Medical Science, Madras, 1977, 48.

30. Mai KS, Xiao LD, Ai QH, Wang XJ, Xu W, Zhang WB, et al. Dietary choline requirement for juvenile cobia, Rachycentron canadum. Aquaculture 2009;289(1-2):124-128. doi:10.1016/j.aquaculture.2009.01.016

31. Chen YJ, Liu YJ, Yang HJ, Yuan Y, Liu FJ, Tian LX et al. Effect of dietary oxidized fish oil on growth performance, body composition, antioxidant defence mechanism and liver histology of juvenile largemouth bass Micropterus salmoides. Aquaculture Nutrition 2012;18(3):321-331. doi:10.1111/j.1365-2095.2011.00900.x

32. Begum G. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish Clarias batrachus (Linn.) and recovery response. Aquatic Toxicology 2004;66(1):83-92. doi:10.1016/j.aquatox.2003.08.002

33. Hidalgo MC, Urea E, Sanz A. Comparative study of digestive enzymes in fish with different nutritional habitus. Proteolytic and amylase activities. Aquaculture 1999;170(3, 4):267-283. doi:10.1016/s0044-8486(98)00413-x

34. Deng J, Chen L, Mai K, Mi H, Zhang L. Effects of replacing soybean meal with rubber seed meal on digestive enzyme activity, nutrient digestibility and retention in tilapia (Oreochromis niloticus × Oreochromis aureus). Aquaculture Research 2016;48(4):1767-1777. doi:10.1111/are.13014

35. Klahan R, Areechon N, Yoonpundh R, Engkagul A. Characterization and Activity of Digestive Enzymes in Different Sizes of Nile Tilapia (Oreochromis niloticus L.). Kasetsart J. (Nat. Sci.). 2009;43(1):143-153.

36. Pavasovic A, Anderson AJ, Mather PB, Richardson NA. Effect of a variety of animal, plant and single cell-based feed ingredients on diet digestibility and digestive enzyme activity in red claw crayfish, Cherax quadricarinatus (Von Martens 1868). Aquaculture 2007; 272(1-4):564-572. doi:10.1016/j.aquaculture.2007.08.027

37. Jiang TT, Feng L, Liu Y, Jiang W-D, Jiang J, Li S-H, et al. Effects of exogenous xylanase supplementation in plant protein-enriched diets on growth performance, intestinal enzyme activities and microflora of juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture Nutrition 2014;20(6):632-645. doi:10.1111/anu.12125

38. Baragi V, Lovell RT. Digestive Enzyme Activities in Striped Bass from First Feeding through Larva Development. Transactions of the American Fisheries Society 1986;115(3):478-484. doi:10.1577/1548-8659(1986)115<478:deaibh>2.0.co;2

39. Harper HA, Rodwell VW, Mayes PA. Review of Physiological Chemistry. Large Medical Publications, California 1977.

40. Mukhopadhyay PK, Dehadrai PV, Banerjee SK. Studies on intestinal protease: Isolation, purification and effect of dietary proteins on alkaline protease activity of the air-breathing fish, Clarias batrachus (Linn.). Hydrobiologia 1978;57(1):11-15. doi:10.1007/bf00018622

41. Tiwari S, Singh A. Control of common freshwater predatory fish, Channa punctatus, through Nierium indicum leaf extracts. Chemosphere 2003;53(8):865-875. doi:10.1016/s0045-6535(03)00595-2

42. Maitra S, Ray AK. Inhibition of digestive enzymes in rohu, Labeo rohita (Hamilton), fingerlings by tannin: an in vitro study. Aquaculture Research 2003;34(1):93-95. doi:10.1046/j.1365-2109.2003.00792.x

43. Mandal S, Ghosh K. Inhibitory effect of Pustia tannin on digestive enzymes of Indian major carps: an in vitro study. Fish Physiology and Biochemistry 2010;36(4):1171–1180. doi:10.1007/s10695-010-9395-6

44. Yaghoubi M, Mozannazad MT, Marammazi O, Gisbert PK, Dehadrai PV, Banerjee SK. Effect of dietary replacement of fish meal by soy products (soybean meal and isolated soy protein) in intestine enzyme activities and microflora of juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture Nutrition 2014;20(6):632-645. doi:10.1111/anu.12125

45. Mandal S, Ghosh K. Inhibitory effect of Pustia tannin on digestive enzymes of Indian major carps: an in vitro study. Fish Physiology and Biochemistry 2010;36(4):1171–1180. doi:10.1007/s10695-010-9395-6

46. Villanueva J, Vanacore R, Goicoechea O, Anthauer R. Intestinal alkaline phosphatase of the fish Cyprinus carpio: Regional distribution and membrane association. The Journal of Experimental Zoology 1997;279(4):347-355. doi:10.1002/(sici)1097-0100x(19971011)279:4<347::aid-ajplegacy.100.co;2-o

47. Pradhan J, Das BK. Effects of Supplementation Diet Containing Microcystis Aeruginosa on Haemato logical and Biochemical Changes in Labeo Rohita Infected with...
Aeromonas Hydrophila. Journal of Aquaculture Research & Development 2015;6(3):1-5. doi:10.4172/2155-9546.1000315

48. Saha S, Kaviraj A. Effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish Heteropneustes fossilis. Chemosphere 2009; 74(9):1254-1259. doi:10.1016/j.chemosphere.2008.10.056

49. Sharma RM. Effect of endosulfan on acid and alkaline phosphatase activity in liver, kidney, and muscles of Channa gachua. Bulletin of Environmental Contamination and Toxicology 1990;44(3):443-448. doi:10.1007/bf01701227

50. Verma AK, Pal AK, Manush SM, Das T, Dalvi RS, Chandrachoodan PP et al. Persistent sub-lethal chlorine exposure elicits the temperature induced stress responses in Cyprinus carpio early fingerlings. Pesticide Biochemistry and Physiology 2007;87(3):229-237, doi:10.1016/j.pestbp.2006.08.001

51. El-Sayed YS, Saad TT. Subacute Intoxication of a Deltamethrin-Based Preparation (Butox®5% EC) in Monosex Nile Tilapia, Oreochromis niloticus L. Basic & Clinical Pharmacology & Toxicology 2008;102(3):293-299, doi:10.1111/j.1742-7843.2007.00157.x

52. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed. Association of Official Analytical Chemists, Inc., Andlid 2012.