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Supplementation of choline chloride directly in the pond water induced enhanced growth performance in Indian major carps and air-breathing fishes

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Abstract

The present study intended to investigate the effects of supplementation of choline chloride under direct field pond application in addition to farm-made-aqua-feed on two air-breathing species, *Clarias batracus* (Magur) and *Anabas testudineus* (Koi) as well as two Indian Major Carps (IMCs), *Catla catla* (Catla) and *Labeo rohita* (Rahu), cultured in a ratio of 2:5:1:1(Catla : Rahu: Magur : Koi) in three ponds: one normal pond (N) with farm-made-aqua-feed only and two with (P1 and P2: considered as T) farm-made-aqua-feed plus choline chloride. Choline chloride was applied directly into the pond water at the rate of 350 g bigha^{-1} per 15 days into the P1 and P2 ponds under a semi-intensive system of 90-d culture during the breeding season (June to August). A comparative study on growth performances based on growth parameters was considered. Growth parameters showed a significantly enhanced pattern in % WG (weight gain), SGR % (specific growth rate), survivability rate, PER (protein efficiency reatio), FE (feed efficiency), CF (condition factor), RLG (relative length of gut), and TP (total production), but a reverse pattern was observed in FCR (food conversion ratio), VSI (viscero somatic index) and IPF (intra- peritoneal fat ratio) both in IMCs and Air-breathing fishes under choline supplementation. An increasing trend in HSI (hepato-somatic index) and ISI (intesto-somatic index) in case of IMCs and declining trend of the same in air-breathing fishes were depicted under choline-applied (CA) groups of fishes. Under direct administration of choline into the pond water, the experimental fish species showed improved growth performances, which can subsequently cause an economic profit to the farmers due to increased production.

Keywords: choline chloride; growth performances; Indian major carps and air-breathing fishes

1. Introduction

Choline chloride ($C_5H_{14}NO.Cl$), an organic, thick, colour less and strongly alkaline substance is ascribed to be an essential vitamin (grouped within the B-complex family) for fishes [1, 2]. It is considered as an essential nutrient for cellular structure and function, recognized as the most essential dietary component to the growing and young vertebrates including fish and its requirement ranges from 50 to 100 or up to 3000 mg kg^{-1} in fish species [3]. It also functions to act as a precursor of betaine (methyl group donner) to synthesize methylated metabolites, acetylcholine (neurotransmitter), and phosphatidylcholine, while, it is revealed to be a major constituent of some plasmalogens, lecithin, and sphingomyelins and various plant and animal cells show its wide abundance in nature [4,5]. Requirement of choline in the animal body was indicated previously as it provides structural integrity of the cell maintenance, having certain metabolic functions, signaling functions of cell membranes; it directly affects the cholinergic neurotransmission, involving the transmission of nerve impulses across synapses, and also engaged in lipid transportation from the liver and normal muscle functioning [6, 7, 8]. It was reported that the decreased lipid content in liver and increased lipid in muscle in fish was found when the diet contains choline [9], whereas, the increased lipid content in liver was recorded by Shiau and Lo (2000) [10] and in whole-body by Wu *et al.* (2011) [11] under increasing dietary choline level; again, some authors opined that there is no significant influence of liver lipid content when the fishes fed with the diet containing choline [12, 13, 14]. Nevertheless, it is nothing but a lipotropic factor that prevents to accumulate the lipid abnormally to generate fatty liver syndrome [3, 15, 16]. Moreover, the hepatic lipidosis, haemorrhagic condition in the renal system, and intestine, anorexia have been reported by many researchers in the choline-deficient diet [17, 18]. It is observed from various studies that the choline-deficient diet influences the retardation of growth and poor feed efficiency, poor survivability and increased concentration of lipid in the in yellow perch (*Perca flavescens*) [13], juvenile cobia (*Rachycentron canadum*) [19], common carp (*Cyprinus carpio*) [2], channel catfish (*Ictalurus punctatus*) [20] and also in lake trout (*Salvelinus namaycush*) [15]. Poor digestion capacity and lowered capability of absorption resulted retarted growth in the fish, fed without choline [21, 22]. The present work is designed to assess the responses of direct supplementation of choline chloride into the pond water in an addition to farm-made-aqua-feed on the fish faunas (*Catla catla, Labeo rohita, Clarias batracus* and *Anabas testudineus*) of different food habits occupying different trophic strata cultured in the semi-intensive fish ponds. Analysis of growth performance, based on growth parameters was considered to disclose the mechanism behind it.

2. Materials and Methods

2.1. Experimental design at the field

The 90-d field experiment (Fig. 1) was fixed up at Khano village, Purba Bardhaman for in the breeding season, *i.e.*, from June to August where, two air-breathing species, *Clarias batracus* (Magur) and *Anabas testudineus* (Koi) as well as two Indian Major C*arps* (IMC) *Catla catla* (Catla) and *Labeo rohita* (Rahu) were taken as test fishes, having a culture ratio of Catla: Rahu: Magur: Koi = 2: 5: 1: 1. About 900 fish-species in the noted ratio were released per experimental pond (Three numbers of experimental ponds and each having effective water area (EWA): 0.20 acre *i.e.*, 0.05 bigha) The desired weight (g) and length (cm) was also maintained during stocking of fishes in each pond (Table 1).

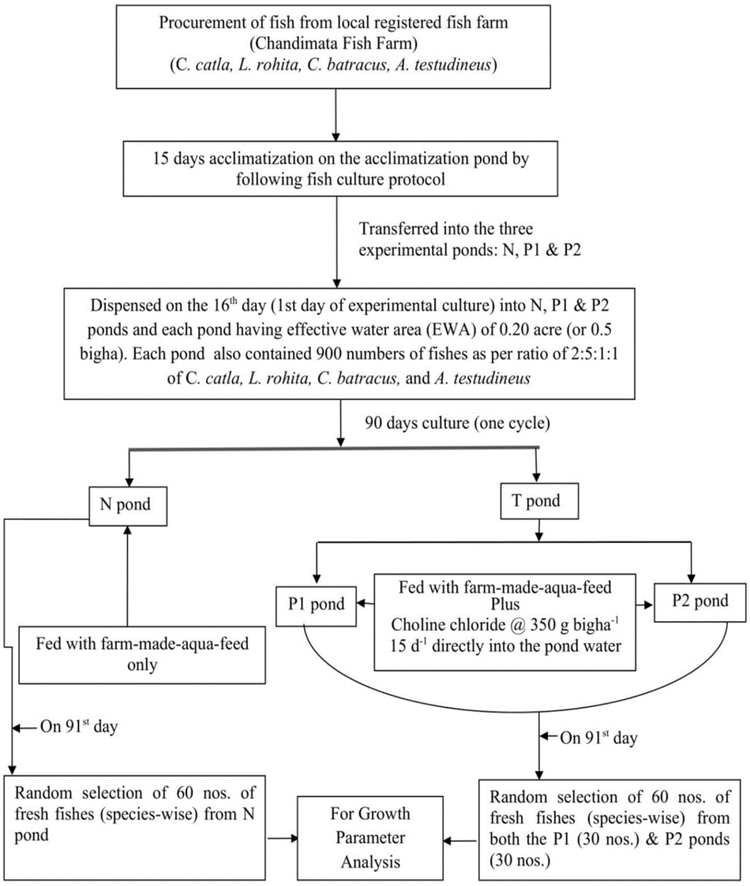

Fig 1: Experimental design at field

2.2. Collection of fish and culture practices

Species-wise sevral fishes as per ratio were taken for the present experiment from the nearby registered fish farm and acclimatization was done in the 'acclimatization pond' (a sepetate pond, other than experimental ponds) for a period of 15 days. To run the experiment smoothly and to obtain the desired stocking ratio, more than 30% of total required fish species were stocked for partial mortality in the acclimatization pond and also for getting desired stocking ratio during the course of experimentation. Before the experiment, selected ponds [both acclimatization (one) and three experimental [one normal (N) and two treatment (T) ponds, *i.e.*, P1 & P2)] were manured and fertilized as per the protocol of aquaculture [23, 24, 25, 26, 27], maintaining a definite depth of 5 ft. Experimental culture practice was conducted in these three experimental ponds *i.e.*, in N, P1 & P2 (Fig. 2) after transferring the species-wise fishes on the basis of desired culture ratio on 16th day of acclimatization from the acclimatization pond. The 16th day of acclimatization was considerd as the 1st day of experimental culture. Fishes of the both normal pond (N) and treatment (T: P1 & P2) ponds were fed with 35% protein containing 'farm-prepared' 'farm-made-aqua-feed', (Table 2) at the rate 4% of total biomass day^{-1} during the entire course of experimentation. Choline chloride of feed-grade (purity: 98%) quality (Meden Pharma Pvt. Ltd., Boisar-401506, Maharashtra) was administrated directly into the treatment ponds' water (P1 & P2) only at the rate of 350 g bigha^{-1} fortnightly. Furthermore, under this pond culture system, the experimental fishes fed with 'farm-made-aqua-feed' and also exposed to choline chloride in the treatment (T) ponds were denoted as CA (choline-applied) groups of fishes while, the fishes fed only with 'farm-made-aqua-feed' at the normal pond (N) were termed as CNA (choline-not-applied) groups of fishes for the convenience of description. Water quality parameters were monitored regularly (15 days of interval time) in all three experimental ponds under field conditions [28] and expressed in mean value. Average value was also considered in case of treatment ponds also (T as avg. of P1 & P2) [Table 3]. Before releasing the fish species into the respective ponds, the following conventional measurements of each fish individual were taken into account like initial total length (TLI), initial standard length (SLI), and initial mean live-body-weight (MWI).

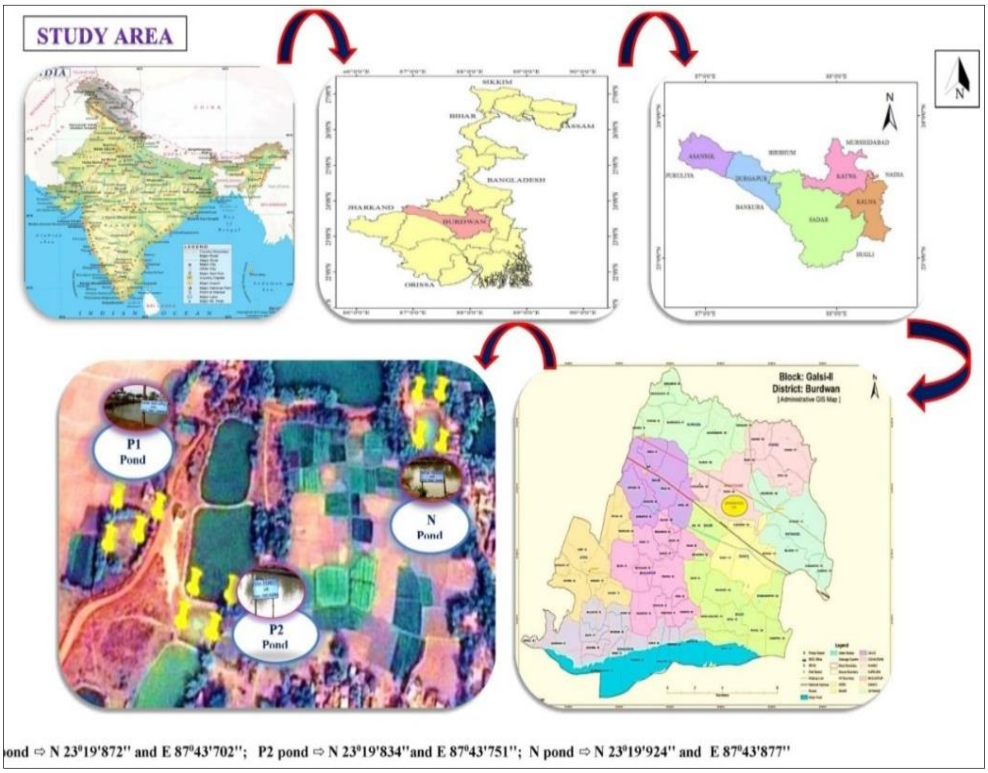

Fig 2: Experimental site at field condition

Table 1: Description of the experimental ponds and stocking composition of fish-species in the experimental ponds

Sl No	Status of the pond	Area: Total Area (TA)	Area: Effective Water Area (EWA)	Latitude & Longitude	Species cultured & Initial weight (g) during the liberation	Species stocking in each pond (number)	Approx. age of fish fauna during liberation into the experimental ponds (days)
1	Normal Pond (N)	0.56 Acre	0.20 Acre ≈0.08 Ha ≈ 0.50 Bigha	N $23^0$19'924" E $87^0$43'877"	*Labeo rohita* (Rahu): TLI:16.00±1.04 cm; SLI: 15.00±1.41 cm MWI: 60.00 ±1.14 g *Catla catla* (Catla): TLI: 17.00±0.90 cm; SLI: 15.20±1.00 cm MWI: 70.00 ±0.47 g *Clarias batrachus:* TLI: 8.80±0.92 cm; SLI: 7.50±0.85 cm (Magur) MWI: 18.00±2.93 g *Anabas testudineus* TLI:5.80±0.53 cm; SLI: 5.00±0.55 cm (koi) MWI: 10.00±1.54 g	*L. rohita*-500 *C.catla*-200 *C. batrachus*-100 *A. testudineus*-100	*L. rohita*-(58-63) *C. catla*-(65-70) *C. batrachus*-(68-73) *A. testudineus*-(45-50)
2	P1	0.62 Acre	0.20 Acre ≈ 0.08 Ha ≈ 0.50 Bigha	N $23^0$19'872" E $87^0$43'702"	*Labeo rohita* (Rahu): TLI:16.00±1.04 cm; SLI: 15.00±1.41 cm MWI: 60.00 ±1.14 g *Catla catla* (Catla): TLI: 17.00±0.90 cm; SLI: 15.20±1.00 cm MWI: 70.00 ±0.47 g *Clarias batrachus:* TLI: 8.80±0.92 cm; SLI: 7.50±0.85 cm (Magur) MWI: 18.00±2.93 g *Anabas testudineus* TLI:5.80±0.53 cm; SLI: 5.00±0.55 cm (koi) MWI: 10.00±1.54 g	*L. rohita*-500 *C.catla*-200 *C. batrachus*-100 *A. testudineus*-100	*L. rohita*-(58-63) *C. catla*-(65-70) *C. batrachus*-(68-73) *A. testudineus*-(45-50)
3	P2	0.70 Acre	0.20 Acre ≈ 0.08 Ha ≈ 0.50 Bigha	N $23^0$19'834" E $87^0$43'751"	*Labeo rohita* (Rahu): TLI:16.00±1.04 cm; SLI: 15.00±1.41 cm MWI: 60.00 ±1.14 g *Catla catla* (Catla): TLI: 17.00±0.90 cm; SLI: 15.20±1.00 cm MWI: 70.00 ±0.47 g *Clarias batrachus:* TLI: 8.80±0.92 cm; SLI: 7.50±0.85 cm (Magur) MWI: 18.00±2.93 g *Anabas testudineus* TLI:5.80±0.53 cm; SLI: 5.00±0.55 cm (koi) MWI: 10.00±1.54 g	*L. rohita*-500 *C.catla*-200 *C. batrachus*-100 *A. testudineus*-100	*L. rohita*-(58-63) *C. catla*-(65-70) *C. batrachus*-(68-73) *A. testudineus*-(45-50)

P1and P2: treatment (T) ponds - 1&2; N=North, E=East, TLI- total length (initial), SLI-standard length (initial), MWI- mean live-body-weight (initial) Ha=Hector, 1 Ha =2.47 Acre = 6.25 Bigha, Data are presented in Mean ± SD (n=60); 1 Bigha=40 Decimal=0.40 Acre

Table 2: Ingredients (g kg^{-1}), used to formulate the 'farm-made-aqua-feed', fed to the experimental fishes and proximate composition of the basal diet

Ingredients	g kg^{-1}	Nutritional content (g kg^{-1})		
Fish meal *	195	Dry matter	:	982
Soya meal*	130	Crude protein	:	350
Groundnut 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 and mineral premix **	80			
Sodium chloride *	10			

Crude protein, crude fat, crude ash and moisture content were measured value [41].

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 3: Comparative analysis of physicochemical parameters of water between choline-applied and choline-not-applied conditions

Parameter	Unit	N Pond	T Pond (Avg. of P1 & P2)
Trans	cm	20.74±0.57	18.00±0.20**
Temp	^{0}C	29.00±0.52	31.50±0.10**
EC	µS/cm	668.60±4.15	680.00±2.50**
pH		7.60±0.28	8.60±0.50**
CO_2	mg/l	5.10±0.35	4.55±0.40**
DO	mg/l	6.30±0.57	7.20±0.40**
TA	mg/l	319.40±1.39	342.50±2.60**
PO_4^{3-}	mg/l	0.80±0.02	1.30±0.10**
TH	mg/l	186.00±1.33	185.00±1.00**
Cl^-	mg/l	50.00±0.74	60.00±0.50**
NH_4^+-N	mg/l	0.81±0.07	1.22±0.10**
NO_3^--N	mg/l	0.36±0.04	0.75±0.15**
Na^+	mg/l	72.40±1.23	82.00±1.75**
K^+	mg/l	15.50±1.17	21.20±1.20**

Trans-Transparency, Temp-Temperature, EC-Electrical conductivity, DO-Dissolved oxygen, TA-Total alkalinity, TH-Total hardness, NH_4^+-N-Ammonical nitrogen, CO_2-Carbon-di-oxide, NO_3^--N- Nitrate nitrogen, Cl-Chloride Data are represented as mean ± SD (n=6). Samples taken 15 days interval (D15, D30, D45, D60, D75, and D 90). D=day. Mean Value of each datum of D15, D30, D45, D60, D75, and D90 is reflected in the table both for N and T ponds. For T pond: the average value of P1 and P2 is taken and represented. ['t'- test (Paired two sample of means) conducted; significance '**', when P<0.01 and '*', when P<0.05]. N pond = Fed with farm-made-aqua-feed only; T Pond= Fed with farm-made-aqua-feed + choline chloride into the pond water

2.3. Sampling

After end of the 90-d experiment, species-wise experimentated fishes were taken through ramdom selection with utmost care from N, P1, and P2 (n=60; 60 from N, 30 from P1 and 30 from P2 for analysis of growth performance. The final total length, final standard length and final live body weight were measured at each pond embankment immediately after catch. Data on liver weight, viscera weight, intestinal total length, intestinal wet weight, hepato-somatic index (HIS), viscera-somatic index (VSI), relative length of gut (RLG), intesto-somatic index (ISI), intra-peritoneal fat ratio (IPF), protein efficiency ratio (PER), specific growth rate (SGR%), weight gain (%WG), survivability rate, food conversion ratio (FCR), feed efficiency (FE), condition factor (CF) and finally the total production (TP) were registered [11, 29, 30, 31, 32].

2.4. Calculations and statistical Analysis

2.4.1 Growth parameter calculation

The growth parameters calculated in this experiment were determined by as follows:

Weight Gain (%WG) = 100 × [{Mean live body weight (final)-Mean live body weight (initial)} / Mean live body weith (initial)]

Specific Growth Rate (SGR % per day) = 100 ×[($ln W_1$-$ln W_0$/ (T_1-T_0)]

Survivability % = 100 × (Number of survived fish/Total number of fish stocked)

Protein Efficiency Ratio (PER) = Live weight gain (g)/Protein intake

Hepato-Somatic Index (HIS %) = 100 × [Liver weight (g)/ Final livebody weight (g)]

Food Conversion Ratio (FCR) = Dry weight of feed given (g) / Live weight gain (g) (W_1-W_0)

Feed Efficiency (FE) = 100 × [Wet weight gain (g)/feed intake (g)]

Condition Factor (CF) = [Final body weight (g) × {(body length (final)}^{3}×100] [33]

Viscero-Somatic Index (VSI %) = 100× [Viscera weight (g)/Final live body weight (g)]

Intra Peritoneal Fat ratio (IPF %) = 100× [Intra Peritoneal Fat weight (g) /Final body weight (g)] [29]

Relative Length of Gut (RLG %) = 100 × [Intestine length (final) (cm)/ Total body length (TL-final) (cm)]

Intesto-Somatic Index (ISI %) = 100 × [Wet Intestine Weight (final) (g)/Wet body weight (final) (g)]

Total Production (TP $Cycle^{-1}$ $bigha^{-1}$) (kg) = Live weight gain of total survived fishes $bigha^{-1}$
× number of cycles

Where, W_1 and W_0 are the final and initial live body weight (g) of fish and T_1 and T_0 are the final and initial time of culture (day); 1 cycle=90days

2.4.2. Statistical Analysis

Data were analyzed by using the statistical software SPSS package (version 22.0). 't'- test (Paired two sample of means) was carried out to compare the significant difference between choline supplemented with farm-made-aqua-feed and farm-made-aqua-feed only fed group of fishes. Data are represented as mean ± SD.

3. Results and observations

After 90 days of culture, the growth status of *L. rohita* and *C. catla* revealed a marked change significantly in the 'choline-applied'(CA) ponds under treatment condition, whereas, in *C. 38atrachus* and *A. testudineus* depicted lowered 38atrachu [Table 4].

In CA group of fishes, % WG, SGR %, PER, FE, CF, and TP showed maximum in *L. rohita* and minimum in *A. testudineus*. Furthermore, the survivability rate revealed higher in air-breathing fish species than Indian Major Carps (IMCs). FCR, VSI, and IPF values were decreased gradually showing highest in *L. rohita* and lowest in *A. testudineus*. Finally, the TP became maximum in *L. rohita* and decreased significantly in *A. testudineus* in comparison to their 'choline-not applied' (HIS) groups. Under choline supplementation, *L. rohita* showed significant (p<0.01) variation in % WG, SGR %, PER, FE, CF, HIS, TP, FCR, VSI and IPF and a moderate to low variation in RLG, survivability rate and ISI compared to its HIS groups. Under CA condition, the *C. catla* in comparison to *L. rohita*, showed significant (p<0.01) less values in % WG, SGR %, PER, FE, CF, HIS, TP, FCR, VSI, IPF, RLG and ISI, finally resulted higher production of *L. rohita*. Moreover, in CA condition, the air-breathing fish species indicated a reduced production compared to the choline supplemented IMCs. In the CA condition, in *C. 38atrachus*, there was a marked change in FE, IPF, and TP, but moderate to low variation in %WG, SGR%, PER, FE, CF, survivability rate, and RLG. Although, in choline-applied *A. testudineus* under treatment condition, there was more significant (p<0.01) variation in FE and TP, moderate in survivability rate, RLG, and comparatively low variation in %WG, SGR%, PER, CF were recorded. Results on FCR, VSI, and IPF showed significant (p<0.01) gradual reduction, but in *L. rohita* the FCR showed the maximum percentage of reduction, and in *C. catla, C.batracus* and *A.testudineus* it was also decreased as compared to the HIS group of fishes. VSI and IPF were dropped in all the choline supplemented fishes, but the *L.rohita* ascribed maximal reduction. Significantly, HIS and ISI depicted a statistical significance at p<0.01 level with an elevating trend in the case of Indian Major Carps and were maximized in *L. rohita* and minimized in *C. catla.*While these were declined significantly (p<0.01) in air-breathing fishes under choline supplementation [Table 5].

Table 4: Comparative analysis of growth performances in L. *rohita, C. catla, C. batracus* and *A. testudineus* between 'choline-applied' (CA) and 'choline-not-applied' (CNA) conditions

Sl No	Parameter	*L. rohita*		*C. catla*		*C. batracus*		*A. testudineus*	
		N	T	N	T	N	T	N	T
1	% weight Gain (% WG)	400.00±2.80	583.33±2.24 [a]	337.86±2.34	457.14±3.62 [a]	66.67±3.51	83.33±2.83 [a]	100.00±1.48	110.00±1.25 [a]
2	Specific Growth Rate (SGR % per day)	1.79±0.06	2.14±0.22 [a]	1.64±0.02	1.91±0.48 [a]	0.57±0.07	0.67±0.14 [a]	0.77±0.07	0.82±0.06 [a]
3	Survivability %	75.00±1.00	90.00±1.74 [a]	75.00±0.55	90.00±1.43 [a]	70.00±0.53	85.00±0.78 [a]	70.00±0.36	85.00±0.51 [a]
4	Protein Efficiency Ratio (PER)	10.13±0.57	13.87±0.45 [a]	25.86±1.80	32.99±1.35 [a]	5.42±0.78	5.91±0.61 [a]	3.62±0.55	3.76±0.31 [ns]
5	Hepato Somatic Index (HSI %)	2.67±0.08	3.90±0.18 [a]	2.77±0.78	3.97±0.35 [a]	6.67±0.23	4.55±0.18 [a]	5.00±0.18	3.57±0.23 [a]
6	Food Conversion Ratio (FCR)	0.93±0.02	0.53±0.02 [a]	0.96±0.04	0.59±0.07 [a]	19.05±1.79	12.55±1.30 [a]	22.86±4.19	17.11±2.96 [a]
7	Feed Efficiency (FE)	1.07±0.09	1.88±0.09 [a]	1.04±0.05	1.69±0.15 [a]	0.05±0.01	0.08±0.01 [a]	0.04±0.01	0.06±0.01 [a]
8	Condition Factor (CF)	5.72±0.51	22.50±1.35 [a]	8.63±1.54	21.23±1.38 [a]	0.09±0.05	0.12±0.06 [a]	0.02±0.01	0.02±0.00 [a]
9	Viscero Somatic Index (VSI, %)	8.33±0.49	4.27±0.22 [a]	9.14±2.88	5.38±0.25 [a]	26.67±4.91	20.61±3.39 [a]	32.50±4.72	26.19±4.03 [a]
10	Intra Peritoneal Fat ratio (IPF, %)	0.67±0.11	0.24±0.01 [a]	0.98±0.05	0.47±0.06 [a]	2.50±0.45	1.21±0.05 [a]	4.75±0.86	2.62±0.59 [a]
11	Ralarive Length of Gut (RLG, %)	524.15±15.14	592.11±14.11 [a]	203.81±6.19	224.27±12.91 [a]	63.38±7.84	77.42±8.38 [a]	23.00±3.34	26.00±4.08 [a]
12	Intesto Somatic Index (ISI, %)	1.67±0.06	2.20±0.44 [a]	1.63±0.53	2.05±0.81 [a]	5.00±0.28	3.03±0.18 [a]	4.00±0.27	2.38±0.19 [a]
13	Total Production (TP $Cycle^{-1}$ $bigha^{-1}$) (kg)	180.00±2.36	315.00±4.31 [a]	70.95±2.43	115.20±3.22 [a]	1.68±0.21	2.55±0.18 [a]	1.40±0.09	1.87±0.13 [a]

Data are reported as Mean ± SD. ['t'- test (Paired two sample of means) conducted; significance 'a', when P<0.01 and 'ab', when P<0.05] (n= 60), N-Choline-not-applied condition, T-choline-applied condition; 1 cycle=90d, 1 bigha=40 decimal=0.40 acre; ns-non-significant

Table 5: Responsive analysis of percent increase (+) or decrease (-) of different growth parameters in *L. rohita, C. catla, C. batracus* and *A. testudineus* between 'choline-applied' (CA) and 'choline-not-applied' (CNA) conditions (N vs T)

Sl. No		Parameters	*L. rohita*	*C. catla*	*C. batracus*	*A. testudineus*
1	Growth	% WG	45.83	35.30	24.99	10.00
2		SGR % per day	19.55	16.46	17.54	6.49
3		Survivability %	20.00	20.00	21.43	21.43
4		PER	36.92	27.57	9.04	3.87
5		HSI %	46.07	43.32	-31.78	-28.60
6		FCR	-43.01	-38.54	-34.12	-25.15
7		FE	75.70	62.50	60.00	50.00
8		CF	293.36	146.00	33.33	0.00
9		VSI %	-48.74	-41.14	-22.72	-19.42
10		IPF %	-64.18	-52.04	-51.60	-44.84
11		RLG %	12.97	10.04	22.15	13.04
12		ISI %	31.74	25.77	-39.40	-40.50
13		TP $Cycle^{-1}bigha^{-1}$ (kg)	75.00	62.37	51.79	33.57

4. Discussion

Inclusion of choline chloride into the pond water attributed a positive effect in the gwowth performance which has been indicated by the results of improved growth of the test fishes under choline supplementation, the findings of ourselves resembled like in juvenile red drum (*Sciaenops ocellatus*) **[34]**, on juvenile hybrid tilapia [10] and juvenile Jian carp (*Cyprinus carpio* var. Jian) [11]. In the present study, the %WG, SGR%, PER, FE, CF, and TP depicted highest in *L. rohita* and lowest in *A. testudineus* under choline exposure. On the other hand, HSI and ISI showed an increasing trend and were maximized in *L. rohita*; minimized in *C. catla*, while, the same was declined in air-breathing fishes under choline supplementation. Moreover, RLG depicted the elevating trend and was maximised in *C. batracus;* minimised in *C. catla* under choline exposure in comparison to their CA groups. This phenomenon occurred in the present study may be due to improved intestine condition and higher utilization of intake-food; as depicted in juvenile Jian carp (*C. carpio*) with the elevation of dietary choline [11]. In addition to that, the survivability rate was observed higher in air-breathing fish species than Indian Major Carps (IMC) under CA condtion. FCR, VSI, and IPF values were gradually declined, showing maximum in *L. rohita* and minimum in *A. testudineus*; finally, the TP displayed highest in *L. rohita* and decreased significantly in *A. testudineus* in comparison to their CNA groups. Addition of choline in the diet showed persistant weight gain, survivability rate, SGR% and FE in *C. carpio* [11], hybrid striped bass (*Morone saxatilis* × *Monrone chrysops*) [18] and in yellow perch [13]. So, it can be inferred that the live weight may be gained with the proper intake of protein and PER may also be enhanced in CA group of fishes due to its proper utilization [35]. Decreased IPF,VSI in all experimented fishes in the CA condition were obtained due to reduced lipid deposition in viscera, and body lipid accumulation for the abundance of phosphatidylcholine [31, 36], thus, resulting into depletion of FCR and elevation of PER and thus, finally, the total production (TP) was gained, similar to *Totoaba macdonaldi* when fed with increasing level of dietary soybean meal [32]. Similarly, enhanced fish growth has been attributed to *C. carpio, var*. Jian, when fed with xylanase exogenously [30] and probiotics (*Bacillus* strains) added L. *rohita* [37, 38]. Significant increased HSI, ISI in IMCs, and decreased HSI, ISI in air-breathing fishes under CA condition were the marked findings at field condition which may be due to supplementation of choline that enhanced hepatopancreas growth and development, leads to hike in HSI index in experimental-IMCs alike in *C. carpio*, *var*. Jian, fed with exogenous xylanase up-to an optimum level [30] and/or increase in pancreatic protein content alike boiler [39], and/or some metabolic and physiological acceleration in spawning phase as found in blunt snout bream (*Megalobrama amblycephal*), exposed to choline [31]; improved intestinal growth as found in *C. carpio var*. Jian, fed with choline [11]. Whereas, depletion of HSI and ISI index in case of air-breathing fishes under choline exposure possibly due to acceleration of oxidation rate of fatty acids in different tissues, leading to increased catabolism of lipid and able to reduce hepatic lipid accumulation, alike in choline supplemented juvenile cobia (*Rachycentron canadum*), in its diet [19], and/or reduced liver size owing to partially liver steatosis, as found in silver perch [40] after L-carnitine supplementation; partial flaccidity and thinner layer in the intestine, alike *Totoaba macdonaldi*, fed with high Soyabean meal [32].

5. Conclusion

Application of choline chloride directly into the pond water resulted into maximum production of *L. rohita* which was evidenced from its maximum feed intake, lowest FCR, highest SGR and CF values based on the emphasis on the experimental ratio of culture. Finally, it can be established that the fishes provided with this additional progenitor as supplementation into the pond water, resulted in an increased growth through higher SGR%, better survivability rate, better feed efficiency and decreased FCR, VSI and IPF index. So, the choline chloride administration into the ponds' water directly under field trial through water attributed congenial water quality and improved fish health with a higher yield and this attempt (*i.e.*, application procees of choline) was maiden in nature. Due to its ready bio-degradability, there would be no risk of excessive accumulation in any stage of the tropic level.

6. Conflict of Interest

Nil

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