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Evaluation of the energy budget of the juvenile fish *Cyprinus* carpio in acid waters

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Abstract
In the present study, the effect of aquatic acidification on the rates of energy consumption (Cr), absorption (Ar), conversion (Pr), and metabolism (Mr) were investigated on the juvenile fish *Cyprinus carpio* (10.18 ± 0.109 gr). The experimental fish *C. carpio* were tested in different acid waters (pH of 5.0, 5.8, 6.6, and control 7.2). The length of the experiment was 21 days for the evaluation of bio-energetics. The fish were nourished by the fresh beef liver. The experimental acidic (pH) media were maintained carefully throughout the research, after the experimentation the fish were subjected to the estimation of the bio-energetics. The IBP formula was followed to calculate the energy budget. The results have shown the declined Cr (69.75 J/g/day) and ($F_{3, 68}$ $p<0.05$); Ar (96.28 (J/g/day); and ($F_{3, 38,}$ $p<0.05$); Pr (-30.76 J/g/day); and ($F_{3, 54;}$ $p<0.05$), the hyper-Mr (5.09 Energy metabolized KJ/animal) and ($F_{3, 59}$ $p<0.05$) was obtained in the fish exposed to low pH 5.0, 5.8, and 6.6 respectively. The acidic waters adversely impair the Cr, Ar, Pr, and Mr of the fish were exposed to pH 5.0; 5.8; and 6.6. But the severe effects were found in the experimental fish, which were exposed to pH 5.0 and 5.8.

Keywords: *Cyprinus carpio*; low ph; aquatic acidification; growth and bioenergetics

Introduction
The acidic environs drastically altered the amount of food consumption, absorption, and conversion, and metabolism of the *Oreochromis mossambicus* (Ibrahim 2003) [25]. Acid waters severely damaged the major energetic components of the carbohydrate, lipids, and proteins of the fish (Ibrahim 2003). The acidic waters impaired various tissues of the fish *C. carpio* (Ibrahim 2020) [26]. The effect of acid waters affected the nutritional physiology of the fish (Ibrahim 2003, Vasco Motal *et al* 2018) [25, 62]. The acidic pH waters disturb the standard and routine metabolisms of the fish *O. mossambicus* (Ibrahim 2003) [25] *O.niloticus* (Mota *et al*., 2014), Environmental stress in the form of high acidic levels have resulted in decreased growth rates (Ibrahim, 2003; Beamish *et al*., 1975; Ryan and Harvey, 1977) [25, 6, 52]. Growth inhibition appears to be a common response in some fish species to acid stress and such inhibition is usually reported as an actual decrease in body weight (Ibrahim 2003; Beamish *et al*., 1974) [25, 4]. Though many works are available on the effect of low pH on the growth of brook trout (Fromm, 1980; Wood 1989) [18, 64]; yellow perch (Ryan *et al* 1980) [51]; Rainbows (Frost, 1940) [19]; brown trout (Richard & Einar Snekvik (1978) [49] and in rock bass (Schofield, 1976) [56]. McKim and Benoit (1971) [42] reported that food consumption was found to decrease in rainbow trout Salmo gairdneri when exposed to pH 6.0. Lacorix *et al*. (1985) [34] exhibited the reduced food consumption of Atlantic salmon when exposed to environmental low pH. Lemly and Smith (1985) [36] disclosed the declined food consumption of Fathead minnows at pH 5.5. Cleveland *et al*. (1989) [11] showed the decreased food consumption in brook trout. Tam *et al*. (1988) [59] reported the reduced food consumption on brook trout when exposed to pH 4.54 and Denny Buckler (1995) [15] also reported the reduction in food consumption in Atlantic salmon when exposed to pH 4.5, Rosseland (1980) reported accumulation of uneaten food when Atlantic salmon was exposed to pH 5.2.

Materials and Methods
The juvenile fish *Cyprinus carpio* (10.18 ± 0.109 gram) were tested in four different acidic pH environs, which were pH 5.0, 5.8, 6.6, and 7.2 (control). The experimental fish have been introduced into the troughs with a capacity of 20 lit. Each trough consists of experimental acidic media and three fish; a similar experimental design has followed in control also. The experiments were carried out in triplicates and conducted at room temperature (29 ± 1°C). Each day the experimental media were changed, the fresh acidic pH media were prepared and the experimental animals have transferred carefully on the daily basis.
The sacrifice method of Maynard and Loosli (1962) [41] was followed in the present investigation to estimate fish growth. Before commencing the experiments, the fish were subjected to starve for 24 hrs to empty the alimentary canal (Mohanty, 1990 a) [43]. Healthy fish were selected for the experimentation, the wet weight of the experimental fish were weighed by electronic analytical balance (@ 0.1mg accuracy), the weight has determined at the beginning of the experiment. The feces collection method of Pallab Kumer Sarker *et al* (2007) [45] was followed. The feces of the fish were collected and oven-dried. The beef liver was chosen as the food for the experimental fish. The beef liver was kept frozen during the experiment. Every day the frozen beef was taken out, defrosted, and the known quantity was weighed and cut into tiny pieces. Fish were fed with a known quantity of beef liver. The collection of uneaten food was done before changing the experimental media. The remnants of the uneaten food (beef liver) were collected from the respective experimental troughs, and they were oven-dried to calculate the dry weights of the unfed. Thus the dry weight of food consumed can be calculated, which was the difference between the dry weight of food given and that of uneaten food.

Every day the feces were collected (once), dried, pulverized, and kept in a desiccator for energy estimation. All the experiments lasted for three weeks. After the experimental period, the fish were starved for a period of 24 hrs. The final weights of the individual fish of each experimental series were taken and the fish were oven-dried. The dried fish were powdered and subjected to estimation of the energy.

Preparation of acid (low pH) media

The pH of the experimental freshwater (control) has gradually reduced to pH 6.6, 5.8, and 5.0 by adding 5% Sulfuric acid (H2So4), The prepared pH experimental media have stirred well by an electric stirrer, and the pH was measured exactly by a high sensitive digital pH meter (Labtronics tabletop pH meter- Model Number: Lt 5001) and the medium was under periodical test with a pen pH meter (Panomex) and ensured the constantly desired pH without the fluctuations. The pH was monitored vigilantly. Already several experiments were conducted on the effect of acidity and acidic trauma on the various physiological modifications in laboratory and field animals. In the laboratory observations, researchers have used acids, such as sulphuric acid (H_2So_4), hydrochloric acid (HCL), and nitric acid (HNo_3) to reduce the pH of the water medium into acid nature. The majority of the researchers used sulphuric acid as it is a mineral acid pollutant in the wild (Ibrahim 2003, 2020) [25, 26] Beamish and Harvey, (1972) [5]; Schofield (1976) [56].To reduce the water pH sulphuric acid was used by Fromm (1980) [18]; Ultsch (1981) [61]; Louise Milligan and Wood (1982) [37]; Hunn *et al.* (1987) [24]; Dheer *et al.* (1986) [16]; Gunn and Noakes (1987) [21], and Tam *et al.* (1988) [59]. Witters (1986) [63] used nitric acid to reduce the water pH and hydrochloric acid was used to reduce the water pH by Smith and Haines (1995) [58]. According to the researchers' views and their methodology about the preparation of low pH, media were as followed in the present experiments. In this investigation, sulphuric acid was used to prepare various experimental pH media. Based on the earlier reports in the present investigations also, sulphuric acid was used to prepare various experimental pH media (6.6, 5.8, and 5.0).

Acid tolerant bioassay

Preliminary experiments were conducted to find the effect of acidity on the selected fish *C. carpio*, Based on the acute lethality bioassay, it was found that the lethality bioassays were found to be not relevant for the present study. The range of acid tolerance was very minimum. When the experimental fish *C. carpio* were exposed to below pH 4.9, the mortality begins, at the minimum level, but it gradually increased when the pH was decreased to pH 4.7. For instance, the percentage of mortality in the fish were exposed to pH 4.80, 4.85, and 4.90 were 100%, 70%, and 30% respectively (Table 1 and Fig 1) in 24 hrs. The experiments revealed that juvenile fish *C. carpio* could tolerate the acidic stress above the pH above 4.9. The results have shown that pH 4.9 to 4.7 were acutely lethal to the test fishes. Based on this, different acidic pH media were selected (pH 5.0, 5.8, and 6.6) to investigate the effect of aquatic acidic pH on the bio-energetic parameters of the fish *C. carpio*, Further, it was found that at pH 5.0 and above, there was no mortality for 4 weeks of the experimental period.

Table 1: The percentage of mortality of *C. carpio* exposed to different acid pH media for 24, 48, 72, and 96 hrs.

Percentage of Mortalities				
pH	**24 Hrs.**	**48 Hrs.**	**72 Hrs.**	**96 Hrs.**
4.75	100	0	0	0
4.80	80	20	0	0
4.85	50	50	0	0
4.90	0	10	20	20
4.95	0	0	0	0

Energy estimation

Energy estimations for fish tissue samples were done by plain jacket oxygen bomb calorimeter (Toshniwal, India) and feces energy was estimated by wet combustion method, dried fish samples were blended into a homogeneous mixture for energy estimation, The necessary corrections were made for the wet combustion method as suggested by Job and Gerald (1969) [29]. The energy values are represented here as Joules or Kilo Joules (KJ).

Oxygen bomb calorie meter

The oxygen bomb calorimeter used in the present investigation is a plain jacket calorimeter (Craig *et al* 1977) [13] and Wet Combustion Method (Karzinkin and Tarkovskaya 1964) [31].

Energy budget

The energy budget followed here is the slightly modified IBP formula (Petrusewicz and Macfadyen, 1970) [47] represented as C = P + R + F, where C is the energy consumed, P the growth (Conversion), R the energy lost as heat due to metabolism and F the feces.

Energy consumed

It was estimated by subtracting the unfed from the energy supplied. Energy absorbed**;** was calculated by subtracting the feces energy from that of energy consumed. Energy metabolized; Energy metabolized was estimated by subtracting the energy converted from the energy absorbed.

Energy converted

Energy converted was determined by subtracting the energy of fish at the commencement of the experiment from the energy of fish after the termination of the experiment. The Cr; Ar; Pr and Mr were calculated by dividing the respective quantities of the products of the initial weight of fish (g) and the duration of the experiment (21 days).

- Consumption rate (KJ / g / day) = Energy consumed (KJ) / Initial wet wt. of fish (g) × days
- Absorption rate (KJ / g / day) = Energy absorbed (KJ) / Initial wet wt. of fish (g) × days
- Metabolic rate (KJ / g / day) = Energy metabolised / Initial wet wt. of fish (g) × days
- Conversion rate (KJ / g / day) = Energy converted (KJ) / Initial wet wt. of fish (g) × days

Efficiencies of absorption and conversion

- Absorption efficiency (Ae) (%) = Energy absorbed / Energy consumed × 00
- Gross conversion efficiency (K1) (%) = Energy converted / Energy consumed × 100
- Net conversion efficiency (K2) (%) = Energy converted / Energy absorbed × 100

- Gross conversion efficiency (K1) (%) = Energy converted / Energy consumed × 100
- Net conversion efficiency (K2) (%) = Energy converted / Energy absorbed × 100

Proximate analysis (Carcass)

Carcass body composition of the experimental fish were determined as follows: protein by Lowry *et al*., method (1951) [51], lipid by Bragdon method (1951) [7] and carbohydrate by Anthrone method (Carrol *et al*., 1956) [56]. and Automatic Proximate Analyser (Model APA2)

Statistical analysis

The obtained bioenergetics data were statistically analyzed by using Microsoft Excel statistical software and PAST (Paleontological Statistics- Hammer *et al* 2001) [22]. Data are presented as mean ± standard deviation. Statistical analyses were performed by one-way ANOVA which was applied to identify the differences between pH, whereas the significant differences were indicated at the 5% level.

Results

Lc_{50} value for *C.* bioassay was found to be not relevant for the present study. The tolerance of acidity (low pH) was in minimum ranges, when the experimental fish of *C. carpio* were exposed to below pH 4.9, the mortality begins, at the minimum level, but it gradually increased when the pH was decreased to pH 4.7. For instance, the percentage of mortality in the experimental fish were exposed to pH 4.80, 4.85, and 4.90 were 100%, 70%, and 30% respectively (Table 1 and Fig 1) in 24 hours. The experiments revealed obviously that the experimental fish could tolerate above pH 4.9. The results revealed that pH 4.9 to 4.7 was acutely lethal to the test fish. *C. carpio* were determined by the lab experiment which shows no mortality above pH 4.95 for 96 hrs, (fig. 1)

Bray-Curtis similarity index illustrates the analogous sway on the existence of the experimental fish.

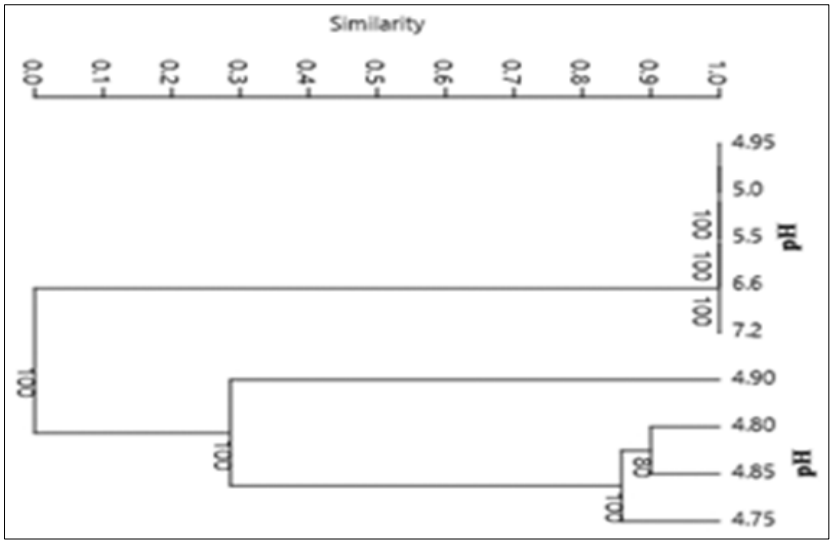

Fig 1: Bray-Curtis similarity index

Consumption rate (Cr)

The rate of energy consumption varied with different degrees of acidic pH. The observed results showed that fish exposed to low pH (pH 5.0; Table 2) exhibited a decrease in consumption rate than those exposed to other pH media (5.8 and 6.6; Table 2). Cr of *C. carpio* exposed to pH 5.0, 5.8, and 6.6 had lost the energy consumption were 69.75; 63.02 and 11.43 respectively J/g/day (Table 2). The statistical analysis (ANOVA, Table 6) revealed that the energy consumption rate (j/g/day) of the experimental animals exposed at four different pH are varying significantly ($F_{3, 68}$ $p<0.05$; table 6)

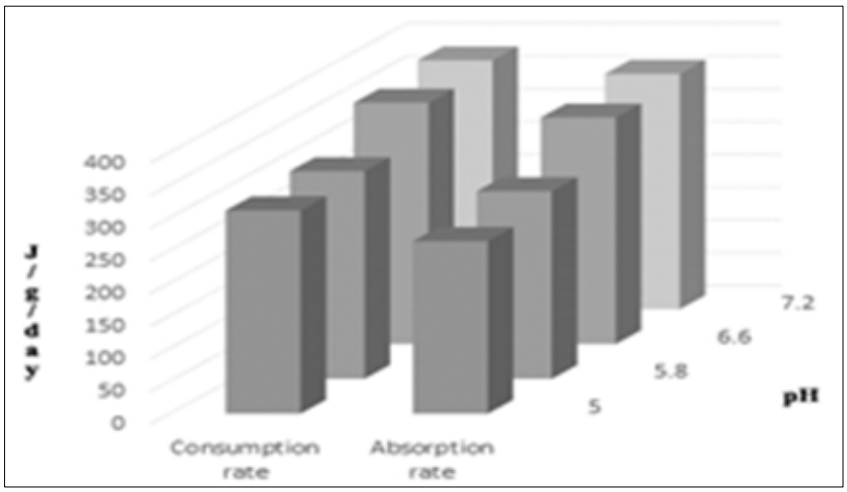

Fig 2: The consumption and absorption rates of *C.Carpio*, exposed to various pH (5.0, 5.8, 6.6, and 7.2)

Table 2: Effect of acidity on the rate of energy consumption of *Cyprinus carpio*, fed with beef for 21 days, Each value represents mean ± At least three replications

pH	Initial wet wt (g/animal)	Initial dry wt (mg/animal)	Consumption (mg/animal)	Energy consumed (KJ/animal)	Rate of energy consumption J/g/day
5.0	10.07 ± 0.32	2285.0 ± 0.19	3801.5 ± 150.61	65.85 ± 2.61	311.19 ± 2.50
5.8	10.27 ± 0.50	2362.0 ± 0.11	3961.5 ± 23.26	68.62 ± 4.03	317.92 ± 4.45
6.6	10.28 ± 0.14	2374.0 ± 0.18	4605.0 ± 5.65	79.77 ± 0.97	369.51 ± 4.02
7.2 (Control)	10.11 ± 0.10	2325.0 ± 0.16	4669.0 ± 9.89	80.87 ± 1.71	380.94 ± 2.39

Absorption rate (Ar)

Acidic environments affected the Ar of fish exposed to different acidic pH mediums (5.0, 5.8, and 6.6). The rate of absorption followed the trend of energy consumption. The rate of energy absorption linearly decreased (r=0.98) with increasing acidic pH. The results exhibited the decline of the Ar were 96.28; 73.10; and 13.46 (J/g/day) respectively when the fish were tested with pH 5.0, 5.8, and 6.6 (Table 3). The statistical analysis (ANOVA) revealed that the energy absorption rate (j/g/day) of the experimental animals exposed at four different pH are varying significantly ($F_{3, 38}$, $p<0.05$; table 6)

Table 3: Effect of acidity on the rate of energy absorption of *Cyprinus carpio*, fed with beef for 21 days, Each value represents mean ± At least three replications

pH	Initial wet wt (g/animal)	Energy consumed (KJ/animal)	Feces energy (KJ/animal)	Energy Absorbed (KJ/animal)	Rate of energy absorption (J/g/day)	Absorption efficiency (%)
5.0	10.07 ± 0.32	65.86 ± 2.61	9.96 ± 0.51	55.89 ± 3.12	264.06 ± 6.40	85.54 ± 0.95
5.8	10.27 ± 0.50	68.62 ± 4.03	6.63 ± 1.05	61.98 ± 2.97	287.24 ± 0.94	90.35 ± 0.96
6.6	10.28 ± 0.14	79.77 ± 0.97	4.88 ± 0.37	74.88 ± 2.80	346.88 ± 2.04	93.87 ± 1.46
7.2(Control)	10.11 ± 0.10	80.87 ± 1.71	4.37 ± 0.16	76.50 ± 0.51	360.34 ± 2.97	94.73 ± 0.14

Absorption efficiency (Ae)

The efficiency of absorption was significantly influenced by the acidic media of the experimental fish. The absorption efficiency has ranged from 85.54 ± 0.95 to 94.73 ± 0.41% in the experimental fish (Table 3).

Metabolic rate (Mr); the maximum metabolic rate was found in the fish tested in pH 5.0, the minimum was found in controls. The maximum metabolic rate of 294.83 ± 3.97 J/g/day was obtained in the fishes, which were exposed to pH 5.0. The metabolic rate increased linearly (r=0.94) with increasing acidity. The metabolic rates of the experimental fish exposed to pH 7.2, 6.6, 5.8 and 5.0 were 269.99 ± 4.40; 273.23 ± 7.45; 289.79 ± 1.30; and 294.83 ± 3.97J/g/day respectively (Table 4). The statistical analysis (ANOVA) revealed that the metabolic rate (j/g/day) of the experimental animals exposed at four different pH are varying significantly ($F_{3, 59}$ $p<0.05$, table 6).

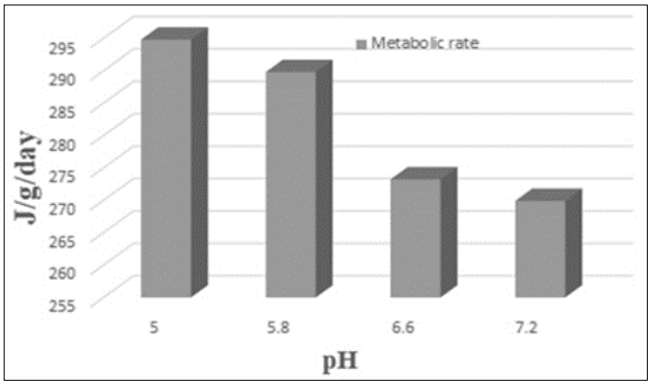

Fig 3: Metabolic rates of *C. Carpio*, exposed to pH 5.0, 5.8, 6.6, and 7.2

Table 4: Effect of acidity on the rate of energy metabolized of *Cyprinus carpio*, fed with beef for 21 days, Each value represents mean ± At least three replications

pH	Initial dry wt (mg/animal)	Final dry wt (mg/animal)	Initial energy (KJ/animal)	Final energy (KJ/animal)	Energy converted (KJ/animal)	Energy absorbed (KJ/animal)	Energy metabolized (KJ/animal)	Rate of energy metabolized (J/g/day)
5.0	2285.0 ± 0.186	2201.0 ± 28.23	35.49 ± 1.04	28.99 ± 0.20	-6.50 ± 0.31	55.89 ± 3.12	62.39 ± 2.81	294.83 ± 3.97
5.8	2362.0 ± 0.106	2356.5 ± 18.30	36.70 ± 1.65	36.15 ± 1.55	0.55 ± 0.31	61.98 ± 2.97	61.54 ± 3.07	289.79 ± 1.30
6.6	2374.0 ± 0.184	2798.0 ± 33.94	36.93 ± 0.20	52.67 ± 1.48	15.74 ± 1.68	74.88 ± 2.80	58.99 ± 2.17	273.23 ± 7.45
7.2(Control)	2325.0 ± 0.160	3090.5 ±134.50	35.62 ± 0.37	54.81 ± 0.60	19.19 ± 0.53	76.50 ± 0.51	57.30 ± 0.55	269.99 ± 4.40

Conversion rate (Pr)

Acidic trauma adversely affected the conversion rates in the experimental fish also. Fish exposed to pH 5.0 and 5.8 invariably showed a decline in conversion rates, For instance, the conversion rate in the experimental fish exposed to pH 7.2, 6.6, 5.8, and 5.0 were 90.41 ± 2.50; 72.94 ± 8.50; -2.54 ± 0.36 and -30.76 ± 2.43 J/g/day respectively (Table 5). The statistical analysis (ANOVA) revealed that the energy consumption rate (j/g/day) of the experimental animals exposed at four different pH are varying significantly ($F_{3, 54;}$ $p<0.05$)

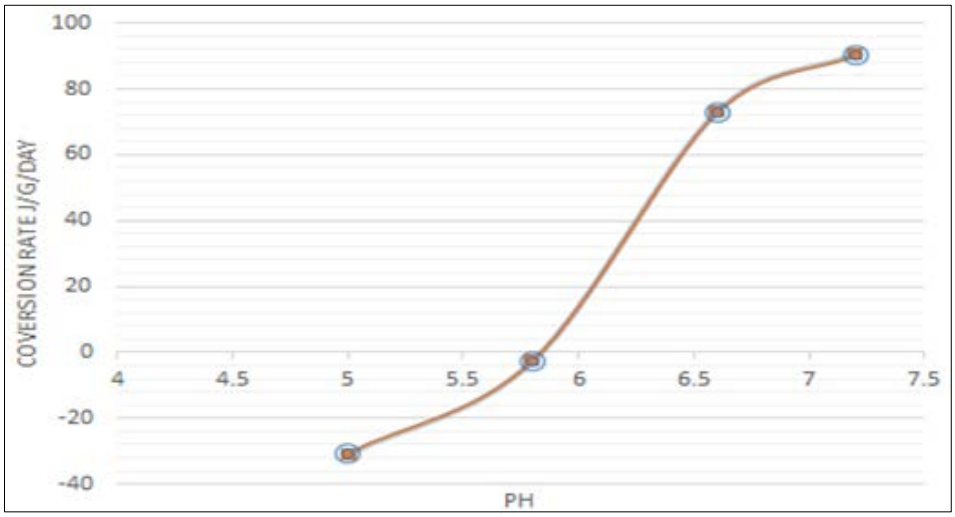

Fig 4 Conversion rates (Pr) of *C.carpio* were exposed to pH 5.0; 5.8; 6.6 and 7.2

Table 5: Effect of acidity on the rate of energy conversion of *Cyprinus carpio*, fed with beef for 21 days, Each value represents mean ± At least three replications

pH	Initial wet wt (g/animal)	Energy converted (KJ/animal)	Energy consumed (KJ/animal)	Energy absorbed (KJ/animal)	Rate of energy conversion (J/g/day)	Gross conversion efficiency K_1 (%)	Net conversion efficiency K_2 (%)
5.0	10.07 ± 0.32	-6.50 ± 0.31	65.85 ± 2.61	55.89 ± 3.12	-30.76 ± 2.43	-	-
5.8	10.27 ± 0.50	-0.55 ± 0.101	68.62 ± 4.03	61.98 ± 2.97	-2.54 ± 0.36	-	-
6.6	10.28 ± 0.14	15.74 ± 1.68	79.77 ± 0.97	74.88 ± 2.80	72.94 ± 8.50	19.73 ± 2.10	20.37 ± 1.95
7.2 (Control)	10.11 ± 0.10	19.19 ± 0.50	80.87 ± 1.71	76.50 ± 0.51	90.41 ± 2.50	23.72 ± 2.18	25.08 ± 2.44

Gross and net conversion efficiencies (K1 and K2)

Acid pH media have affected the gross and net conversion efficiencies of the fishes. Fish exposed to pH 5.0 and 5.8, invariably showed negative conversion efficiencies (K1 and K2), But the fish exposed to pH 6.6 and 7.2 exhibited their conversion efficiencies. The gross conversion efficiencies of the test fish exposed to pH 6.6 and 7.2 were 19.73 ± 2.10 and 23.72 ± 2.18% respectively (Table 5). The net conversion efficiencies of the fish exposed to pH 6.6 and 7.2 were 20.37 ± 1.95 and 25.08 ± 2.44% respectively (Table 5).

Biochemical (Proximate Analysis)

The acid waters influence the Protein, Lipid, and carbohydrates quantity of the experimental fishes, the obtained results were *Hypoproteinemia*, *Lipolysis*, and *Hypoglycemia* (Table 7 and 8) when the experimental fish were experienced with the acid environments (pH 5.0; 5.8; and 6.6) shown the decline in the percentage of the protein was 25.40; 13.40; and 2.00 respectively, similarly the same trends of reduction were found in both lipid and carbohydrate constituents (table; 7 and 8), So the experiments ascertained that different concentrations of the acidic pH have suppressed the growth and bioenergetics parameters of the fish living in the acidic environs.

Discussion

Despite ad libitum supply of the food, the acidity of the experimental media affected the Cr, Ar, Pr, and Mr. All the bioenergetics parameters except Mr which increased linearly with increasing acidity. In the present study, *C. carpio* exposed to different pH media (5.0, 5.8, and 6.6) exhibited a significant ($P < 0.05$) (ANOVA table 6) reduction in Cr. The least Cr was noticed in pH 5.0 followed by 5.8 and 6.6. The observed results clearly showed that low pH considerably upset the Cr in fishes. Like the observed results reduction of Cr in fish exposed to low pH is not rare in literature (Vasco C. Motal *et al* 2018) [62]. Ibrahim (2003) [25] has registered the results of the decline in the food consumption rate of tilapia, *O. mossambicus* when exposed to pH 5.0, 5.8, and 6.6. McKim and Benoit (1971) also reported that food consumption was found to decrease in rainbow trout *Salmo gairdneri* when exposed to pH 6.0. Lacorix *et al*. (1985) [34] exhibited the reduced food consumption of Atlantic salmon when exposed to environmental low pH. Lemly and Smith (1985) [36] disclosed the declined food consumption of fat head minnows at pH 5.5. Cleveland *et al*. (1989) [11] showed the decreased food consumption in brook trout. Tam *et al*. (1988) [59] reported the reduced food consumption on brook trout when exposed to pH 4.54 and Denny Buckler (1995) [15] showed the reduced food consumption in Atlantic salmon when exposed to pH 4.5. Rosseland (1980) reported accumulation of uneaten food when Atlantic salmon was exposed to pH 5.2. The observed results of the present study have highly supported the views of the researchers. Food intake constitutes secondary stress which modulates the direct effect of acid stress (Leivestad and Muniz, 1976) [35]. Effect of feeding inhibition especially reduced survival of life stages (Baker *et al*., 1990) [2]. Dennis Lemly (1986) [14] worked on fat head minnows and reported that acidity affected even visual feeding behavior and could be affected by an impairment of chemoreception. This may be the initial point for normal feeding responses because even if the selection of food items is based entirely on visual feeding cues, inadequate gustatory stimulation can lead to the rejection of food. Lemly and Smith (1985) [36] suggested that aquatic acidification can affect the chemoreception and modify the normal appropriate behavioral responses of fish to natural stimuli such as food odors. Behavioral studies indicate that the normal attraction of fish to chemical feeding stimuli can be eliminated when pH levels drop by a rather

modest pH (from 7.0-8 to 5.5-6.0). Aquatic acidification disrupts chemically mediated feeding behavior long before other more obvious symptoms of acid stress occur. The observed results of decreased consumption may be due to inhibition in chemoreception and chemical communication which form a very important component of the environmental physiology of fish. The trend obtained for absorption rate (Ar) also paralleled that of energy consumption rate in the tested fishes. Ar was highly affected in the experimental fish exposed to pH 5.0, followed by 5.8. the data showed a significant ($P < 0.05$) (ANOVA Table 6) reduction from the control when fish were tested in acidic medium pH 5.0. The decline in Ar may be due to physiological stress. The acidic stress the assaults the tissues of the digestive system, especially the intestine showed the abnormalities of chronic inflammation of laminapropria, which led to a massive accumulation of macrophages, necrosis, and atrophy of the intestinal villi when exposed to pH 5.8 experimental media exhibited, the hyper vacuolations of the intestinal mucus membrane, mucosal necrosis of absorptive cells and submucosal edema and fish were exposed to pH 5.0 test media showed massive sloughing off mucosal epithelium and accumulation of macrophages. This leads to the impairment of the function of the intestinal villi, and the absorptive area of the intestine has failed to absorb the nutrients (Ibrahim 2020) [26], Fish under acidic stress exhibited hypersensitivity in their physiological process. Jones *et al*. (1985) [30] reported in Arctic char (*Salvelinus alpinus*) subjected to the exposure of pH 5.5 showed hyperactivity in response to acid exposure. Absorption efficiency has been used by previous workers as energy extraction efficiency or assimilation efficiency (Ibrahim 2003). Absorption efficiency varied between 85.54 to 94.73% in individuals of C. *carpio*. The results of the present investigation were very closer to the reports of Arunachalam (1985) [1] and Sakthivel and Sampath (1989) [53] Ibrahim (2003) [25]. However many others have reported that Ae values ranged from 20-98% for different fishes. Absorption efficiency was 20% in *Ctenopharyngodon idella* (Fischer, 1972 a) [17], 65.30 to 78.30% in carp, (Haniffa and Arulselvan, 1992) [23], 88.2% in *Cirrhinus mrigala* (Ramachandran 2003) [48] 85.5% in trout, (Brocksen *et al*., 1968) [8], 86.44 to 97.88% in *Megalops cyprinodes*, and 87.07 to 94.30% in *H. fossilis*, (Marian *et al*., 1982) [40], 89.90 to 91% in *Macropodus cupanus*, (Manoharam, 1984) [39], 89 to 92.3 in *C. straiatus*, (Sampath, 1985) [54] 89.76 to 91.65% in *H. fossilis*, (Arunachalam *et al*., 1985) [1], 90.33% *in S. fontinalis* (Job, 1960) [28], 92.77% in Anguila species (Tarr and Hill, 1978) [60], 93.66 to 96.23% in Tilapia mossambica (Narayanan, 1980) [44] and 95.43% in *C. carpio* (Jeyaseeli, 2000) [27] 65.16 % to 93.88%, *O.mossambicus* (Ibrahim2003) [25], The severe acidic stress fetch up the reduced growth rates in the fish exposed to the different acidic media of pH 5.0 and 5.8.In addition, the proximate analysis also being evident for the impact of acidity on the growth. The observed results from the experiments revealed the fall in growth. In acidic stress, fish are supplied with an ad libitum diet. But the fish did not consume adequately. So the intake of energy is very less, it was inadequate to maintain their physiological process. In this stressful situation, fish have to spend more of their energy to tackle or overcome the acidic stress. But the consumed energy is very less. To compensate for the depletion of energy from consumption, the fish get their energy from their body reserves and lassitude. Depletion of energy from the body leads to lessened growth. The obtained results of the present study corroborated with previous results. Ibrahim (2003) [26] registered the declined growth of *O. mossambicus* tested in pH of 5.0; 5.8 and 6.6. Baker and Schofield (1982) [3]; Saunders *et al*. (1983) [55]; Lacorix *et al* (1985) [34] and Perry (1990) [46] reported the reduced growth of Atlantic salmon exposed to pH 4.5; Cleveland *et al*. (1986, 1989, 1991) [10, 11, 12] reported the decreased growth of brook trout when exposed to the acidic environment; Hunn (1987) [24] reported the reduced growth of brook tout exposed to low pH; Geen *et al*. (1985) [25] reported the decreased growth of Chinook salmon, Saunders *et al*. (1983) [55] reported the reduction in the growth of Atlantic salmon, Lacorix (1985) [34] suggested the decreased growth of Atlantic salmon (Salmo salar), in increased acidity Perry (1990) [46] reported the reduced growth of Atlantic salmon in the experiments of chronic effects of low pH. Beamish (1974) [4] reported the declined growth in white suckers (*Catostomus commersoni*) similarly, Ryan and Harvey (1980) [51] also reported the growth of yellow perch was reduced in acidified lakes. Beamish *et al*. (1975) [6] reported that growth inhibition appeared to be a common response in some fish species to acidic stress. Such inhibition is usually reported as an actual decrease in body weight. Beamish *et al*. (1975) [6] reported in white sucker (*Catostomus commersoni*) was documented that weight loss was associated with linear growth. Inhibition of linear growth suggests a lack of somatotropin and weight loss implies abnormalities in nutrient metabolism. The loss of metabolites from the body leads to the loss of weight. The loss of growth in the present investigation may be due to the loss of metabolites. Again growth may also decrease because of the increased metabolic rate due to the acidic stress. The experimental fish attained the maximum rate of metabolism when they were exposed to pH 5.0. The rate of absorption followed the trend of consumption in both species whereas the rate of metabolism increased with increasing acidity. The pH has influenced the metabolic rate of the animal through consumption. Thus the fluctuation in consumption rate is more apparent than those observed in conversion rates. This again reinforces the results of both species. The influence of pH is more predominant on the various bio-energetics parameters. Smith *et al*. (1995) [58] reported that Atlantic salmon showed the increased metabolic rates when exposed to acid environmental pH 5.2 - 5.4. The observed results were corroborated with the results of Smith *et al*. (1995) [58].

Table 6: ANOVA for Consumption rate (Cr), Absorption rate (Ar), Metabolic rate (Mr), and Conversion rate (Pr) as a function of acidity of *C. carpio*

Source of variation	SS	df	MS	F	P-value
	Consumption rate				
Between pH	14848.460	3	4949.487	68.677	P < 0.05
Error	432.410	6	72.068		
Total	16990.830	11			
	Absorption rate				
Between pH	33089.040	3	11029.680	38.210	P < 0.05
Error	1731.922	6	288.653		
Total	34891.530	11			
	Metabolic rate				
Between pH	3011.831	3	1003.944	59.037	P < 0.05
Error	102.030	6	17.005		
Total	6335.501	11			
	Conversion rate				
Between pH	54814.540	3	18271.510	54.068	P < 0.05
Error	2027.608	6	337.935		
Total	59785.57	11			

Table 7: Effects of acidity on the carcass body composition (proximate) of *C. carpio*. The Values are given as the percentage dry weight. Each value represents mean ± SD of at least three replications

pH	Protein %	Lipid %	Carbohydrate %
5.0	40.23 ± 0.166	8.62 ± 0.240	1.74 ± 0.750
5.8	46.70 ± 0.179	09.22 ± 0.069	2.11 ± 0.025
6.6	52.83 ± 0.166	10.60 ± 0.034	2.30 ± 0.025
7.2 (Control)	53.91 ± 0.101	10.93 ± 0.115	2.56 ± 0.061

Table 8. ANOVA for the carcass body composition (Protein, Lipid and Carbohydrate) as function of the acidity of *C. carpio.*

Source of variation	SS	df	MS	F	P-value
	Protein				
Between pH	6.557	2	3.278	2.305	P < 0.05
Error	8.531	6	1.421		
Total	530.040	11			
	ipid				
Between pH	0.995	2	0.497	127.615	P < 0.05
Error	0.023	6	0.003		
Total	12.992	11			
	Carbohydrate				
Between pH	0.094	2	0.047	17.908	P < 0.05
Error	0.0160	6	0.003		
Total	1.530	11			

Conclusions

The experiments were conducted to study the effect of acid waters (low pH) on the bioenergetics of the juvenile fish Cyprinus carpio. The pH media prepared for the present study, such as 5.0, 5.8, and 6.6 were prepared by mixing sulphuric acid with water. The rate of energy consumption decreased linearly with increasing acidity in the media in the experimental fish. Percentage reduction in energy consumption in C. Carpio was exposed to pH 5.0. The rate of energy absorption sincerely followed the rate of energy consumption. The absorption was highly affected in the experimental fishes exposed to pH 5.0 followed by pH 5.8. Acidic media severely affected the absorption efficiency, particularly, when the fishes were exposed to pH 5.0 and 5.8. Experiments clearly showed that the reduction in growth was due to decreased consumption, absorption, and absorption efficiency in the experimental fish. The maximum rate of metabolism was noticed in the experimental fishes when they were exposed to pH 5.0 and followed by pH 5.8. The rates of absorption have followed the trends of consumption rate, whereas the rate of metabolism increased with increasing acidity.

Abbreviations

C. carpio-Cyprinus carpio

Ar - absorption rate

Cr - energy consumption rate

KJ - Kilo Joules

Mr - metabolism rate

Pr - conversion rate

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  This research program didn't get any funds from any institution.
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  The authors declare that they have no competing interests
- Availability of data and materials
  Not applicable
- Code availability (software application or custom code)
  Microsoft Excel statistical software and PAST (Paleontological Statistics- Hammer *et al* 2001) [22]
- Authors Contribution
  Ibrahim (The first author) designed and coordinated this research and drafted the manuscript, Narayanan carried out the experiments and Ramachandran has involved in the statistical analysis
- Ethics approval and consent to participate
  Not Applicable
- Consent to participate (include appropriate statements)
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- Consent for Publication
  Not applicable

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"Guru Brahma, Guru Vishnu, Gurudeva Maheshwara, Guru Satshath Parabrahma Tasmi Shree Guruve Namah" is a mantra to honor the teacher in Indian tradition, In Sanskrit, Guru stands for the teacher who is the remover of darkness and is the harbinger of enlightenment. Guru Brahma - Guru is Brahma, who is the Lord of Creation, also called as Generator, Guru Vishnu means Guru is Vishnu (Vishnu is the Lord who is called organizer), Guru Devo Maheshwarah means Guru is the Maheshwara (Shiva or the destroyer), Guru Sakshat Parabrahma means Parbrahma *viz*. the supreme god or almighty. Since Guru leads to a path of light, Guru is that Para Brahma. Tasmai Shree Guruve Namah means we bow to that Guru the guru referred to earlier.

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