Evaluation of the Energy Budget of the Fish Cyprinus Carpio in Acid Waters

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Abstract

The present study was focused on the influence of acid waters on the energy budget of the fish Cyprinus carpio. The experimental fish were tested in the experimental media (pH 5.0, 5.8, 6.6, and control 7.2) for 21 days for the bioenergetics evaluation. The fishes were fed by the fresh beef liver. The pH was upheld vigilantly throughout the investigation, after the experimental period, the results revealed the declined rates of food consumption (69.75 J/g/day), absorption (96.28 (J/g/day), conversion (-30.76 Rate of energy conversion (J/g/day), and the hyper-metabolic rate (5.09 Energy metabolized KJ/animal) was observed in the fishes exposed to low pH 5.0, 5.8, and 6.6 respectively.

Introduction

The acidic environs drastically altered the amount of food consumption, absorption, and conversion, of the *Oreochromis mossambicus* (Ibrahim 2003). The hyper acidic stress diminishes the energetic matters of the carbohydrate, lipid, and proteins of the tissues of fishes (Ibrahim 2003). The deleterious feat of acidity adversely upsets the various tissues of the fish *C.Carpio* (Ibrahim 2020) The effect of acid waters affects the nutritional physiology of the fishes (Ibrahim 2003, Mota et al 2018). The acidic pH waters disturb the standard and routine metabolisms of the fish *O.mossambicus* (Ibrahim 2003) *O.niloticus* (Mota et al., 2014), Environmental stress in the form of high acidic levels has resulted in decreased growth rates (Ibrahim, 2003, Beamish et al., 1975, Ryan and Harvey, 1977, Abbink et al., 2012, Kennedy & Picard, 2012). 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). Though many works are available on the effect of low pH on the growth of brook trout (Fromm, 1980, Wood and Mc Donald, 1982) (Ryan, 1980) yellow perch, (Frost, 1940) Norwegian salmonids (Jenson Snekvik, 1972) brook charr (Schofield, 1976) and in rock bass (Ryan and Harvey, 1977), McKim and Benoit (1971) reported that the food consumption was found to decrease in rainbow trout Salmo gairdneri when exposed to pH 6.0. Lacorix et al. (1985) exhibited the reduced food consumption of Atlantic salmon when exposed to environmental low pH. Lemly and Smith (1985) disclosed the declined food consumption of Fathead minnows at pH 5.5. Cleveland et al. (1989) showed the decreased food consumption in brook trout. Tam et al. (1988) reported the reduced food consumption on brook trout when exposed to pH 4.54 and Denny Buckler (1995) also reported the reduction in food consumption in Atlantic salmon when exposed to pH 4.5 and growth reduction of 40% for seabass cultured at a pH of 5.5 (Lemarié et al. 2000) also reported. Rosseland (1980) reported accumulation of uneaten food when Atlantic salmon was exposed to pH 5.2.

Materials And Methods

Cyprinus carpio (10.18 ± 0.109 gram) were subjected to four different acidic environments, namely pH 5.0, 5.8, 6.6, and 7.2 (control). The capacity of the experimental trough was 20 L each. All the experiments were carried out in triplicates and conducted at room temperature (29 ± 1°C). The sacrifice method of Maynard and Loosli (1962) was followed in the present investigation to estimate fish growth. After the complete evacuation of their alimentary canal by starving them for at least 24 hrs (Mohanty, 1990) healthy fishes were selected and the wet weight of the experimental fishes were determined at the beginning of the experiment by electronic analytical balance (@0.1mg accuracy). The feces released by the fishes were filtered and oven-dried. The beef liver was kept frozen during the experiment. Every day the frozen beef was taken out, thawed, and the known quantity was weighed and cut into pieces. Fishes were fed with a known quantity of beef. The remnants were collected from the respective experimental troughs the next day before changing the water medium and 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. Feces collected every day (once) were dried powdered and kept in a desiccator for further analysis. 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 fishes were oven-dried. The dried fishes 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) Beamish and Harvey, 1972, Schofield, 1976). To reduce the water pH sulphuric acid was used by Fromm (1980), Ultsch (1981), Louisemilligan and Wood (1982), Hunn *et al.* (1987), Dheer *et al.* (1987), Gunn and Noakes (1987), Sadler and Lynam Wood (1987), Tam *et al.* (1988) and Vanduk *et al.* (1993). Witters (1986) used nitric acid to reduce the water pH (1986) and hydrochloric acid was used to reduce the water pH by Smith and Haines (1995). 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 acidic (low pH) stress on the selected experimental fishes *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 acidity tolerance was very minimum. When the experimental fishes 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 for 24 hrs in *C. carpio* exposed to pH 4.80, 4.85, and 4.90 were 100%, 70%, and 30% respectively (Table 1 and Fig 1). 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 fishes. Based on this different pH media were selected (pH 5.0, 5.8, and 6.6) for studying the influence of low pH on the various physiological parameters of experimental animals. Further, it was found that at pH 5.0 and above, there was no mortality for 4 weeks of the experimental period.

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). 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 1978) and Wet Combustion Method (Karzinkin and Tarkovskaya 1964 and Craig et al 1978).

Energy budget

The energy budget followed here is the slightly modified IBP formula (Petrusewicz and Macfadyen, 1970) 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, 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. Rates of energy consumption, absorption, conversion, and metabolism 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 × 100

- 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 fishes were determined as follows: protein by Lowry et al., method (1951), lipid by Bragdon method (1950) and carbohydrate by Anthrone method (Carrol et al., 1956).and Automatic Proximate Analyzer (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). 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 range of acidity tolerance was very minimum. When the experimental fishes 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 for 24 hrs in *C. carpio* exposed to pH 4.80, 4.85, and 4.90 were 100%, 70%, and 30% respectively (Table 1 and Fig 1). 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 fishes. *carpio* was 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.

Consumption rate (Cr)

The rate of energy consumption varied with different degrees of acidic pH. The observed results showed that fishes 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)

Absorption rate (Ar)

Acidic environments affected the Ar of fishes 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 fishes were tested with pH 5.0, 5.8, and 6.6 (Table 2). 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)

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 fishes 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 fishes 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 and Fig 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).

Conversion rate (Pr)

Acidic trauma adversely affected the conversion rates in the experimental fishes also. Fishes exposed to pH 5.0 and 5.8 invariably showed a decline in conversion rates, For instance, the conversion rate in the experimental fishes 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 and Fig 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 ($F_{3, 54,}$ $p<0.05$)

Gross and net conversion efficiencies (K1 and K2)

Acid pH media have affected the gross and net conversion efficiencies of the fishes. Fishes exposed to pH 5.0 and 5.8, invariably showed negative conversion efficiencies (K1 and K2), But the fishes exposed to pH 6.6 and 7.2 exhibited their conversion efficiencies. The gross conversion efficiencies of the test fishes 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 fishes 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 fishes 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 fishes 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 fishes exposed to low pH is not uncommon in literature (Mota et al 2018). Ibrahim (2003) 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) exhibited the reduced food consumption of Atlantic salmon when exposed to environmental low pH. Lemly and Smith (1985) disclosed the declined food consumption of fat head minnows at pH 5.5. Cleveland et al. (1989) showed the decreased food consumption in brook trout. Tam et al. (1988) reported the reduced food consumption on brook trout when exposed to pH 4.54 and Denny Buckler (1995) 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 highly support the views of the researchers. Food intake constitutes secondary stress which modulates the direct effect of acid stress (Leivestad and Muniz, 1976). Effect of feeding inhibition especially reduced survival of life stages (Baker et al., 1990). Dennis Lemly (1986) 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) 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 cause disruption of 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 fishes 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 fishes 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 fishes 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), Fishes under acidic stress exhibited hypersensitivity in their physiological process. Jones et al. (1985) strongly 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). Pandian and Delvi (1973) conveniently distinguished the energy extraction efficiency from absorption/assimilation efficiency and the discussion of the efficiency of absorption was followed here as it was by Pandian and Delvi (1973). 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) and Sakthivel and Sampath (1989) Ibrahim (2003). However many others have reported that Ae values ranged from 20 - 98% for different fishes. Absorption efficiency was 20% in *Ctenopharyngodon idella* (Fischer, 1972), 65.30 to 78.30% in carp, (Haniffa and Arulselvan, 1992), 88.2% in *Cirrhinus mrigala* (Ramachandran 2003) 85.5% in trout, (Brocksen et al., 1968), 86.44 to 97.88% in *Megalops cyprinodes,* and 86.58 to 93.33% in *Ophiocephalus straiatus*, (Pandian, 1967, a, b), 87.07 to 94.30% in *H. fossilis*, (Marian et al., 1982), 89.90 to 91% in *Macropodus cupanus,* (Manoharam, 1984), 89% in mirror carp Ivlev (1939), 89 to 92.3 in *C. straiatus*, (Sampath, 1985) 89.76 to 91.65% in *H. fossilis*, (Arunachalam et al., 1985), 90.33% *in S. fontinalis* (Job, 1960), 92.77% in Anguila species (Tarr and Hill, 1978), 93.66 to 96.23% in Tilapia mossambica (Narayanan, 1980) and 95.43% in *C. carpio* (Jeyaseeli, 2000) 65.16 % to 93.88% *O.mossambicus* (Ibrahim2003), The severe acidic stress effect attributed the reduced growth rates in the fishes 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, fishes are supplied with an ad libitum diet. But the fishes did not consume adequately. So the intake of energy is very less, it was inadequate to maintain their physiological process. In this stressful situation, fishes have to expend 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 fishes 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 corroborate prior findings. Ibrahim (2003) registered the declined growth of *O. mossambicus* tested in pH of 5.0, 5.8 and 6.6. Bucker et al. (1995) reported the significant reduction in the growth of Atlantic salmon, exposed to pH 4.5 and 5.0, Baker and Schofield (1982), Saunders et al. (1983), Lacorix and Townsend (1987) and Perry (1990) reported the reduced growth of Atlantic salmon exposed to pH 4.5, Cleveland et al. (1986, 1989, 1991) reported the decreased growth of brook trout when exposed to the acidic environment, Hunn (1987) reported the reduced growth of brook tout exposed to low pH, Geen et al. (1985) reported the decreased growth of Chinook salmon, Saunders et al. (1983) reported the reduction in the growth of Atlantic salmon, Lacorix and Townsend (1985) suggested the decreased growth of Atlantic salmon (Salmo salar), in increased acidity Perry (1990) reported the reduced growth of Atlantic salmon in the experiments of chronic effects of low pH. Beamish (1974 a, b), Ryan and Harvey (1980) reported that the growth of white suckers was reduced in acidified lakes. Beamish et al. (1975) 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) 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 highest 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) reported that Atlantic salmon showed the increased metabolic rates when exposed to acid environmental pH 5.2 - 5.4. The observed results corroborate with the results of Smith et al. (1995).

Declarations

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Conflicts of interest /Competing interests, The author sturdily declared that there is no conflict with any scholars, institutions, etc, this presented research article free from total skirmishes.

Ethics approval/declarations, there is no need for ethical clearance for this submitted article. This experimental study is involved with edible fish

Consent to participate, nil

Consent for publication (include appropriate statements), nil

Availability of data and material/ Data availability, nil

Code availability (software application or custom code), nil

Authors contributions

- Ibrahim Abdul Azeez, concept, and design of this study, final approval of the manuscript
- Narayanan Muthuswamy, Literature collection, preliminary manuscript
- Ramachandran Sethuraman, Data collection and computation of data and statistical analysis

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Tables

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

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

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

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 ± 128.23	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 ± 118.30	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

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

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

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			
	Lipid				
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			

Figures

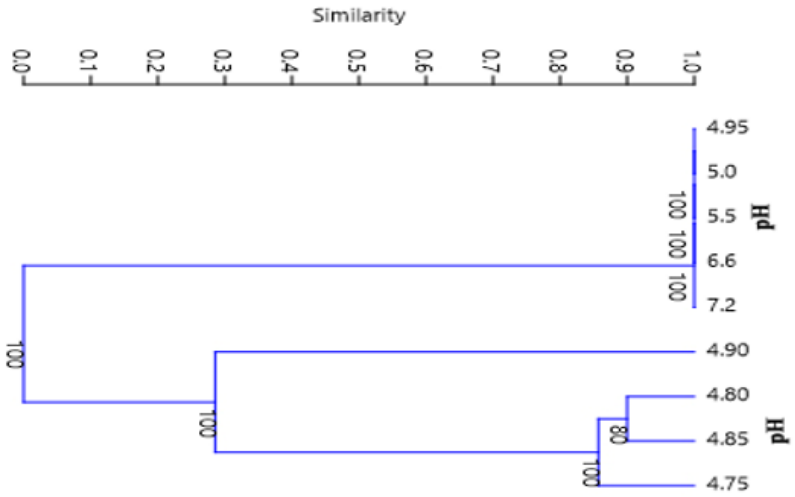

Figure 1

Bray- Curtis similarity index

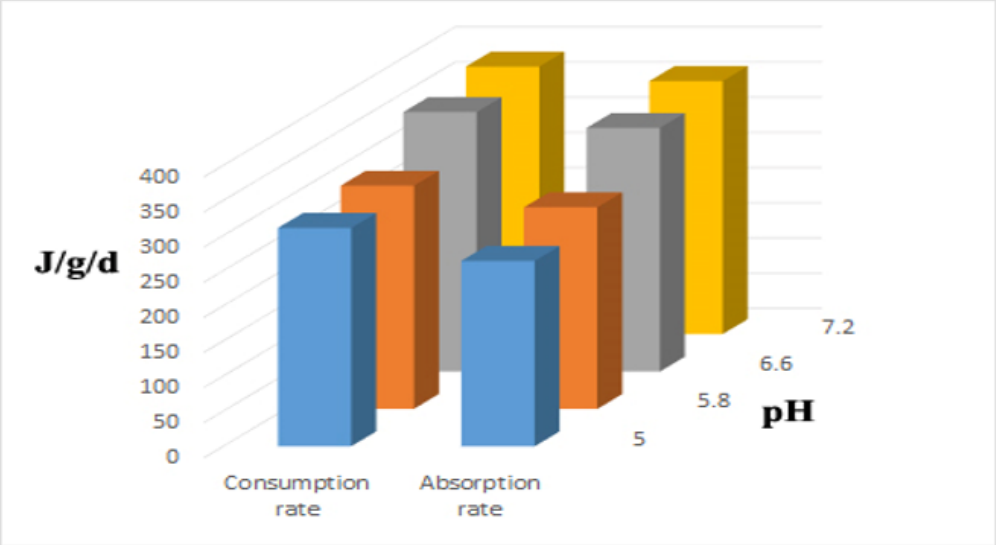

Figure 2

The consumption and absorption rates of C.Carpio, exposed pH 5.0, 5.8, 6.6, and 7.2

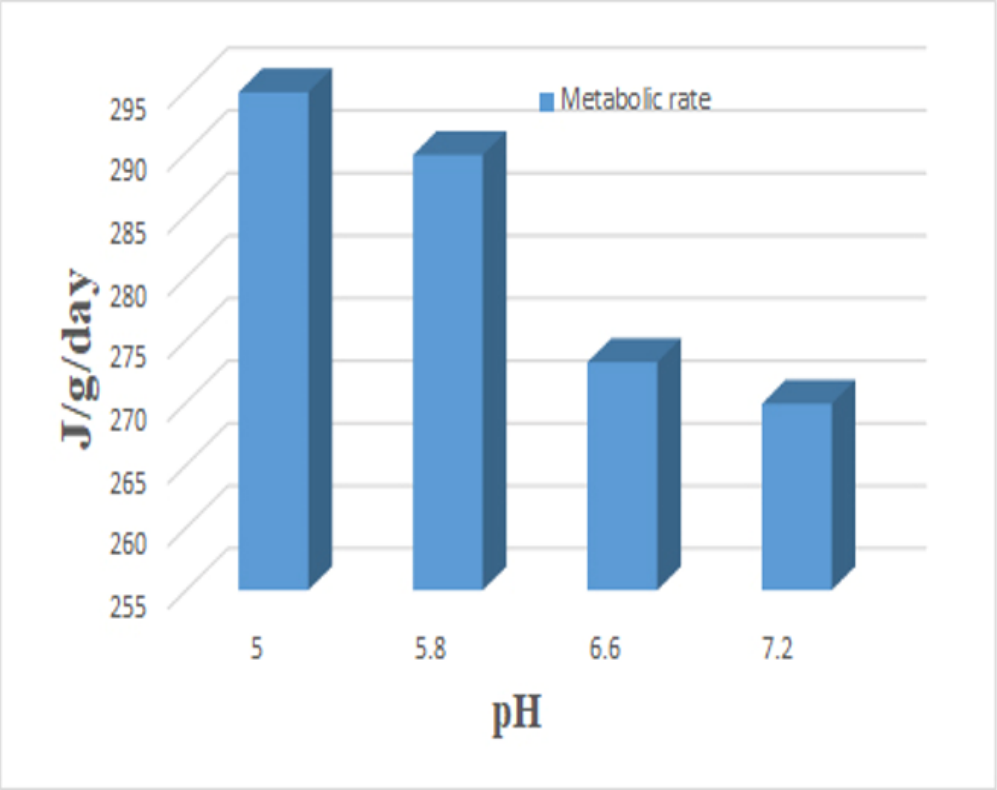

Figure 3

Metabolic rates of C.Carpio, exposed to pH 5.0, 5.8, 6.6, and 7.2

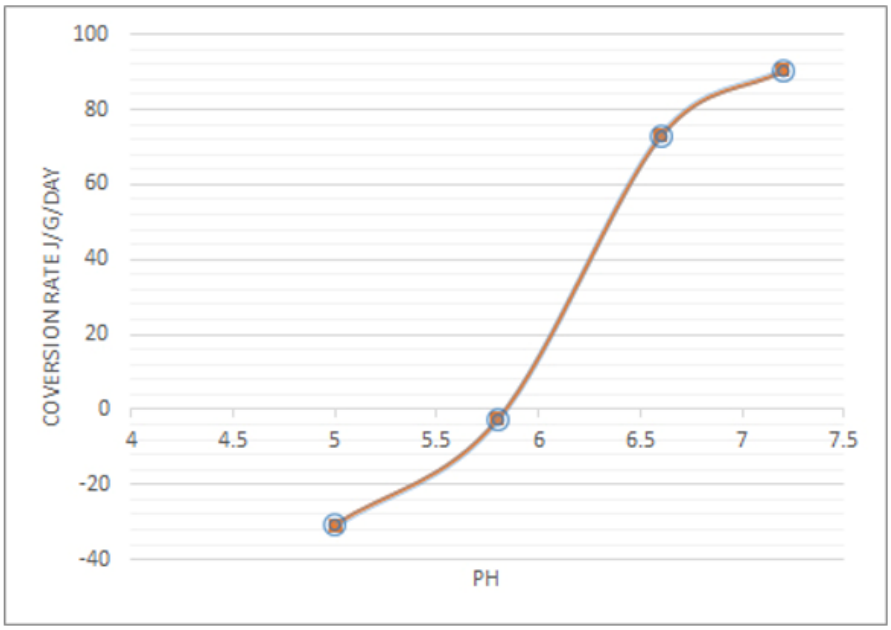

Figure 4

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