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Comparative evaluation of two iso-nutritional feeds (mash and pellet) upon the environmental health and growth potential of two Indian major carps (Rohu, *Labeo rohita;* Mrigal, *Cirrhinus mrigala*) under mixed culture condition

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Abstract: *Comparative evaluation of two iso-nutritional feeds (crude protein- 22%) viz. mash and pellet upon the water quality and effectiveness in terms of survival and production of two Indian major carps namely column- bottom feeder rohu (Labeo rohita) and bottom feeder Mrigal (Cirrhinus mrigala) under mixed culture condition was carried out in outdoor experimental tanks (180 L) for 96 days. Though, increase in body weight of the test fishes was not influenced by the feed types; water and soil quality parameters, feeding efficiency, survivability and total fish biomass significantly varied. Though, feeding efficiency remained lower in mash feed (23.97%) than pellet (83.81%), net weight gain of the test fishes remained slightly higher in mash (108.60%) than pellet (106.52%) fed treatments. However, the percentage in fish biomass gain in mash fed treatment (65.92%) was lower than pellet (83. 81%). The environmental health of the treatments with respect to water and sediment quality subjected to mash and pellet feed application under identical stocking composition of fish did not differed significantly. N: P ratio in both mash and pellet fed treatments tended to increase overtime and remained congenial (4 to 6.4). Increase in available phosphorous and organic carbon of sediment under both the feed types supported by the physico-chemical exchange and microbial mineralization process was slightly higher in mash fed treatment as evidenced from higher presence of heterotrophic load (72.57 CFU x 10^2 ml^{-1}) than pellet (61.86 CFU x 10^2 ml^{-1}) fed treatments. Therefore, mash type of feed was highly competitive and economic under judicious management practices of carp polyculture conditions.*

Keywords: *Environmental health, feeding efficiency, Indian major carps, mineralization, Iso-nutritious feed.*

I. Introduction

Aquaculture in Asia has established itself as an important farming sector contributing 50% of the global food fish consumption [1]. During the last two decades, Indian aquaculture has registered over six fold growth where freshwater aquaculture contributed over 95 percent of the total aquaculture production. Fish production in India has increased from 4.16 million tonnes in 1991-92 to 8.67 million tonnes in 2011-12 in which Indian major carps, namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contributed the bulk of freshwater production with over 1.8 million tonnes [2]. Fisheries sector contributes significantly to the national economy while providing livelihood to approximately 14.49 million people in the country. Fishery being one of the promising sectors of agriculture and allied activities in India, annual growth target rate of 6 per cent was fixed.

Freshwater aquaculture in India is primarily based on improved traditional type low input culture system with moderate level of intensity in operation. Organic manures and agricultural by-products are extensively used which stimulate the growth of natural food by providing essential elements to increase the planktonic biomass. Besides, fertilization stimulates both the autotrophic and heterotrophic levels which increase fish production [3] by increasing primary production, dissolved oxygen, pH and total phosphorus [4]. In recent times, application of processed supplementary feed in the form of pellet is gaining momentum replacing the traditional mash type feed in the polyculture ponds where carps of different ecological and feeding niches are co-stocked for optimal use of the resources of the system. Polyculture is preferred, based on the assumption that each species stocked has its own feeding niche that does not completely overlap with the feeding niche of other species [5]. Species with different feeding niches stocked at different densities can influence the natural food availability positively (e.g. by releasing nutrients from the pond bottom) or negatively [6]. In consequence, a larger fraction of the natural food available in the pond is used in multi-species systems. In some cases, one species enhances the food available for other species, thus increasing further the total fish yield per unit area [7].

Since, a single type of feed either as mash or as pellet is being provided by the fish farmers to an assemblage of cultivable carps with different feeding niches and habitat, an attempt has been made to evaluate the impact of iso-nutritional feeds with different physical forms upon the water quality and effectiveness in terms of survival and production of two Indian major carps namely rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*).

II. Materials and methods

Nine outdoor experimental cylindrical cement cisterns (180 L) were thoroughly washed, sun dried, provided with soil base of 15 cm. and then filled with ground water (pH-7.5). All the tanks were applied with cow manure @10,000 kg/ha. as practiced in traditional pond preparation for fish farming in the locality. The requisite amount of cow manure were mixed with water and dispensed in the form of slurry into the tanks. The tanks were also applied with lime @ 200 kg/ha after seven days of manure application and kept undisturbed for another seven days. When the water colour turned greenish blue they were then randomly grouped into three batches in triplicate. A fixed level of water was maintained in the experimental tanks by periodic addition of ground water to compensate the losses due to evaporation and sampling.

2.1. Stocking of fish

Healthy fingerlings of Rohu (*Labeo rohita*) (8.1 ± 0.2 cm; 12.75 ± 0.21g) and Mrigal (*Cirrhinus mrigala*) (7.08 ± 0.2cm; 13.31 ± 0.21g) were procured from Naihati Fish Seed Market and acclimatized in identical experimental tanks for 7 days. Stocking of fish was done in two of the three batches of tanks @15 nos./tank (10 nos. of *Rohu* and 5 nos. of *Mrigal*) two weeks after application of cowdung when the colour of the water changes to greenish blue indicating development of planktonic organisms. They were reared for 96 days.

2.2. Preparation of feed

Supplementary mash feed of 22% protein content was prepared by using the Double Pearson's Square method [8]. Freshly collected fish meal (45 % protein) and mustard oil cake (30 % protein) were used as animal and plant sources of protein inputs. Total protein input was equally distributed into animal and plant sources. Rice polish was used as carbohydrate source as well as filler, whereas, equal mixture of groundnut oil and cod liver oil @ 6 % was used to supplement essential fatty acids. Different ingredients for protein and carbohydrate supplementations as required upon calculations were weighed, powdered by using a mixer-grinder and mixed thoroughly. The mash was then fortified with vitamin-mineral mixture @ 1%.

For preparation of pelleted feed, the mash was prepared with the same method and formulations and then mixed with boiled tapioca starch as binder @ 2 % with addition of required quantity of boiled water. The dough was cooked in a pressure cooker for 10 minutes, cooled and passed through an automated pelletizer machine with dye size of 2 mm. diameter. The pellet was then sprayed with required quantity of oil and vitamin-mineral mixtures by using a hand sprayer; air dried and packaged in polythene bags with proper sealants for future use.

2.3. Feeding

Fish in the first and second batches of cisterns were fed with mash (M) and pellet feed (P) respectively @ 5% of body weight once daily between 9.00 a.m. to 10 a.m. The required amount of feed was broadcasted over the water surface whereas, the third batch of cisterns were not applied with any kind of feed which served as control (C).

2.4. Collection of water and soil samples

Water samples were collected at 15 days interval from each of the tanks at a fixed hour of the day (9.30 a.m.) by completely dipping the collection bottle at 15 cm depth for physico-chemical analyses. During collection of water samples, cautions were taken so as to prevent air bubbling, which might influence water quality parameter like dissolved oxygen. Soil samples from each of the cisterns were collected from two different places of the soil bed using hand sampler. They were then mixed, air dried, pulverized with pestle and mortar, sieved through 150 µm mesh sieve and stored in labeled polythene packets for analyses.

III. Analysis of samples

3.1. Water quality

3.1.1. Temperature, pH and Dissolved oxygen

The water temperature was measured using a centigrade thermometer on spot and expressed as ^{0}C. pH of water samples was estimated by a digital pH meter (Systronics-VI) on spot. For estimation of dissolved oxygen, Winkler's method was followed [8].

3.1.2. Total Alkalinity and total hardness

Carbonate alkalinity of water samples were analyzed by titrating the samples against N/50 H_2SO_4 using phenolphthalein as indicator. Bicarbonate alkalinity was determined against N/50 H_2SO_4 using methyl orange indicator [9]. Total hardness of water samples was measured by titrating the samples against EDTA (Ethylene Di-amine Tetra Acetic acid) after adding ammonia buffer and Eriochrome Black T as indicator [9].

3.1.3. Inorganic nitrogen

Ammonia-nitrogen (NH_3-N) was estimated through a double beam UV-vis-Spectrophotometer (CECIL CE-4002) at 640 nm wavelengths using phenol solution, sodium nitropruside solution and oxidizing solution following modified phenate method [10]. Similarly, nitrite-nitrogen (NO_2-N) was measured at 543 nm using α-napthylamine and sulphalinic acid [10] and nitrate-nitrogen (NO_3-N) by UV-spectrophotometric method at 220 and 275 nm using 1 (N) hydrochloric acid [9].

3.1.4. Orthophosphate

The orthophosphate level of water was determined colorimetrically through a double beam UV-vis-Spectrophotometer (CECIL CE-4002) at 690 nm wavelengths following the stannous chloride method [9].

3.2. Soil quality

The pH was determined with a digital pH meter (Systronics-VI) using 1:2 suspensions of soil and water [9]. For estimation of organic carbon, air-dried powdered sediment sample (1 g) was digested with 1 N $K_2Cr_2O_7$ (10 ml) and concentrated H_2SO_4 (20 ml) and kept for 30 minutes at dark. The digested sample was then diluted with 200 ml distilled water and 10 ml ortho-phosphoric acid and 1 ml diphenyl amine indicator was added. It was the titrated against 1 N ferrous ammonium sulphate (Mohr's salt) until brilliant green colour appeared [11]. Available phosphorus was determined using 1:20 soil to Olsen's extractant (0.5 $NaHCO_3$ adjusted to pH 8.5) [12] followed by [13] Chlorostannous reduced molybdophosphoric acid blue colour method in hypochloric acid system as described by [14].

IV. Enumeration of total aerobic heterotrophic bacteria

Total aerobic heterotrophic bacterial load of water was estimated at fortnightly intervals. Collection of samples was performed as per the method of [15] and [16]. Population of heterotrophic bacteria was grown in standard nutrient agar medium having the following composition [8]:

Peptone: 10 g	Beef extract: 1.5 g
Sodium Chloride 2.0 g	Bacto agar: 20 g
Distilled water: 1000 ml	

The medium was sterilized in the autoclave at 15 lbs. pressure for 15 minutes.

V. Feeding efficiency

Feeding efficiency (%) was calculated as:

$$\frac{\text{Weight gain in treatment – weight gain in control}}{\text{Weight gain in control}} \times 100$$

VI. Statistical analyses

All the results were subjected to statistical analysis. One way analysis of variance (ANOVA) were applied to test the significance among the treatments followed by paired two sample t-test to find out significance in difference between every possible pair of treatment combinations. Correlation co-efficient (r) test was applied to establish relationship between selective parameters using appropriate software and where significant, selective variables were fitted with appropriate models to find out the nature and intensity of dependency.

VII. Results

Water temperature and pH did not differ significantly among the treatments and ranged between 26 - 30^0C and 7.31 to 8.56 respectively during the period of investigation. The temporal trend in pH exhibited a gradual decline till day 56 followed by a fall after which, it declined sharply in all the three systems (Fig. 1). Total alkalinity of water, in general, continued to decline throughout the period of investigation (Fig. 2). The overall mean value of total alkalinity was maximum (201.56 mg l^{-1}) in P followed by (186.51 mg l^{-1}) M and lowest (105.45 mg l^{-1}) in C with high level of significance ($F_{2, 38} = 42.45$; $P < 0.001$). Likewise total alkalinity, total hardness of water tended to declined gradually. The overall mean value was highest in M (1219.81 mg l^{-1}) followed by P (1112.85 mg l^{-1}) and lowest in C (976.15 mg l^{-1}) (Fig. 3). Overall treatment difference was significant ($F_{2, 38} = 13.67$; $P < 0.001$). The overall mean value of dissolved oxygen was highest in M (11.15 mg l^{-1}) followed by P (10.45 mg l^{-1}) and lowest in C (9.89 mg/L) with maximal temporal variation in P (39.85%)

followed by M (27.33%) and minimal in C (8.84%) (Fig. 4). However, differences among the systems were insignificant ($P > 0.05$).

Ortho-phosphate concentration of water tended to increase over time in both P and M (Fig. 5). The overall mean value of ortho-phosphate (0.09 mg l^{-1}) was maximal in M closely followed by P (0.08 mg l^{-1}) and minimal in control (0.05 mg l^{-1}). The overall difference remains insignificant ($P > 0.05$) up to day 32 after which, differences became significant ($F_{2, 38} = 18.58$; $P < 0.001$).

Ammonia and nitrate-nitrogen followed identical trends to that of ortho-phosphate, but the magnitude of difference among the treatments and control was much higher (15.9 % to 19.27 %). Overall difference among the systems in both the cases remained highly significant ($F_{2, 38} \geq 31.07$; $P < 0.001$) (Fig. 6, 7). However, nitrite-nitrogen concentration of water either remained absent during several dates of observation or exhibited in fairly low concentrations in both the treatments (Fig. 8) with average value of 0.013 mg l^{-1} (P) and 0.012 mg l^{-1} (M) ($P > 0.05$). The N:P ratio tended to increase over time and remained congenial (4 to 6.4) both in M and P during most part of the period of investigation with distinctly lower value in C (Fig. 9).

The population of aerobic heterotrophic bacteria exhibited a more or less stationary trend in all the systems during the first month of investigation followed by an increasing trend thereafter both in M and P. In contrast, a fall was observed in the control after day 48. The highest mean value (72.57 CFU x 10^2 ml^{-1}) encountered in M was closely followed by P (61.86 CFU x 10^2 ml^{-1}) (Fig. 10). Differences among the systems were distinctly pronounced ($F_{2, 20} = 4.56$; $P < 0.05$) during the second half of the investigation.

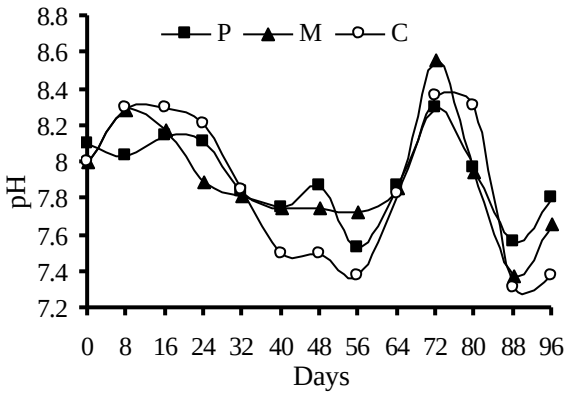

Fig. 1.Temporal changes of pH of water in different treatments employed.

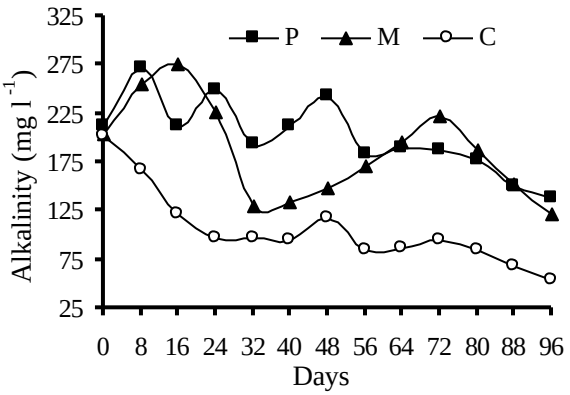

Fig. 2.Temporal changes of alkalinity of water in different treatments employed.

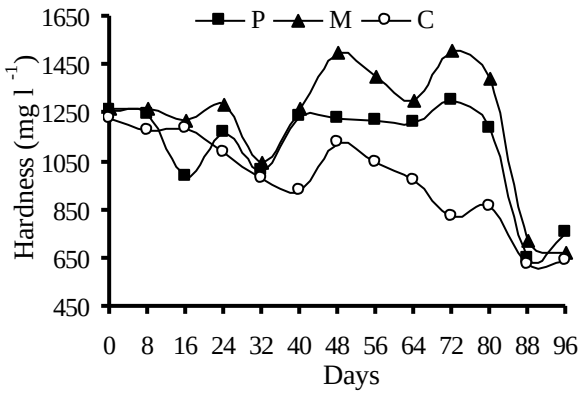

Fig. 3.Temporal changes of hardness of water in different oxygen of treatments employed.

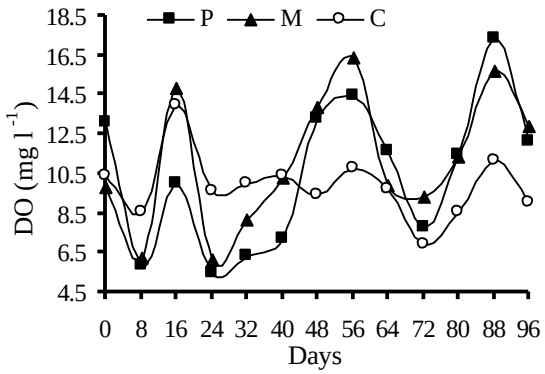

Fig. 4.Temporal changes of dissolved water in different treatments employed.

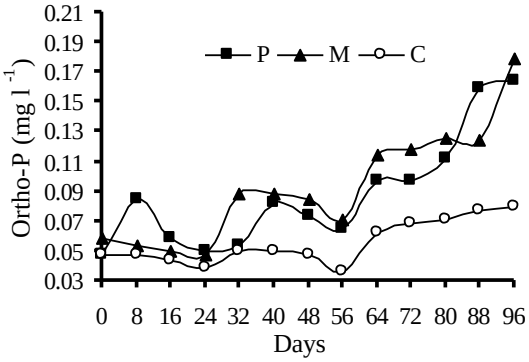

Fig. 5. Temporal changes of Ortho-P concentration of water concentration in different treatments employed.

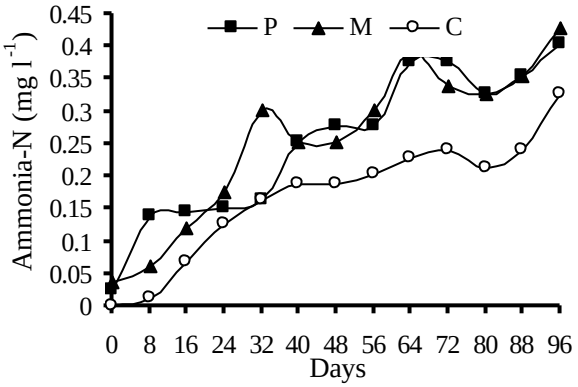

Fig. 6. Temporal changes of NH_3-N of water in different treatments employed.

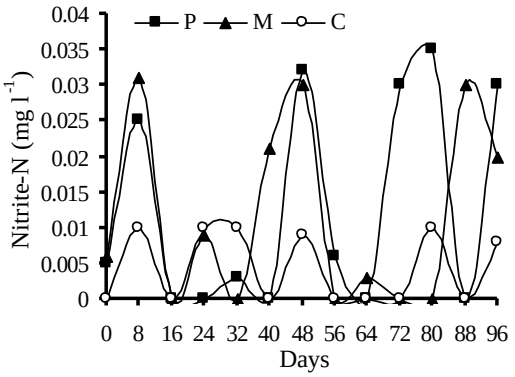

Fig. 7.Temporal changes of NO_2-N concentration of water in concentration different treatments employed.

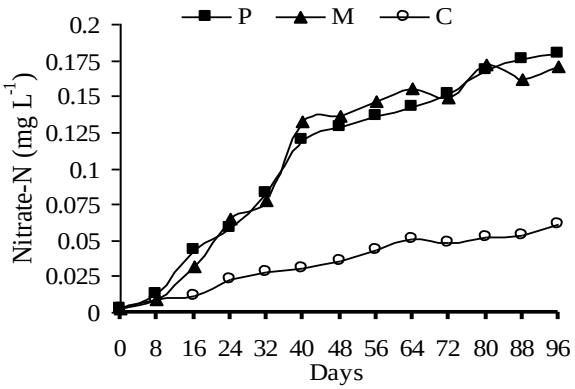

Fig. 8.Temporal changes of NO_3-N of water in different treatments employed.

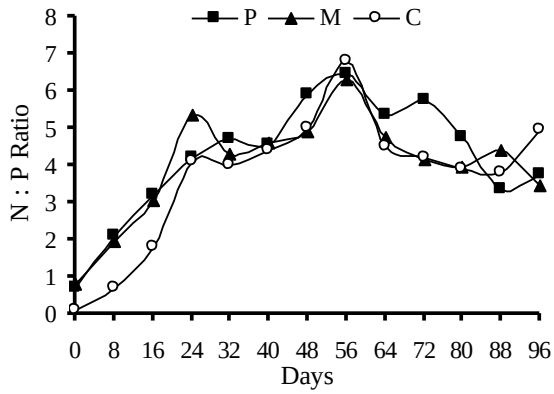

Fig. 9. Temporal changes of N: P ratio of water in different treatments employed.

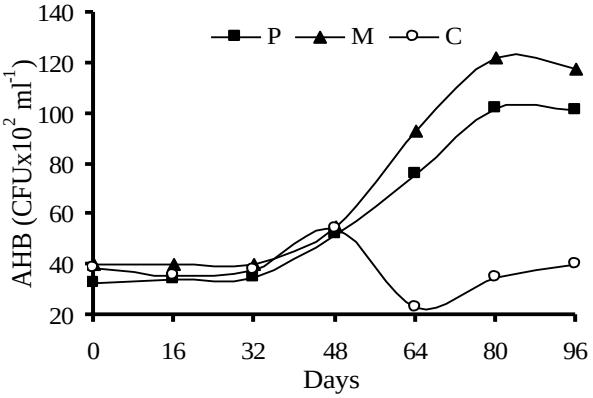

Fig. 10. Temporal variation of aerobic heterotrophic bacteria of water in different treatments employed.

Survival rate of fish was maximal (85%) in P compared to M (80%), however, both are lower than C (90%). Though, average weight gain of fish was slightly higher (27.18 g) in M than P (26.91), rohu gained higher weight in M (118.43%), whereas, it was mrigal (103.83%) in P. In contrast, gain in total biomass of the fish was higher (83.81%) in P than M (65.92%). Moreover, feeding efficiency was distinctly higher (57.63%) in the former than in the later (23.97%) (TABLE 1, 2).

TABLE- 1. Performance in weight gain and survivality of the test fishes under different treatments employed.

	Treatment						Control (C)		
	Mash (M)			Pellet (P)					
	Rohu	Mrigal	Mean	Rohu	Mrigal	Mean	Rohu	Mrigal	Mean
Initial weight (g)	12.75	13.31	13.03	12.75	13.31	13.03	12.75	13.31	13.03
Final weight (g)	27.85	26.51	27.18	26.68	27.13	26.91	22.0	22.05	22.03
Net weight gain (g)	15.1 (118.43%)	13.2 (99.1%)	14.15 (108.60%)	13.93 (109.25%)	13.82 (103.83%)	13.88 (106.52%)	9.25 (72.55%)	8.74 (65.66%)	9.0 (69.07%)
Survivability (%)	80	80	80	90	80	85	90	90	90(%)

TABLE- 2. Biomass gain and feeding efficiency under different treatments employed.

	M	P	C
Initial fish biomass	194.05	194.05	194.05
Final fish biomass at harvest	321.96	356.69	297.23
Gain in fish biomass	127.91 (65.92%)	162.64 (83.81%)	103.18 (53.17%)
Feeding efficiency (%)	23.97%	57.63%	-

Soil pH gradually declined and remained alkaline throughout the period of investigation with values range from 7.33 to 8.06 in M and P, and 7.21 to 7.86 in control (Fig. 11). Organic carbon tended to decline during the first month of observation followed by a gradual increase in both M and P. The average value did not varied much between P (1.739 mg g^{-1}) and M (1.754 mg g^{-1}) and remained higher than the control (1.359 mg g^{-1}) (Fig. 12). The overall difference exhibited significance ($F_{2, 38} = 27.74$; $P < 0.001$). Soil available phosphorous continued to increase both in P and M during the period of investigation. The overall mean value was highest in M (0.13 mg g^{-1}) followed by P (0.12 mg g^{-1}) and lowest in control (0.08 mg g^{-1}) (Fig. 13). The overall difference among the systems remained highly significant ($F_{2, 38} = 18.69$; $P < 0.001$).

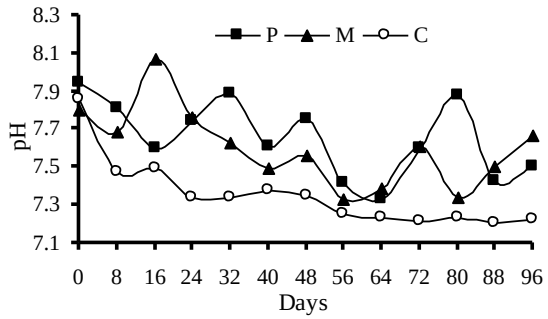

Fig. 11. Temporal changes of pH of soil in different treatments employed.

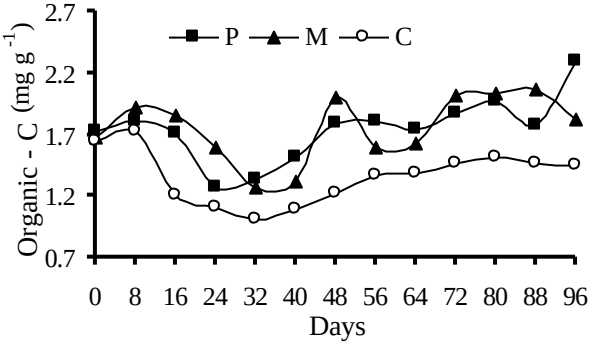

Fig. 12. Temporal changes of organic-carbon of soil in different treatments employed.

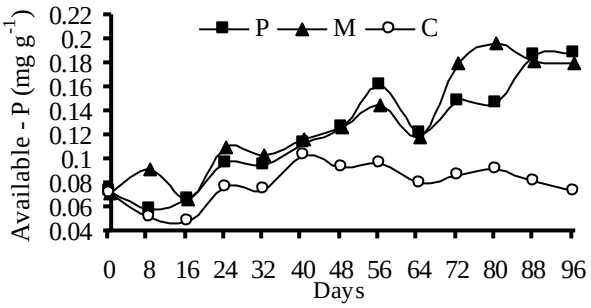

Fig. 13 Temporal changes in available phosphorus of soil in different treatments employed.

VIII. Discussion

Though, the sediment pH declined gradually in both the treatments, in both the feed types tested, it remained alkaline (Fig. 11) in spite of no application of lime during the post stocking management period which suggests that decomposition of the organics primarily from the faeces of the test fishes and uneaten feed

proceeded under oxygenated condition by the aerobic heterotrophs thereby not reducing the pH. Because of the aging of the culture system in both the treatments, the sediments gained organic carbon as evidenced from Fig. 12 which in turn supported the heterotrophic community by acting as energy source. Several literatures emphasized the role of carbon as energy source for the microbial population operating in the mineralizing process [17]; [18].

The steady increase of available-P of the sediment in either of the treatments might be explained as the resultant product of the physico-chemical transformation and the mineralization of the feed remnants and other organic loading as the period of investigation progressed. [19] studied the leaching process of different fractions of P from salmonid diet and [20] quantified that average amount of organically bound phosphorous fraction in feed and faeces released to the water was 80% and 60% respectively. [21] opined feeding management with high quality feed in a manner that ensures essentially complete consumption of feed by fish which could lower phosphorus inputs in ponds.

As expected, the body weight of fish has established strong positive correlation with the nutritional parameters and the primary productivity as well in any of the treatments employed (TABLE -2).

TABLE- 2. Correlation between body weights of fish with nutritional parameters in different treatments employed.

Parameter	Body weight of fish					
	M (Rohu)	P (Rohu)	M (Mrigal)	P (Mrigal)	Overall P	Overall M
NH_3-N	0.93 [c]	0.97 [c]	0.84 [b]	0.96 [c]	0.96 [c]	0.94 [c]
NO_3-N	0.98 [c]	0.99 [c]	0.98 [c]	0.98 [c]	0.99 [c]	0.99 [c]
NO_2-N	0.93 [c]	0.97 [c]	0.93 [c]	0.97 [c]	0.97 [c]	0.93 [c]
Ortho-P	0.88 [b]	0.79 [b]	0.80 [b]	0.75 [a]	0.77 [a]	0.80 [b]
AHB	0.90 [b]	0.85 [b]	0.80 [b]	0.81 [b]	0.83 [b]	0.80 [b]
SAP	0.90 [c]	0.88 [b]	0.94 [c]	0.85 [b]	0.84 [b]	0.84 [b]

[a] Level of significance = 5 % ; [b] Level of significance = 0.1 %; [c] Level of significance = 0.01 %

Higher abundance of orthophosphate and total ammonia in any of the treatments compared to the control (Fig. 5, 6) is because of the metabolites and the mineralization product of the feed remnants. Breakdown of protein in fish acts as an important source of ammonia [21] and the relationship between them as: total ammonia (production rate in g) = 56 P (decimal fraction of protein in diet) has been documented by [22]. However, such process of N addition favoured the N: P ratio both in mash and pellet fed treatments where it ranged from 4 to 6.4. [23] suggested optimum N: P ratio of 4: 1 to 8: 1 in pond culture system.

The environmental health of the treatments with respect to water and sediment quality subjected to mash and pellet feed application under identical stocking composition of fish did not differed significantly as evidenced from the results of the investigation. This is more pertinent when especially referred with pH, dissolved oxygen, alkalinity of water and, pH and organic carbon of the sediments. Also, this is true with respect to the total aerobic heterotrophic load of both the treatments were considered. This implied that difference in the physical forms of feed did not cause any environmental degradation particularly with mash feed application in the present investigation. This might be explained as the difference in the physical forms of feed did not cause any environmental degradation particularly with mash feed application in the present investigation as pellet feeds are considered superior in this respect [24]. Also, both the feed types besides directly acting as sources of nutrition supported both the autotrophic and heterotrophic pathway substantially. Therefore, mash type of feed is highly competitive and economic under judicious management practices under carp culture conditions. This is because, pelleting the mash adds to the input cost and ultimately the production cost, in general [25]. However, higher survival rate, total biomass gain and feeding efficiency in pellet fed treatment established it's superiority over mash feed (TABLE 1, 2).

IX. Conclusion

The present investigation clearly indicated that under Indian carp culture condition, iso-nutritious mash form of feed was equally effective in maintaining water quality and growth performance of rohu and mrigal to that with pelleted feed. However, considering survival rate and increase in biomass indicated higher production potential with pellet form of feed.

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