Effect of Poultry Litter on Water Quality and Isolation of Bacteria From Cyprinus Carpio

Abstract

The present study was conducted to evaluate the effect of poultry litter on water quality (temperature, pH, dissolved oxygen, free CO₂, alkalinity, total solids, nitrates and phosphates), and investigation of pathogenic bacteria viz. Salmonella sp., E. coli and Pseudomonas sp. in manures used for treatment and gills and skin of exposed fish Cyprinus carpio. 210 fingerlings of C. carpio obtained from Government Fish Hatchery, Jammu were acclimatized for one month with conventional feed and daily water exchange. The experiment tubs were cleaned, fresh soil was spread to 5 cm height and treated with lime @ 250 kg/ha. After three days of liming, the tubs were filled with water at 40 cm depth. Experimental water in tubs were treated in duplicates with poultry litter at low (TP1) @ 8000 kg/ha, medium (TP2) @10000 kg/ha and high (TP₃) @ 12000 kg/ha doses, respectively along with control. Water quality parameters were within tolerable range for C.carpio. At the end of the experiment, E. coli was detected in fishes from TP₂ and TP₃ while presence of Salmonella was found in fishes from all the treatments of poultry manure. However, samples of poultry litter were also positive for E. coli and Salmonella. Pseudomonas was neither detected in treated fish samples nor in samples from poultry litter. These observations indicate poultry manure at lower doses is effective to stimulate productivity with conducive range of water quality with lesser pathogenic hazards.

Keywords :Poultry Manure, Water Quality, Pond Water, Fish Health, Cyprinus Carpio

Introduction

India has vast resource of livestock and poultry, which play a vital role in improving the socio-economic conditions of rural masses. India ranks first in respect of buffaloes, second in cattle & goats, third in sheep, fifth in ducks & chickens and tenth in camel population in the world. Livestock wastes including animal manure and poultry byproducts, which are a menace to the environment, are sources of wealth creation in fish farming (Adewumi et al. 2011). Livestock manure contains considerable quantities of nutrients for fish production. Protein content ranges between 10-30 percent, energy between 1100-1400 kilocalories per kilogram manure and soluble vitamins are synthesized in high concentration (Tuleun, 1992). It also contains non digested feed, metabolic excretory products and residues resulting from microbial synthesis. The livestock wastes such as cow-dung, poultry and pig excreta, goat and sheep pellets in fish culture are useful in enhancing the production of fish food organisms as well as in cutting down the expenditure on costly feeds and fertilizers.

Reducing amount of feed is a means of lowering costs if production is not reduced. In attempts to reduce feed costs, integration of chicken with fish farming might be an economically viable and productive system for both rural farmers and commercial entrepreneurs. Poultry production wastes have inherent qualities that make them particularly valuable for fish production compared to other livestock wastes. Poultry manure is a complete fertilizer, with the characteristics of both organic as well as inorganic fertilizers and fresh chicken manure contains 1.6% nitrogen, 1.5% phosphorous and 0.9% potassium (Woynarovich, 1980). However, Excessive use of manures may present a risk related to the creation of unbalanced conditions in water ecosystem, such as oversupply of nutrients, eutrophication and decay of organic matter and presence of enteric pathogens. Thus, bacterial contamination, especially pathogenic species is a potential hazard in all forms of aquaculture.

Sahar Masud

Associate Professor(Fisheries) Div.of Livestock Production and Management, Faculty of Veterinary Sciences & AH, Sher-e Kashmir univ. of Agriculture Sciences & Technology –Jammu, Jammu & Kashmir

Asma Khan

Faculty of Veterinary Sciences & AH, Sher-e Kashmir univ. of Agriculture Sciences & Technology –Jammu, Jammu & Kashmir

Md. Rasheed

Faculty of Veterinary Sciences & AH, Sher-e Kashmir univ. of Agriculture Sciences & Technology –Jammu, Jammu & Kashmir P: ISSN No. 2231-0045 E: ISSN No. 2349-9443

Contamination levels in farmed fish depend on bacterial levels in the pond environment and the quality of input water. Shehzad *et al.* (2012) isolated almost the same type of bacterial species from organic manures, pond water and organs of fish. Sustainable and successful freshwater fish culture depends upon scientific basis principally the use of adequate, economically viable and environmental friendly techniques. Among the techniques, manure usage at different rates and quality may significantly influence water quality and assist in defining the optimal conditions for continuous culture of plankton.

Materials and Methods

Live specimen (fingerlings) of C. carpio, average length 8.77 cms (range 7.8-10.2 cms) and average weight 31.5g (range 21-45g), were obtained from the Government Fish Hatchery, Jammu and were acclimatized in 1000 litres capacity tank for one month before transferred to the experimental tubs. During acclimatization, the fish were fed with conventional fish feed (rice bran and mustard oil cake in 1:1 ratio) at the rate of 5% body weight. Daily replenishment of water was done during acclimatization. The study was conducted in fourteen plastic tubs of 100 litres capacity with 40 cms depth divided into 7 groups of treatments each in duplicates and each experimental tub was having 15 fingerlings. Before starting the test, all the experimental tubs were cleaned and disinfected with KMnO₄ solution. Fresh soil was laid on the bottom of completely dried tubs upto a thickness of 5 cms. The soil was treated with quick lime at the rate of 250 kg/ha. Three days after the application of lime, the tubs were filled with water. Water depth was maintained at 40 cm during the experimental period. The water in the tubs was allowed to stabilize for two days before fertilization. Experimental water in twelve tubs was treated with low. medium and high doses of poultry manure (deep litter) each in duplicates. Two tubs without fertilization served as the control $(TO_a \text{ and } TO_b)$ with alternate day water exchange and conventional feeding (rice bran and oil cake in 1:1 ratio at 5% body weight) two times a day. Half of the amount of the total calculated dose of poultry manure was used 15 days prior to stocking of fish in tubs. Subsequently, fertilization was carried out at 2week intervals using 1/10th of the initial quantity. Fingerlings of C. carpio were stocked uniformly at 15 numbers in each of the experimental tubs.

The 60 days experiment consisted of following treatments along with control:

Control

No fertilizer was offered. The ration treatment included the daily application of conventional feed i.e. rice bran and oil cake (1:1) @ 5% body weight **Treatment 1**

Poultry manure (deep litter) was applied at three doses of low (TP₁) @8000 kg/ha, medium (TP₂) @10000 kg/ha and higher (TP₃) @12000 kg/ha. Half of the total quantity was applied as basal dose 15 days prior to stocking of fingerlings. The remaining amount was applied in $1/10^{th}$ equal splits at bimonthly intervals

Water from each tub was tested for temperature and pH (daily), for dissolved oxygen (DO) and free CO_2 (bi-weekly) and for alkalinity, total solids, nitrates and phosphates (weekly). All determinations were carried out according to the Standard Methods of

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American Public Health Association (APHA, 1998). Mean weight and length of the fishes were measured initially and at the end of the experiment to determine specific growth rates and condition factor. Fish were also observed daily for any mortality to calculate the survival rate.

Samples from fish (skin and gills) and manures used for the treatments were taken for investigating pathogenic bacteria species viz. *Salmonella* sp., *Pseudomonas* sp. and *Escherichia coli*. Sampling from fish (skin and gills), using sterile cotton swabs was done initially at the beginning of the experiment and then, weekly from each tub taking five fishes from each tub at random. Sampling from manure was done every time before fertilization at an interval of 15 days.

Procedure followed for selective plating and further analysis of both fish and manure samples E.coli

Streaking was done on Mac Conkey's Agar in sterile Petri plates under laminar flow and the plates were incubated for 24 hours at 37^oC. Characteristic small, round and pink coloured colonies were taken from the positive culture media after 24 hours of incubation and inoculated on nutrient agar and again incubated for 24 hours at 37^oC. The colonies with typical characteristics of the bacteria were further confirmed by biochemical tests using HiMViCTM test kit (Hi-Media, Mumbai, India; Code No. KB 001).

Salmonella Sp

Streaking was done on Brilliant Green agar in sterile Petri plates under laminar flow and the plates were incubated for 24 hours at 37^oC. Characteristic pink colonies surrounded by red medium were taken from the media after 24hours of incubation and inoculated on triple sugar iron agar and again incubated for 24 hours at 37^oC. The colonies with typical characteristics of the bacteria were further confirmed by biochemical tests using HiMViCTM test kit (Hi-Media, Mumbai, India; Code No. KB 001).

Pseudomonas Sp

Pseudomonas isolation agar is based on medium A described by King *et al* (1954). This medium is selective and formulated to enhance formation of blue or blue- green pyocyanin pigment by *Pseudomonas*. The pigment diffuses into the medium surrounding growth. Medium in sterile petri plates was inoculated using the streak plate method to obtain isolated colonies. Then, these petri plates were incubated for 18-48 hours at 35^oC and examined for good growth. *Psuedomonas* colonies should be blue to blue-green with pigment that diffuses into the medium, in positive cases.

Results and Discussion

Physico-Chemical Parameters of Water pH (Hydrogen ion concentration)

As shown in table & figure 1, the pH during the experiment ranged between 7.24 to 7.68 in different treatments including control. According to Boyd *et al.* (1998) optimum pH for growth and health of most fresh water fish is in the range of 6.5 to 9.0. Sub-optimal pH can cause stress, increased susceptibility to diseases and poor growth in cultured fish. Brezonic *et al.* (1983) suggested that high water pH values promoted growth of phytoplanktons and resulted in bloom. In this study, there were no great swings in pH and were generally

P: ISSN No. 2231-0045 E: ISSN No. 2349-9443

within the optimum range recommended for warm water aquaculture.

Temperature

Each aquatic organism has a range of temperature for its optimal growth and performance. Jhingran (1983) observed that carps thrive well in the temperature range of $18.3-37.8^{\circ}$ C.Water temperatures in this study were generally within this range. As shown in table & figure-1, the mean water temperature in all the treatments was optimal for carp rearing throughout the period of this experiment. Common carp occur within the temperature range of $3-35^{\circ}$ C (Froese and Pauly, 2011). The optimum water temperature for growth and propagation is $20-25^{\circ}$ C.

Dissolved Oxygen

According to Swann (1990), dissolved oxygen (DO) is by far the most important chemical parameter in aquaculture. Low-dissolved oxygen levels are responsible for more fish mortalities, either directly or indirectly. Warm water fish require DO \geq 5 mg/l for good growth and reproduction (Swingle, 1969).

As shown in table & figure -1, the values of dissolved oxygen in PT1, PT2 and control were within the safe level. However, in PT3 it was less than the benchmark (i.e. $DO \ge 5 \text{ mg/l}$). According to Boyd (1998), oxygen concentration of less than 3.5 ppm is fatal to carps within duration of 24 hours. Brown (2006) also reported that dissolved oxygen in the range of 5.2 mg/l is optimum level for favorable fish growth. But above that threshold or below, the growth rate would decrease. Lower dissolved oxygen values (4.66mg/l) in TP₃ may be attributed to decomposition of poultry manure. The results of the present study were also in agreement with findings of Garg and Bhatnagar, 1999; Garg 1996 and Bhatka et al, 2006 as they observed that fertilizer dose influences the level of dissolved oxygen and increases with increasing the fertilization level up to a certain limit and then declines with higher doses. Shevgoor et al. (1994) also reported that increasing level of fertilization is reported to raise all water quality parameters in suitable range except dissolved oxygen which showed the variation at dawn by the application of high manuring rate.

Free Carbon Dioxide (CO₂)

The results of the present study revealed that the level of free CO₂ was within the desirable limit and it is also depicted that different magnitude of manure significantly influencing the free CO2 level during experimental period of 60 days (table & figure- 1) which in long term with the extent of exposure to higher level of manure may have cumulative effect on water qualities. According to Santhosh and Singh (2007), the free carbon dioxide in water supporting good fish population should be less than 5 mg/l which support the present study as highest mortality was observed in the TP₃ might be due to the periodic release of carbon dioxide (CO_2) from decomposition of manure. Bhatnagar et al. (2004) suggested 5-8 ppm is essential for photosynthetic activity; 12-15 ppm is sublethal to fishes and 50-60 ppm is lethal to fishes. During decomposition of organic manure, carbon dioxide is released. In aquatic system organic manures contribute a great amount of combustible matter, which is oxidized and produce carbon dioxide that helps in the algal photosynthesis and also helps in the reduction of dissolved atmospheric

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nitrogen to ammonia. The decomposition of organic fertilizer is carried out by bacteria, fungi, actinomycetes (Persson *et al.*, 1980; Gaur *et al.*, 1995; Boyd, 1995) which also add CO_2 in the water.

Alkalinity

Boyd et al. (1998) stated that total alkalinity is important environmental variable in aquatic an ecosystem because it interacts with other variables that affect the health of aquatic animals or the fertility of the ecosystem. Boyd and Lichtkoppler (1979) suggested that water with total alkalinities of 20 to 150 mg/l contain suitable quantities of carbon dioxide to permit plankton production for fish culture. According to Wurts and Durborow (1992) alkalinity between 75 to 200 mg/l, but not less than 20 mg/l is ideal in an aquaculture pond. According to Santhosh and Singh (2007) the ideal value for fish culture is 50-300 mg/l. Based on above suggestions value of alkalinity in different treatment as shown in table & figure-1 were within the optimum range throughout the experimental duration. However, lowest mean value of total alkalinity was recorded in control T₀ (156.01 mg/l). Study of Boyd (1990) also support to the present study as reported that alkalinity increases with organic fertilization because bacterially generated CO2 from manure decomposition dissolves calcium and magnesium carbonate in pond water into calcium and magnesium bicarbonate. Diana et al. (1994) reported that fertilization alone led to low alkalinity.

Nitrates and Phosphates

Nitrates get into water bodies through nitrification, surface fertilizer run-off, sewage, animal manure and fish wastes discharges. Nitrates stimulate the growth of plankton and water weeds that provide food for fish. It is toxic to fish only at high levels and excess nitrate is converted by bacteria to nitrogen (N₂) and carbon dioxide (CO₂) gases. According to Stone and Thomforde (2004), nitrate is relatively non toxic to fish and do not cause any health hazard except at exceedingly high levels (above 90 mg/l). Santhosh and Singh (2007) described the favorable range of 0.1 mg/l to 4.0 mg/l in fish culture water. As shown in table & figure-1 the values of nitrate were within the safe limit and favorable for productivity. Control T0 without any manure showed lowest value.

Almost all of the phosphorus (P) present in water is in the form of phosphate (PO₄⁻) and in surface water mainly present as bound to living or dead particulate matter. It is an essential plant nutrient as it is often in limited supply and stimulates plant (algae) growth and its role for increasing the aquatic productivity is well recognized. According to Stone and Thomforde (2004) the phosphate level of 0.06 mg/l is desirable for fish culture. Bhatnagar et al. (2004) suggested 0.05-0.07 ppm is optimum and productive; 1.0 ppm is good for plankton. Bhatnagar and Devi (2013) suggested that 0.03-2 mg/l is acceptable range; however fish comes in stress when phosphorus level reaches more than 3 mg/l. As shown in table & figure-1, phosphate concentration were higher in all the three treatment of poultry manure with highest value in TP₃ and lowest values were observed in T₀ where no manure was added.

The results of the present study are in accordance with findings of Sayed and Ebiary (1998) as they reported that although there was no nutrient limitation in experimental pond, the nitrates and phosphates contents had higher levels in poultry manure treatment than cow manure. Burns and Stickney (1980) also found increase in the levels of nitrate and phosphate with increasing the number of hens raised on fish ponds.

Total Solids

During this investigation, total solids showed the maximum value in higher dose treatment and minimum was shown in control (T_0) (table & figure 1). These results were corroborated with the findings of Sayeed *et al.* (2007), Afzal *et al.* (2007) and Anetikhai *et al.* (2005) who suggested that basic macro and micro nutrients in the pond sediments can be enhanced by the application of organic and inorganic fertilization. Cattle manure and nitrophos caused a marked increase in planktonic biomass which is an indication of good pond productivity. Similar observations were recorded by Lane (2000).

Bacteriological Study

The observations of bacteriological study are depicted in table 2. The Salmonella and E.coli existed in almost all the samples collected from manure and fish skin and gill mucus. The occurrence of salmonella and E.coli in the poultry manure supports the findings of Quines, 1988; Pell, 1997; Guan and Holley, 2003 as they stated that microbiological analyses revealed presence of various pathogenic microorganisms in manure in addition to the common micro flora of animal intestine. Presence of E. coli in this study has indicated that fish cultured in organic waste fertilized water are susceptible to infection with pathogenic bacteria as the presence of the coliform group of bacteria in fish and fish products presents a health hazard to humans. Fish from the non fertilized water (T₀) had no bacterial detection. Salmonella spp. was detected in all the samples of poultry manure and samples from fish gills and skin in all the poultry manure treated fish. Disease outbreaks would thus be relatively less common in the non-manured water as it has less stressful environments, even though the pathogens and host species may be present. Presence of Salmonella in all the samples of manure all the treatment of poultry manure is in agreement with the study of Reilly and Twiddy (1992) as they stated that Salmonella has also been found in shrimp pond sediment and shrimp throughout Southeast Asia as the cause attributed to the use of large amounts of fresh chicken manure. The presence of Salmonella in manure or human waste is risky because it has several serotypes which are pathogenic to man and animals. It is worthy to note that pig is well-known reservoir of pathogenic Salmonella, so using porcine manure for fertilization should be done with care. Furthermore, the occurrence of Salmonella sp. is very common and is known to have the ability to cause variety of human diseases (Ogbondeminu et al., 1994). This study gives the evidence that contamination of pond with these bacteria may have potential human epidemiological hazard. Although, fish are rarely consumed in a raw state, the exposure of fish growers to the infected fish and their culture water may be a predisposing factor in the transmission of potentially enteropathogens to man.

Conclusion

The above study clearly indicates that poultry manure at lower doses is effective to stimulate primary

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productivity with conducive range of water quality and lesser pathogenic hazards.

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E: ISSN No. 2349-9443

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P: ISSN No. 2231-0045 E: ISSN No. 2349-9443

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 Table 1

 Effect of Low, Medium and High Doses of Poultry Manure on Different Water Quality Parameters of

 Experimental Water During 60 Days of Experiment (Mean±S.E).

Parameters	T ₀	TP ₁	TP ₂	TP ₃
Temperature	21.20 ^ª ±0.06	21.23 ^a ±0.06	21.36 ^a ±0.09	21.27 ^ª ±0.05
рН	7.68 ^a ±0.10	7.54 ^a ±0.12	7.36 ^ª ±0.10	7.24 ^ª ±0.11
Dissolved oxygen	5.52 ^b ±0.07	$5.10^{a} \pm 0.14$	5.22 ^{ab} ±0.17	4.66 ^c ±0.07
Free CO ₂	2.49 ^a ±0.08	5.05 ^c ±0.12	5.57 ^d ±0.10	5.57 ^e ±0.12
Alkalinity	154.02 ^a ±2.97	161.21 ^{ab} ±2.92	161.90 ^{ab} ±2.58	161.24 ^{ab} ±3.11
Total solids	58.94 ^a ±1.54	423.45 ^c ±0.30	397.99 ^b ±15.73	424.65 ^c ±0.40
Nitrates	0.015 ^a ±0.001	0.251 ^{bc} ±0.028	0.258 ^{bc} ±0.027	0.265 ^c ±0.017
Phosphates	0.043 ^a ±0.014	0.173 ^b ±0.003	0.347 ^e ±0.005	$0.429^{t} \pm 0.004$

Figure			
Effect of Low, Medium and High Doses of Poultry Manure on Different Water Quality Parameters of			
Experimental Water During 60 Days of Experiment			

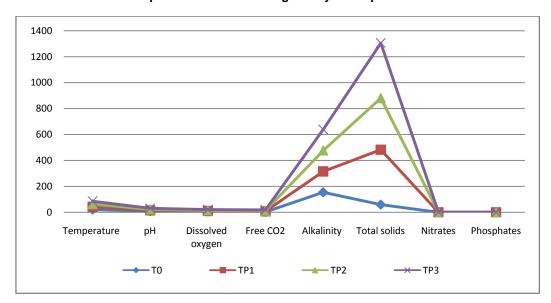


Table 2

Isolation and Identification of Pathogenic Bacteria from Poultry Manure Used for Treatment and Fish (Skin and Gills) Treated With Poultry Manure and Control

Name of the pathogens	Manure	Control (without manure)	PT1	PT2	PT3
E. coli	+	-	-	+	+
Salmonella sp.	+	-	+	+	+
Pseudomonas sp.	-	-	-	-	-