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HYGIENIC ASSESSMENT OF WATER AND FISH QUALITY AND PERFORMANCE FEEDING ON FRESH OR FERMENTED CHICKEN MANURE

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ABSTRACT

The aim of the present study is to provide an overview about the impact of feeding fish on chicken manure (CM) or fermented chicken manure (FCM) on the physicochemical character of water and performance of fish, as well as public health hazards and risks associated with consuming such fish in regard to fish quality. Nile tilapia (*Oreochromis niloticus*) as an experimental fish were stocked into 7 groups (G) with duplicate treatment. Each group received different mixture of CM or FCM with fish ration (FR), 0:100, 25:75, 50:50 and 100:0 (% CM or FCM: % FR). (Physico-chemical character of water and heavy metal load (Cu, Pb and Zn) of water and fish, as well as fish performance were measured and compared with control group received only fish ration (FR). Unionized ammonia (UIA) was significantly high ($P < 0.05$) at groups received FCM resulted in 100% mortality in such groups. Dissolved oxygen level (DO) was significantly low ($P < 0.05$) at both groups received CM and FCM compared with control group. While heavy metals load (zinc, copper, lead) at water samples showed no significant difference among the examined groups, however slight decrease were observed at groups received FCM. While in fish flesh zinc only was detected above permissible limits in all examined samples. Specific growth rate (SGR) percentage were lower at groups received CM and FCM. Results recommended that addition of CM as a feed for fish must be controlled carefully in regard to the amount added and method of application. 25% CM as a part of fish feed is quiet safe for adding according to the above result. In spite of FCM is less in heavy metals than CM, but its ammonia content is higher, so using FCM must be done with great care concerning the level of ammonia and DO.

Keywords: water: fish quality and chicken manure

INTRODUCTION

Fish is a major source of protein for the increasing world population especially in developing countries of Africa, Asia and South America (FAO, 2006a; Gabriel *et al.*, 2007) and the major solution to the dietary protein shortage in such countries is increased fish production (Nnaji *et al.*, 2009a,b). Therefore, the main aim of the Egyptian government is not only to increase the fish production from different resources, particularly from aquaculture, but also to improve the quality of fish product. To meet the growing demand for fish, aquaculture is experiencing a rapid development. Total aquaculture production in Egypt reached 705, 490 tonnes in 2009 (GAFRD, 2010).

The need for low cost system for fish production that will meet the food needs of the rural and urban poor and at the same time maximize the utilization of resources becomes pertinent (Nnaji *et al.*, 2011). Inorganic fertilizer has been promoted as more desirable due to its

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lower loading rates (due to higher nutrient contents) and lower oxygen demand (Yamada, 1986; Coleman and Edwards, 1987). However, manure has often been demonstrated to increase fish yield beyond that expected from the addition of inorganic forms of N and P alone (Schroeder, 1978; Noriega-Curtis, 1979). Studies have shown that there is a significantly higher level of fish yield in ponds fertilized with chicken manure than in ponds fertilized with inorganic fertilizer alone (Diana *et al.*, 1990). Many farmers pile the dry manure on the pond dykes, where it is sprayed with water for a few days before it is washed into the ponds. This process increases the fermentation rate and reduces the time to achieve maximum primary production.

Poultry litter and animal wastes in general contain high concentrations of some trace elements (Jackson *et al.*, 2003; Nnajiet *al.*, (2011) reviewed that CM applied to fish ponds is a mean for possible transfer of heavy metals in to aquatic system. Likely health risks to human beings and fish from heavy metal contamination arising from the use of CM and spilled chicken feed in rearing of fish in integrated chicken –fish system especially when fish reared in such a system and consumed for long periods. They explained the effect of chicken manure on pond /water sediments. They concluded that the persistence and toxicity of heavy metals in man and aquatic organisms calls for the constant monitoring of the incidence of these metals in the environment. According to Alinnorandobiji (2010) the contamination of water bodies and aquatic animals by heavy metals have been a global problem and constant monitoring of the environment for heavy metal contamination is important. This concern is largely due to the persistence, toxicity, bioaccumulative and non-biodegradable nature of such metals (Azmatet al 2008; Bhattacharya *et al.*, 2008). Fish consumption is an important avenue for pathogen and heavy metal exposure to man (Musaiger and D'Souz, 2008; Christopher *et al.*, 2009). Heavy metals concentration in the tissues of fish enter into human beings through food chain and due to their cumulative action causes potential health hazards sometimes even lethal (El-shehawiet *al.*, 2007). Heavy metals like arsenic (As), zinc (Zn) and copper (Cu) are also added feed supplements to boost poultry production and when added above the required levels they may accumulated to high levels, in the animals which are also transferred to aquatic systems when manure used for aquaculture. Organic manuring also leads to severe depletion of dissolved oxygen, high biological and chemical oxygen demand and high ammonia levels (Boyd, 1982), leading to stress in cultured fish (Parker, 1986).

Of all the water quality parameters that affect fish, ammonia is the most important after oxygen, especially in intensive systems. Ammonia in aquatic systems exists in both ionized (NH_4^+) and un-ionized forms (NH_3). The un-ionized form of ammonia present in effluent ponds and systems is toxic to aquatic life (Alabaster and Lloyd, 1980). Harmful effects of ammonia on aquatic organisms have been extensively studied (Adams *et al.*, 2001; Alabaster and Lloyd, 1980; Chen and Lin, 1995; Harris *et al.*, 1998; Noor-Hamid *et al.*, 1994; Rasmussen and Korsgaard (1996). Ammonia causes stress and damages gills and other tissues, even in small amounts. Fish exposed to low levels of ammonia overtime are more susceptible to bacterial infections and have poor growth. Acute toxicity of un-ionized ammonia affects the survival of all aquatic organisms, while long term exposure to un-ionized ammonia (UIA) induces chronic toxicity effects on growth, reproduction and longevity (Andersen and Buckley, 1998; Arauzo, 2003; Arauzo and Valladolid, 2003; Leung *et al.*, 2011). Knud-Hansen *et al.* (1991) found through leaching experiments that about 40% of chicken manure (N) was released as either ammonia or nitrate-nitrite after 6 days' immersion in pond water. Nitrate, the end product of the nitrogen cycle, is considered to be harmless to fish in natural systems and ponds as it is used as a fertilizer by plants, including phytoplankton. In closed systems with little or no water exchange, however, nitrate will accumulate and may be harmful if higher than 250 mg/L. Acute and chronic effects of nitrate have been reported in several fresh water fish species (Camargo *et al.*, 2005; Hamlin, 2006; Kamstra and van der Heul (1998), and marine invertebrates (Camargo *et*



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al., 2005; Hirayama, 1974; Kuhn *et al.*, 2010; Romano and Zeng, 2007).

To the best of author's knowledge most of previous studies concerned mainly with the benefits of using CM as cheap organic fertilizer for fish ponds. Therefore, the aim of the present study is to provide an idea about the impact of using CM on water and fish quality. Two types of chicken manure were used fresh (CM) and fermented under thermophilic condition (55°C), with the aim to compare the effect of this treatment on water and fish quality. Physico-chemical character of pond water, heavy metal load as Cu, Pb, and Zn of water and fish as well as fish performance were measured and compared with control groups receiving only fish ration (FR).

MATERIALS AND METHODS

This laboratory study was carried out for 60 days (from 1st September till 1st November, 2012) at laboratory of department of Hygiene and preventive medicine faculty of vet. Medicine, Kafrelshikh University, Egypt. Nile tilapia (*Oreochromis niloticus*), (was used for this study

Fingerlings with mean average weight of 10g and 7 cm length was obtained from local fish farm (El-Reyad, Kafr El-Shiekh, Egypt). Fish were homogenous in size, body weight and apparently health. The fish were acclimatized for seven days before the experiment. They were fed on the same diet (fish ration composition is illustrated in table 2) used in this study. During this adaptation period, the dead and weak fish were eliminated daily.

Chicken manure (CM) and fermented chicken manure (FCM)

CM was obtained from Kafrelshikh University chicken farm (cage layer system) was collected from deposits directly under chicken cages. Fermented CM (FCM) was prepared by placing CM in a set of 125 ml capacity anaerobic serum vials. The headspaces of in the bottles were purged with N₂ gas, and the vials were sealed with rubber stoppers and crimped aluminum caps. These bottles were incubated anaerobically 55°C. Biogas produced was monitored every day. When gas production stopped, vials were opened; FCM produced was collected and kept under -20°C until used (Abouelenien *et al.*, 2009; 2010). Characteristics of CM and FCM were illustrated in Table 1.

Experimental design

Feeding regime was 3 % of body weight per day was employed throughout the trial. The amount of feed was calculated and readjusted after weekly weighing. The fish aquaria under experiment were divided into 7 groups (G), stocked into 14 aquaria (dimension, 40x50x100cm) at stocking rate of 14 fish per aquaria (60 L water /aquaria). The fish aquaria were supplied with chlorine free tap water with continuous aeration, using an electric aquarium air pump (HaliBAO-Cx (8200 for 60 days with. Two set of manure feeding used, first set received CM as a feed with different ratios (ranged from 0-100%), the second set received FCM, with different ratios (0-100%), and control aquaria which received fish ration (FR), containing 27% crude protein (CP). Composition and chemical analyses of the experimental CM, FCM and FR are shown in table (1) and (2). 2 replicate for each treatment, water changes on daily basis. Detailed description of the groups was illustrated in Table 3.

Sampling

Water sampling was done once every two weeks, with a total number of 4 water samples which done at fixed time (Jha *et al.*, 2004). Water samples were collected by inverting 250 glass bottle 30 cm below the pond water surface. All samples were transferred to the lab. Five Fish were sampled at the same time of water sampling with water sampling by harvested with net



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and put in polyethylene bag. Ten grams of muscle portion of fish along with skin were homogenized for 1 min. with 90 ml of saline (0.85% NaCl) in a stomacher .

CM, FCM and FR were sampled with a rate of 100g, of each. Samples were transferred into a plastic bag and subjected to sample preparation according to APHA (1992; 1998).

Analytical methods

Water temperature, pH and dissolved oxygen were measured in situ with (a Yellow Springs dissolved oxygen (DO) meter Model 54A). Pond water pH was determined using a Suntex digital meter Model Sp-5A. Temperature was recorded by a mercury thermometer. TOC was determined with a TOC analyzer (TOC-5000, Shimadzu). (TS, VS, TKN, NH₃-N, and NO₃-N were measured in accordance with the standard methods (APHA, 1998).

Digestion of water samples, 10ml of nitric acid was added to 50ml of each water sample and heated at 150°C for 30mins. 5ml of nitric acid was then added to each tube and heated for 30minutes at 200°C, to the mixture was added 2ml of hydrogen peroxide before further heating at 200°C for 30minutes. The resulting solutions were allowed to cool at room temperature and then the volume made up to 25ml with distilled water (AOAC, 1995).

Fish samples were analyzed by a modified procedure from the Association of Official Analytical Chemists (AOAC, 1995). For analysis of heavy metals in CM, FCM, and FR samples were prepared according to the technique described by AOAC official method 999.11. In prepared samples of water, fish muscles, CM, FCM, and FR. Lead (Pb), (Zinc) Zn (and Copper) (Cu) using Atomic Absorption Spectroscopy, (Model-analyst 200, PerkinElmer).

The weights of the fish were recorded at the beginning of the experiment and during harvest to the nearest 0.001 g. Dead fish were removed daily, and were not replaced during the course of the study. Differences between the number of fish stocked and the number of fish at harvest were used to calculate mortality percentage in each treatment. The specific growth rate (SGR) was calculated as: $SGR = 100 \frac{\ln W_t - \ln W_0}{t}$; where W_0 and W_t are the initial and final weights of live fish (g) respectively, and t is the culture period in days (Ricker, 1975).

Statistical analysis

Data was presented as means \pm SEM and analyzed by one-way ANOVA using Graph Pad™ prism 5. The Tukey's Multiple Comparison Test was used as a post hoc test when appropriate ($P > 0.05$, ANOVA). The significance level was set at $P > 0.05$.

RESULTS AND DISCUSSION

Table (1), illustrated that the highest amount of TKN was found in CM (87 g-N kg-TS-1) and lowest in FR. Additionally highest amount of TAN was found in FCM (30 g-N kg-TS-1) from this result the amount of CM replaced FR was calculated. The crude protein content in FCM was lower than that in CM. Water temperature was between 24.22°C and 25.02°C during the 8 weeks. However, there was no difference in water temperature from one management regime to another on any particular sampling date (Table 4). The water pH in all the treatments was alkaline (Table 4) with the lowest value in control group (8.26), while the highest value was reported in G7 (8.47). Average values of total ammonia and un-ionized ammonia were illustrated in table 4 which significantly higher in G7 (100% FCM) $P < 0.05$. Highest unionized (15.49 mg/L) ammonia was found in water samples taken from G7 (100% FCM), and lowest value (4.17 mg/L) was in G1 (control). However there was no difference in nitrate values between different groups (1.95 to 2.36 mg/L) (table 4). The values of dissolved oxygen were significantly higher ($P < 0.05$) in G1 (control) treatment, than other treatments (Table 4).

Metal concentrations (Pb, Zn and Cu) in fish and water samples in different treatment groups



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are illustrated in table 5. The mean concentration of Pb, Zn and Cu in water samples were ranged from 0.03 (G7) to 0.11 (G3), 0.79 (G7) to 1.26 (G2) and 0.09 to 0.23 mg/l respectively. Cu and Pb were not detected in fish muscle samples in any treatment; however Zn was detected in a range of 32.13 to 44.13 mg/g (Table 5).

The final body weight of the Nile tilapia under different treatments ranged from 8.85 to 16.38 g with significant difference between groups (Tab. 6). At harvest, maximum final weight was achieved in G2 (25% CM), and lowest in G4 (100% CM). The specific growth rate (SGR %/day) was ranged from 0.4 to 1.92 with highest value in G2 and lowest in G4 (table 6). Survival of Nile tilapia among treatments was ranged from 0 in G7 (100% FCM) to 100% in G1 (control).

Evaluating the impact of introducing organic fertilizers (chicken manure) with or without supplementary feeding on physico-chemical character of pond water, heavy metal load as Cu, Pb, and Zn of water and fish, as well as fish performance were studied using Nile tilapia. CM contains high amount of organic nitrogen in the form of uric acid (Krylova et al., 1997, (with thermophilic anaerobic fermentation ammonia was accumulated) Abouelenien et al., 2010; 2014). (The amount of CM and FCM used in different groups was calculated on dry matter (DM) basis according to its crude protein content. Amount of crude protein measured in the current study either in CM (12.39) and FCM (9.68) was lower than that measured by Rapatsa and Moyo (2013) who found 27.6% CP in CM (Table 1).

Investigating the water quality of experimental ponds is of great importance in regulating and following the suitability of their water for holding and rearing fish. For basic understanding of water quality in studied ponds, water temperature, dissolved oxygen, pH, nitrate and ammonia were determined and are given in Table (4). No significant difference in water temperature between different groups during the experiment. This result was the same as obtained by El-Ebiary (1998) and Priyadarshini et al. (2011). Farmanfarman and Moore (1979) reported that, aquatic organisms could put up with a wider range of temperatures, without sudden or severe fluctuations. Water temperature over 18 °C was best for warm water fish survival and growth and many species suffered and died below 12 °C (Nagel, 1979). The current temperature results were slightly lower than the optimal temperature for growth of tilapia that is 26-34 °C (Saber et al., 2004). (No significant difference in values of pH, which was in the alkaline range throughout the experiment (Table 4). This result could be attributed to the high level of ammonia in all groups received either CM or FCM, and indicating favorable conditions for biological production (Priyadarshini et al., 2011 and Fallah et al., 2013). However, this range is still in the range that optimum for growth of tilapia (Ross, 2000).

Dissolved oxygen (DO) was highest in G1 that not received manure and lowest in G7 that received 100% FCM (Table 4). There is a significant difference between control group and groups from G3-G7 but no significant difference between control group and G2 that received 25% CM. This finding could be attributed to the addition of manure, which contains organisms that decompose the organic matter and thus consuming the DO leading to decrease in its concentration in water (Fallah et al., 2013). However, Tilapia is highly tolerant to low dissolved oxygen concentration (0.1 mg/l) (Magid and Babiker, 1975) and the optimum growth is obtained at concentrations greater than 3 mg/l (Ross, 2000). The measured DO was lower than that measured by Rapatsa and Moyo (2013), who found the mean of DO in ponds received CM was 7.73 mg/l.

Ammonia is a killer when present in higher concentrations, and many unexplained production losses have likely been caused by ammonia (John and Tucker, 2004). UA was calculated from TAN through measuring of temperature and PH. UA is more dangerous than ionized ammonia and in the present study pH was (≥ 8) which intensify the fraction of UA



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)Arauzoand Valladolid, 2003 and Leung et al., 2011). Un-ionized ammonia (UIA) was higher with groups received either FCM or CM compared to control group which is significantly different (Table 4). Where, UIA reach up to 15.09 mg/l in G7 that received 100% FCM and this concentration resulted in 100% fish mortality because of ammonia toxicity. As ammonia is toxic to tilapia at concentrations of 2.5 and 7.1 mg/l as UIA, for blue and Nile tilapia respectively (Redner and Stickney, 1979 and El-Sherif et al., 2008) and depressed feed intake and growth at concentrations as low as 0.1 mg/l (El-Sherif et al., 2008). Optimum concentrations are estimated to be below 0.05 mg/l (El-Sherif et al., 2008). Exposure of fish to different high concentrations of ammonia and/or UIA are known to cause behavioral and physiological changes in fish and have several possible mechanisms of toxicity (Evans et al., 2006). Under the condition of this experiment, exposure of Nile tilapia to high concentration of ammonia and/or UIA was accompanied by decreased swimming activity. At first, the fish settled on the bottom of fish pond with very little movement. With the increase of ammonia concentration at (G6 and G7), the fish moved higher in the water column and reach the surface of fish pond with the highest level of ammonia and UIA (G7). For groups that received CM and FCM (Except 25% CM), fish showed dark discoloration of body and lethargy. This may be attributed to the respiratory difficulties originated from high ammonia and UIA and low DO (Israeli-Weinstein and Kimmel, 1998). The decreased activity of fish resulted in lowering of ammonia production and subsequently reduced its harmful effects. While, under higher concentration of UIA, fish became more lethargic and anorexic which leads to exhaustion, surface swimming and death (Randall and Tsu, 2002).

There is no significant difference in Nitrate concentration between different groups ($P < 0.05$). This result was nearly the same obtained by Priyadarshini et al., 2011) (who studied the effect of using manure as a feed for fish).

According to the Environmental Protection Agency (EPA), lead, copper and zinc are some of the most common heavy metals inducing pollution (Ang, 2008).

With the aim to study the heavy metal impacts through feeding the fish on CM and the potential transfer of metals to human through consumption of fish flesh. Cu, Zn, and Pb were measured in CM, FCM, FR, water and fish muscle samples. The result of the present study showed that no significant difference between different groups and control group ($P < 0.05$ in concentration of tested metals (Cu, Pb and Zn) (Table 5).

The Concentrations of lead (Pb) (in water samples) showed no significant difference between groups ($P < 0.05$ with maximum concentration, of 0.11 mg/l, in G3 which received 50% CM. While the lowest concentration was found in G7 (0.03 mg/l). (It seems that anaerobic fermentation of CM resulted in decreasing in the level of Pb. In addition, this value is less than that of control. The level of Pb in all groups does not exceed the maximum permissible limit MPL of EOS (1993), (which is 0.1 mg/l) (Table 5). The mean concentration of Zn in different groups was not significantly different ($P < 0.05$ with highest in G2 and lowest in G7 with 100% FCM. Moreover, the mean of Zn in all groups was less than the maximum permissible limit recommended by EOS, (1993) which is 5 mg/l) Kebede and Wondimu, (2004). For Cu, there was no significant difference between groups with highest in G1 and G3 (0.23 mg/l), and lowest in G5 (0.09 mg/l), and the values of all groups are less than the MPL (Table 5). The values of Zn and Cu in CM is decreased after thermophilic anaerobic digestion (Table 1), so it is expected that the water samples from groups received FCM have lower concentrations of these metals than others. This result was in agreement with that obtained by Voća et al., (2005).

The result of fish muscle examination showed that, only Zn was detected with highest level in G4 (44.13 mg/kg), and lowest in G1 (control), however no significant difference between groups. The level of Zn in fish muscles was lower than the MPL (EOS, 1993), except for G4 which was higher than the MPL (Table 5). Zinc in aquatic environment is a potential toxicant to



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fish) Everallet *et al.*, 1989) which causes disturbances of acid-base and ion regulation, disruption of gill tissue and hypoxia (Hogstrand *et al.*, 1994). (SenthilMurugan *et al.* (2008) reported that zinc load in fish muscle was low as compared to other organs.

There were significant difference ($P > 0.05$) at the average of feed intake and final body weight between control and different groups received fresh and fermented CM (Table 5). Highest FI and final body weight were recorded in G2 that received 25 % CM while the lowest one in G4 which received 100% CM. This result could be explained on the basis of better water quality of water in this group as lower ammonia content was observed. Feeding and manuring, individually and in combination, improved the growth of fish significantly ($P < 0.05$) in all groups (Table 5).

Highest Specific growth rate (SGR %) was observed in G2 which received 25% CM and the lowest was in G4 that received 100% CM. The average SGR percentage was higher than that obtained by El-Ebiary (1998) who obtained 1.05% SGR for tilapia reared on poultry manure as a fertilizer. Survival rate was highest in control group followed by G2 and lowest was in G7 which received 100% FCM which showed 100% mortality which may attributed to sudden elevation of ammonia level by addition of FCM. As FCM contain higher amount of ammonia than non-fermented) Abouelenien *et al.*, 2010), which caused fish toxicity and death.

CONCLUSION

Ammonia toxicity is the main problem appeared to limit the portion of chicken manure applied to fish aquaculture. UIA concentration was higher than 6 mg/l in all groups received CM or FCM with higher values in Groups received FCM. Fish group that received 100% FCM showed 100% mortality caused by ammonia toxicity. The practice which done by fish farmer which leads to fermentation of CM before applying it to the fish pond cause increase in UIA of CM. Groups which received 25% CM showed better performance of fish and water quality. Fermentation of CM lowers its heavy metal content and consequently decreasing its concentration in water samples. No significant difference found between groups concerning the measured metals) Pb, Zn, and Cu), while their concentration were less than the MPL reported by EOS (1993). Zn was the only mineral detected in fish muscle, reaching its highest concentration in G4, which received 100% CM. From the above results, it is recommended that addition of CM as a fertilizer or feed for fish pond should be controlled carefully concerning the amount added and method of application. Adding of 25% CM as a part of fish feed is quiet safe according to the above result. In spite of FCM is bacteriologically safer than CM and contain more available nitrogen, less heavy metals, but its ammonia content is higher. Therefore, using of FCM should be done with great care to level of ammonia and DO.

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