Egyptian Journal of Aquatic Research (2015) **41**, 93–100

HOSTED BY

ELSEVIER

National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

http://ees.elsevier.com/ejar
www.sciencedirect.com

FULL LENGTH ARTICLE

Impact of using raw or fermented manure as fish feed on microbial quality of water and fish

Nagham Elsaidy [a,*], **Fatma Abouelenien** [a], **Ghada A.K. Kirrella** [b]

[a] *Department of Hygiene and Preventive Medicine, Faculty of Vet. Medicine, Kafrelsheikh University, 33156, Egypt*
[b] *Department of Food Control, Faculty of Vet. Medicine, Kafrelsheikh University, 33156, Egypt*

Received 31 October 2014; revised 19 January 2015; accepted 19 January 2015
Available online 6 March 2015

KEYWORDS

Chicken manure;
Fermented chicken manure;
Total bacterial count;
Total coliform count;
Salmonella

Abstract The microbial water and fish quality was assessed due to feeding of chicken manure (CM) and fermented chicken manure (FCM) to fish in ponds, using Nile tilapia (*Oreochromis niloticus*) which were classified into 7 groups (G). Each group received different mixtures of CM or FCM with fish ration (FR), 0:100, 25:75, 50:50 and 100:0 (%CM or FCM:% FR). The obtained results revealed that total bacterial count (TBC) and total coliform count (TCC) were significantly high at $P \leqslant 0.05$ in CM than both FCM and fish ration (FR). *Escherichia coli* and *Salmonella* were isolated from CM but not from FCM or FR. Additionally, TBC and TCC were significantly high at $P \leqslant 0.05$ at water and fish samples raised at CM ponds followed by FCM ponds in comparison with FR. Both *E. coli* and *Salmonella* were isolated from water and fish raised in ponds receiving either CM or FCM with higher incidence in those with CM. However all water and fish samples examined were free from *E-coli* O157: H7. The obtained results, proved the influence of CM on water and fish quality and recommend the use of FCM as a bacteriologically safe fish pond fertilizer.

Introduction

Worldwide more than 1 billion people rely on fish as an important source of animal proteins (i.e., fish provides at least 30% of their animal protein intakes) (Omojowo and Omojasola, 2013). Fisheries and aquaculture in Egypt are important components of the agricultural sector and a significant source of animal protein (Abo-Elela et al., 2005; El-Naggar et al., 2008). Aquaculture is a real tool in increasing fish production, which was achieved through higher fish stocking density and the application of artificial feeding. Unfortunately, the cost of feeding is enormous; therefore interest has been diverted to other sources of enrichment of the water, such as using of animal manure.

Fish pond manuring is often used in fish farming for the intensification of fish production by balancing the ratio between carbon and other nutrients. The manure is directly consumed by fish, and the released nutrients support the growth of mainly photosynthetic organisms (Moav et al., 1977; Little and Edwards, 1999). Additionally the manures were applied to produce some necessary plant nutrients which serve as a soil fertilizer by adding the organic matter (Sloan et al., 2003).

* Corresponding author. Tel.: +20 1005468643, +20 473243453; fax: +20 473231311.
E-mail address: nagham.elsaidy@yahoo.com (N. Elsaidy).
Peer review under responsibility of National Institute of Oceanography and Fisheries.

http://dx.doi.org/10.1016/j.ejar.2015.01.002

Different kinds of manure can be utilized; cow, poultry dung and semi-liquid pig manure are of the highest interest (Govind et al., 1978; Wohlfarth and Schroeder, 1979). Among manures used, chicken is preferred because of its ready solubility and high level of phosphorus concentrations (Knud-Hansen et al., 1991). However, the use of manure, classified as hazardous organic matter originating from animal feces, poses a risk to the water environment (Mlejnkova and Sovova, 2012). Additionally it represents great public health concern; owing to an increase in the concentrations of pathogenic microorganisms in animal manure which is exaggerated by the confinement of farming system units with little exchange of water (Petersen et al., 2002).

Prithwiraj et al. (2008) tested the effectiveness of introducing live zooplankton against direct manuring in ornamental fish, through comparing four management regimes including poultry manure (PM), live zooplankton , cow dung (CD) and fish feed, and they found that average counts of heterotrophic bacteria in the water of PM and CD ponds were significantly higher than other treatments ($P < 0.05$).

The microbiological analyses revealed the presence of various pathogenic microorganisms in manure in addition to the common microflora of animal intestines. Persistence of pathogens in manures and in the water environment is one of the most important factors for infection transmission. It was found that zoonotic pathogens can survive in such environments up to 4 months, depending on the type of manure, temperature, pH, oxygen level, ammonia concentration and the presence of competing organisms (Jones, 1976; Guan and Holley, 2003).

Under the use of thermophilic fermentation of CM most of pathogens are destroyed. The thermophilic anaerobic process is effective against pathogenic bacteria (such as fecal coliform, *salmonella* and enterococcus) (Watanabe et al., 1997). *Salmonella* and *Mycobacterium paratuberculosis* were inactivated within 24 h under thermophilic conditions, while weeks or even months will be needed under mesophilic conditions (Sahlstrom, 2003).

Information about the effect of manuring on water quality and fecal contamination is scarce. Additionally the usual practice of fish pond owners to keep CM in tightly sealed plastic bags and leave it for some time in the sun, creates a condition resembling to some extent the anaerobic thermophilic fermentation process. So the aim of this study is to clarify the impact of using raw CM in fish farm environments on the occurrence of water pollution indicator bacteria, defining related potential health risks represented by pathogenic bacterial contamination especially the detection of *Salmonella* and *E. coli* O157.

Moreover, the effect of anaerobic treatment of CM under thermophilic conditions on microbial contamination of fish pond water has to our knowledge not been investigated previously.

Material and methods

This study was carried out for 60 days (from 1st September till 1st November, 2012) at the Laboratory of the Department of Hygiene and Preventive Medicine, Faculty of Vet. Medicine, Kafr El-Shiekh University, Egypt. Nile tilapia (*Oreochromis niloticus*), were used for this study.

Fingerlings with a mean average weight of 10 ± 0.58 g and 7 ± 0.68 cm length were obtained from the local fish farm (El-Reyad, Kafr El-Shiekh, Egypt). Fish were homogeneous in size and body weight and apparently healthy. The fish were acclimatized for 7 days before the experiment. They were fed on the same diet 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 the Kafr El-Shiekh University chicken farm (cage layer system) and 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 bottles. The headspaces of the bottles were purged with N_2 gas, and the vials were sealed with rubber stoppers and crimped aluminum caps. These bottles were incubated anaerobically at 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 use (Abouelenien et al., 2009, 2010).

Experimental design

Feeding regime of 3% of Body.wt per day was employed throughout the trial. The amount of feed was calculated and readjusted weekly. The fish aquaria under experiment were divided into 7 groups (G), stocked into 14 aquaria (dimensions, 40 × 50 × 100 cm) at a 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 (Hali BAO-Cx 8200) for 60 days. Two sets of manure feeding were used, the first set received CM as a feed with different ratios (ranged from 0% to 100%), the second set received FCM, with different ratios (0–100%), and the control aquaria which received commercial fish ration (FR). 2 replicates for each treatment were made, with water changes on a daily basis. A detailed description of the groups is illustrated in Table 1.

Sampling

CM, FCM and FR were sampled with weights of 100 g, each sample was transferred into a plastic bag and analyzed to enumerate total bacterial and fecal counts, as well as to determine the presence of *Escherichia coli* and *Salmonella* spp. (APHA, 1992). Water sampling was done once every 2 weeks, with a total number of 4 water samples which was done at a fixed time (Jha et al., 2004). Water samples were collected by inverting a 250 ml sterilized glass bottle 30 cm below the pond water surface. All samples were transferred to the lab in an icebox. Analysis was initiated within 2 h of sample collection. Five fish were sampled at the same time with water sampling by harvesting with a net and put in a sterile polyethylene bag. 10 g of the muscle portion of fish along with the skin was homogenized for 1 min with 90 ml of peptone water in a homogenizer (Polytron®PT-MR 2100).

Bacteriological analysis

Enumeration of total bacterial counts (TBCs)

The total bacterial count of water samples was carried out according to Cruickshank et al. (1972) and Ayandirana et al. (2014).