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HAZARDS ASSOCIATED WITH THE USE OF CHICKEN MANURE IN FERTILIZATION OF FISH PONDS

(With 2 Tables and One Figure)

By

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المخاطر المصاحبة لإستخدام زرق الدواجن في تسميد الأحواض السمكية

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تعتبر الأسماك مصدرا هاما للبروتين الحيوانى ولزيادة الأنتاجية استخدمت العلائق المصنعة في تغذية الأسماك ونظرا لأرتفاع سعر تكلفتها اتجهت الأنظار فى انحاء العالم الى اضافة الأسمدة العضوية من روث الحيونات وزرق الدواجن كعامل مساعد لتغذية الأسماك لرخص ثمنها. وتناولت معظم الدراسات العائد الأقتصادى ولم تتناول المخاطر الصحيةعلى الأسماك من استخدام الأسمدة العضوية. ولذا اجرى هذا البحث لأستبيان مدى خطورة استخدام زرق الدواجن الغير معامل على صحة الأسماك (البلطى النيلى) ودوره فى نقل العديد من الأمراض لها. تم اجراء البحث علي احواض معاملة بالأسمدة العضوية واخرى غير معامله وتم جمع ٥٠ عينة لكل من زرق الدواجن و٥٠ عينة لكل من اسماك و مياه الأحواض المعاملة والغير معاملة. وبالفحص البكتريولوجى لعينات زرق الدواجن وجد ان متوسطات العدد الكلى لميكروبات القولون وميكروبات القولون البرازية والميكروبات السبحية البرازية هى ٤,٥ X $١٠^{٨}$ و ١,٩ X $١٠^{٥}$ و ١,٩ X $١٠^{٦}$ على التوالى بينما كانت فى مياه الأحواض المعاملة ١,٤ X $١٠^{٦}$ و ٢,٦ X $١٠^{٣}$ و ٣,٢ X $١٠^{٣}$ أما عينات مياه الأحواض. الغير معاملة ٦,٠ X $١٠^{٣}$ و ١,٩ X $١٠^{٢}$ و ٢,٨ X $١٠^{٢}$ على التوالى. بالفحص البكتريولوجي لعينات الأسماك فى الأحواض المعاملة كان العدد الكلى لميكروبات القولون ٤,٨ X $١٠^{٤}$ بينما كانت ٣,٥ X $١٠^{٣}$ فى عينات الأسماك فى الأحواض الغير معاملة. كان العدد الكلى لكل من ميكروبات القولون البرازية والسبحية البرازية ٥,٢ X $١٠^{٣}$ و ٥,٤ X $١٠^{٢}$ فى عينات الأسماك فى الأحواض المعاملة بينما كانت ٢,٦ X $١٠^{٢}$ و ١,٢ X $١٠^{٢}$ فى عينات الأسماك فى الأحواض الغير معاملة. تم عزل ٢٨٠ عترة منها ١٣٢ (٤٧,١٪)

عترة من عينات زرق الدواجن و ٨٠ (٢٨,٦٪) عترة من عينات مياة الأحواض و ٦٨ (٢٤,٣٪) عترة من عينات الأسماك. وبتصنيف العترات المعزولة وجد منها ٤٥ (١٦,١٪) لميكروبات السبحية البرازية و٣٧ (١٣,٢٪) للسيدوموناس و ٣٦ (١٢,٨٪) للأريوموناس و ٦ (٢,١٪) للفلاقوبكتريم كولمنارز وهى من العترات المسببه للأمراض فى الأسماك وقد تم مناقشة الأهمية الصحية للميكروبات المعزولة ومدى خطورتها على صحة الأسماك. توصى الدراسة بمعاملة زرق الدواجن قبل استخدام فى تسميد الأحواض السمكية.

SUMMARY

The main aim of this study was to evaluate the bacterial impact of adding untreated chicken manure to fish ponds as an organic fertilizer on fish health. The most probable number (MPN) of Total Coliforms (TC), Faecal Coliforms (FC), and Faecal Streptococci (FS) were estimated from samples of chicken manure and water and fish (Nile tilapia, *Oreochromis niloticus*) from ponds treated with chicken manure and other not treated as control. Results indicated that the mean MPN of TC, FC, and FS of chicken manure samples per gram were 4.5 X 10^8, 1.9 X 10^5, and 1.9 X 10^6, respectively. The mean MPN of TC, FC, and FS of water samples per ml from ponds treated were respectively 1.4 X 10^6, 2.6 X 10^3, and 3.2 X 10^3, while those mean MPN were 6.0 X 10^3, 1.9 X 10^2, and 2.8 X 10^2, respectively in water of control ponds. Fish samples from ponds treated showed mean MPN of TC, FC, and FS per gram to be 4.8 X 10^4, 5.2 X 10^3, and 5.4 X 10^2, respectively, while those of control ponds were 3.5 X 10^3, 2.6 X 10^2, and 1.2 X 10^2 respectively. Major fish pathogens isolated were *Streptococcus faecalis, Pseudomonas spp., Aeromonas hydrophila,* and *Flavobacterium* columnare as 45 (16.1%), 37 (13.2), 36 (12.8%), and 6 (2.1%) strains, respectively. The hygienic significance of fish pathogens isolated was discussed. This study suggests that chicken manure should be treated before applying to fish ponds to lower the possibilities of contamination with pathogenic bacteria.

Key word: Chicken manure, Nile tilapia (*Oreochromis niloticus*), Ponds, and Fertilization

INTRODUCTION

The basic goal of successful fish production operations is high yields with low input, and limited, if any, supplementary feed for fish (Petersen *et. al*, 2002). Nutrients necessary for pond production tend to be in short supply in water used to fill ponds. In practice, this condition is overcome by adding feed or fertilizers to ponds. The use of organic manure to fertilize ponds has become a traditional technique in fish culture in various countries of the world. Organic fertilizer, apart from being utilized as food directly by fish, encourages pond productivity by improving the growth of phytoplankton and also serves as a substrate for production of bacteria and protozoa that serve as food for zooplankton and fish (Schroeder, 1978). Chicken manure has been used successfully as an organic fertilizer for Nile tilapia (*Oreochromis niloticus*) production in many parts of the world (Rappoport and Saring, 1979 and Shevgoor *et al.*, 1994).

For economic and biological efficiency, applications of organic fertilizers to fish ponds should be based on the balance between nutrients required by fish-food organisms and hazards generated from using such fertilizers. The occurrence and persistence of certain pathogenic bacteria and viruses in chicken manure used as fertilizer in fish ponds could be potential health hazard to fish and to consumers of fish grown in such treated ponds (Vaughan and Ryther, 1974). Most of studies that investigated using manure as fish ponds fertilizer focused on the productivity of such treated ponds, water quality issues, or human public health problems as a result of consuming fish grown in ponds treated. On the other hand, only few studies have investigated the potential diseases of fish that may arise from using manure to fertilize fish ponds. Jinyi *et. al* (1987) concluded that numbers of *Aeromonas sp.* And *Pseudomonas sp.* in manured ponds are higher than those in control ponds which could cause fish diseases, and numbers of heterotrophic bacteria isolated from fish skin were related to those in water and depending on the amount of manure applied to ponds. According to a study held by The Livestock Manure Pollution Prevention Pilot Project Working Group (Ontario, Canada 1997), manure spills were the leading cause of fish kills reported from 1988 to 1997 in Ontario, Canda. In addition, accumulation of organic matter in fish pond leads to high concentrations of ammonia and nitrite and low dissolved oxygen reducing the water quality (Boyed, 1982) that may act as a precursor to fish infections (Walters and Plub, 1980).

The present study aims to shed some light on potential threats of causing fish disease outbreaks if untreated chicken manure is used to fertilize fish ponds through the determination of microbial contamination of chicken manure, water, and fish.

MATERIAL and METHODS

The study was conducted in Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, and Animal Healthy Laboratory Research in El-Minia Governorate.

Fish and ponds fertilization:

Samples were collected from different fish ponds treated with chicken manure as organic fertilizater and others non-treated as a control in El-Minia Governorate. Ponds have a clay bottom and average size of about ¼ faddan in surface area with a depth of 1¼m. Fish were daily fed with standard poultry feed at 3% of their weight. Chicken manure was brought directly from poultry houses with no treatment and scattered on the surface of the water to fertilize some ponds during summer season at rate of 15kg/ faddan/day, while other ponds remain with no organic fertilization.

Preparation of samples:

1. Chicken manure sampling

Fifty litter samples were collected in sterile polyethylene bags and delivered to the laboratory without delay for bacteriological examination according to Williams *et al*. (1975). Thirty grams from the well-mixed chicken manure sample were placed in a sterile container with 270ml of sterile distilled water and left for 20 minutes. Samples were shaken well and filtrated through sterile gauze to sterile containers.

2. Ponds water sampling:

Fifty water samples were collected from different locations of the treated and non-treated (control) ponds according to the World Health Organization (WHO, 1971) and American Public Health Association (APHA, 1980) in clean, sterile colorless 500ml bottles closed with sterile glass stoppers. These samples were sent to the laboratory without delay.

3. Fish sampling

Fifty fish samples of Nile tilapia (*Oreochromis niloticus*) were collected from control and treated ponds. Clinical signs were recorded and the fish were, immediately, transferred in sterile plastic bags on ice to the laboratory for bacteriological examination. Twenty-five grams from muscle of fish examined were blended with 225ml of 0.1% sterile peptone water in warring blender at high speed for one minute.

Bacteriological examination of samples:

1. Determination of most probable numbers of Total and Faecal Coliforms counts:

Chicken manure samples were ten-fold serially diluted with sterile saline up to 10^{-12}, while fish and water samples were ten-fold serially diluted with sterile saline up to 10^{-6} for examination. Samples were tested for Total Coliform (TC) and Faecal Coliform (FC) counts using the multiple tube technique to determine the most probable number (MPN) according to Oblinger and Koburger (1975), and APHA (1980).

2. Determination of Faecal Streptococcus count:

Enterococcus Selective Differential media (ESD) was used and all magenta colonies were counted as *Streptococcus faecalis* (Efthymious and Joseph, 1974).

3. Isolation and Identification:

Membrane filter (Sartorious membrane filter pore size 0.45µm) with negative pressure technique was used for isolation of microorganisms. The membrane filter was divided before being immersed in various enrichment media and then cultivated into brain heart infusion agar, trypticase soy agar, MacConkey's agar, Staphylococcus No.110, ESD medium, Cytophaga agar medium and Hsu-Shotts (Austin and Austin, 1984) medium at 28^0C and 37^0C for 24-72 hours. Pure culture were transferred to nutrient agar slants for 24 hours at 37^0C and kept for further biochemical identification. Identification of isolates was carried out according to Anaker and Ordal (1959), Bailey and Scott (1974), Efthymious and Joseph (1974), Cruickshan *et. al.* (1975), International Commission on Microbiological Specifications for Foods (ICMSF, 1978), Speck (1984), Inglis (1993), and Robert *et. al.*, (1998).

RESULTS

Results presented in Table 1 show that the mean value of most probable number (MPN) of Total Coliform (TC) in chicken manure was $4.5X10^8$ colony forming unit (CFU)/g, while it was $1.4x10^6$ CFU/ml and $6.0X10^3$ CFU/ml in water of treated and control ponds, respectively. Fish of treated ponds showed TC of $4.8X10^4$ CFU/g, one log more than that of fish of control ponds which was only $3.5X10^3$ CFU/g. On the other hand, MPN of Faecal Coliforms (FC) isolated from chicken manure was $1.9X10^5$ CFU/g. Most probable number of FC isolated from water of ponds treated, however, was $2.6X10^3$ CFU/ml, whereas, in

Table (1) Analysis of the Most Probable Number (MPN) of bacteriological examination of chicken manure, water, and fish samples.

		Total Coliforms			Faecal Coliforms			Faecal Streptococci		
		Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Manure MPN/g		$1.3X10^{6}$	$5.2X10^{9}$	$4.5X10^{8} \pm 2.9X10^{8}$	$4.0X10^{2}$	$1.2X10^{6}$	$1.9X10^{5} \pm 1.9x10^{3}$	$2.1X10^{3}$	$7.1X10^{6}$	$1.9X10^{6} \pm 6.8X10^{5}$
Pond Water MPN/ml	Treated	$0.7X10^{4}$	$1.8X10^{7}$	$1.4X10^{6} \pm 9.8X10^{5}$	$3.0X10^{2}$	$1.3X10^{4}$	$2.6X10^{3} \pm 9.3X10^{2}$	$3.5X10^{2}$	$1.2X10^{4}$	$3.2X10^{3} \pm 7.5X10^{2}$
	Non treated	$1.2X10^{2}$	$2.3X10^{4}$	$6.0X10^{3} \pm 2.1X10^{3}$	$0.3X10^{2}$	$1.4X10^{3}$	$1.9X10^{2} \pm 0.9X10^{2}$	$0.6X10^{2}$	$1.4x10^{3}$	$2.8X10^{2} \pm 1.1X10^{2}$
Fish MPN/g	Treated pond	$1.8X10^{3}$	$1.6X10^{5}$	$4.8X10^{4} \pm 1.3X10^{3}$	$2.0X10^{2}$	$1.5X10^{4}$	$5.2X10^{3} \pm 1.2X10^{3}$	$1.1X10^{2}$	$2.5X10^{3}$	$5.4X10^{2} \pm 1.1X10^{2}$
	Non-treated pond	$1.2X10^{2}$	$1.3X10^{4}$	$3.5X10^{3} \pm 9.9X10^{2}$	$1.1X10^{2}$	$2.4X10^{3}$	$2.6x10^{2} \pm 1.3X10^{2}$	$0.8x10^{2}$	$2.1X10^{2}$	$1.2X10^{2} \pm 0.1X10^{2}$

SE = Standard error.

water of control ponds it was $1.9X10^2$ CFU/ml. Also, MPN of FC isolated from fish in ponds treated was $5.2X10^3$ CFU/g, while it was $2.6X10^2$ CFU/g in fish of control ponds. In addition, MPN of Faecal Streptococci (FS) in chicken manure was $1.9X10^6$ CFU/g. Most probable number of FS in water of ponds treated was $3.2X10^3$ CFU/ml, but that of control ponds was $2.8X10^2$ CFU/ml. In contrast, MPN of FS in fish of ponds treated was $5.4X10^2$ CFU/g, but that of fish of control ponds was $1.2X10^2$ CFU/g (Fig. 1).

Bacteriological examination of chicken manure resulted in isolation of 132 strains of bacteria, while 80 strains were isolated from water of ponds treated with chicken manure. On the other hand, 68 strains of bacteria were isolated from fish obtained from ponds treated with chicken manure. Isolated bacteria were *Staphylococcus sp.*, *Streptococcus faecalis*, *Enterobacter sp.*, *Klebsiella sp.*, *Escherichia coli*, *Pseudomonas spp.*, *Aeromonas sp.*, *Proteus sp.*, and *Flavobacterium columnare*.

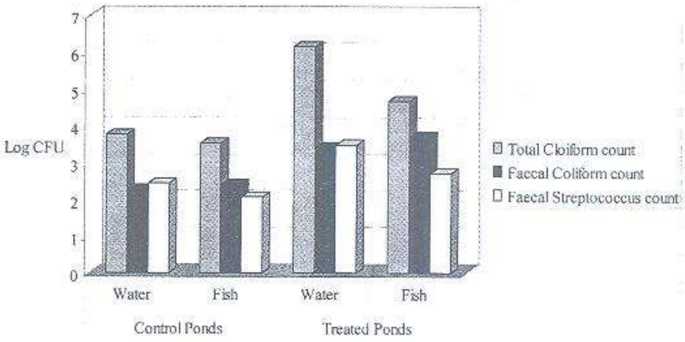

Fig. 1: Comparison of MPN of Total Coliforms, Faecal Coliforms, and Faecal Streptococci counts of water and fish from control and treated ponds.

Numbers and incidence of each isolate from chicken manure, water of ponds treated, and fish raised in such ponds are summarized in Table 2. The incidence of isolation of bacterial strains from chicken manure, pond water, and fish samples showed that the four major fish pathogens, *Streptococcus faecalis*, *Pseudomonas spp.*, *Aeromonas* *hydrophila*, and *Flavobacter columnare*, represent 44.3 % of all isolates, while all other isolates represented 55.7 %.

Clinical examination of fish show that some fish were apparent healthy, while others were manifested by loss appetite, sluggish movement, hard respiration, and brown to yellow brown lesions on their gills, skin and fins. Other fish showed fin rot, sloughing of necrotic tissue, loss of scales, exophthalmia, signs of septicemia with marked hemorrhage on the body surface, and congestion and petechiae hemorrhage seen in the internal organs.

Table 2: Numbers and incidence of each isolate from chicken manure and water and fish of ponds treated.

	Chicken manure		Water		Fish		Total	
Isolates	No.	%	No.	%	No.	%	No	%
Staphylococcus spp.	19	14.4	11	13.75	11	16.2	41	14.7
Streptococcus faecalis	25	18.9	12	15.0	8	11.8	45	16.1
Enterobacter spp.	28	21.3	15	18.8	7	10.3	50	17.9
Klebsiella sp.	6	4.5	0	0	0	0	6	2.1
Escherichia coli	22	16.7	15	18.8	10	14.7	47	16.8
Proteus sp.	8	6.1	3	3.75	1	1.5	12	4.3
Pseudomonas spp.	15	11.4	11	13.8	11	16.1	37	13.2
Aeromonas hydrophila	8	6	12	15.05	16	23.5	36	12.8
Flavobacterium columnare	1	0.75	1	1.25	4	5.9	6	2.1
Total	132	47.1	80	28.6	68	24.3	280	100

DISCUSSION

Present study clearly reveal that using chicken manure to fertilize fish ponds has an impact on total bacterial load either in water or on fish raised in such ponds. *Streptococcus faecalis* and *Pseudomonas spp.* were isolated from chicken manure at a relatively higher rate than that of ponds water and fish. Numbers of such bacteria increased in ponds with application of chicken manure. Streptococcal infections in fish have gained attention in recent years because of the increased incidence of infections and high economic losses caused by these microorganisms in both wild and cultured fish (Tung *et al.*, 1987). Ebtsam (2002) concluded that *Streptococci* caused considerable mortalities among wild tilapia in Egypt and may threat the population. It is generally believed that *Pseudomonas spp.* can be opportunistic pathogens of fish or produce damaging secondary infections (Woo and Bruno, 1999). This bacteria causes mortalities in tilapia fry (Duremdez and Lio-po, 1985) and infect other species of fish (Woo and Bruno, 1999).

In accordance with Jinyi *et. al* (1987), present study sowed that *A. hydrophila* was less in chicken manure than what was found in ponds water and fish (Table 2). This indicates that relatively less amount of *A. hydrophila* was brought to ponds with chicken manure. *Aeromonas hydrophila* causes haemorrhagic septicaemia in fish (Snieszko and Axelrod, 1971). Motile aeromonad infection caused by *A. hydrophila* is one of the most common bacterial diseases that affects most of freshwater fish (Noga, 1996, 1996). *Aeromonas hydrophila* has been recognized as a pathogen not only of fish but also amphibians, reptiles, snakes, cattle, and human (Snieszko and Axelrod, 1971).

On the other hand, *Flavobacterium columnare* was hardly found in chicken manure and water samples, but was relatively more in fish samples. *Flavobacter columnare* can infect most freshwater fish causing columnaris disease with high mortalities (Becker and Fujihara, 1978). The ability of *F. columnare* to cause both acute and chronic disease and its ubiquitous nature in the environment have made it one of the most devastating bacterial pathogens of fish (Woo and Bruno, 1999).

Present study focused on the impact of using organic fertilizers on fish health, however, public health concerns should not be neglected as well. In present study, several bacteria of public health importance and known to be pathogenic to human consumers as *Staphylococcus spp.*, *Streptococcus faecalis*, *Enterobacter spp.*, *Pseudomonas spp.*, in addition to members of *Enterobacteriaceae* as *Klebsiella sp.*, *E. coli*, and *Proteus sp.* were isolated from fish samples. Thomas *et. al* (1983) have shown that fish grown in ponds containing wastewater accumulate faecal bacterial that penetrate into the muscle tissue, and in turn, could represent a threat to consumers. Although, those bacteria are not considered as classical fish pathogens, yet adverse environmental conditions and poor water quality render fish more susceptible to infection with those bacteria. Furthermore, clinical signs of fish infected with those bacteria are not quite different than those of classical fish pathogens (Badran *et. al*, 1994).

Using organic manure to fertilize fish ponds has at least a dual action making fish more vulnerable to infections. It not only increases the bacterial load in water but also decreases water quality. With the application of chicken manure and decomposition of organic matter, water quality deteriorates and weakens fish resistance to diseases (Noga, 1996). Organic matter pollution is one of the important predisposing risk factors for infection with *Pseudomonas spp.*, *A. hydrophila*, and *F. columnare* (Noga, 1996). Fish may become actively infected or subjected to a wide variety of classical and non-classical fish pathogens that may result in serious pathogenic lesion as well as significant economic losses in artificial aquaculture practices (Ghittion, 1972).

Conclusion:

Using organic manure, directly without treatment, as an organic fertilizer to fertilize fish ponds increases bacterial load in water, which are pathogenic to fish, raising risk level of emergence of fish disease outbreaks that may cause economic losses along with public health problems to human consumers. Present study suggests that chicken manure should be treated before applying to fish ponds to lower the possibilities of contamination with pathogenic bacteria.

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