

HAZARDS RESULTED FROM ADDITION OF POULTRY DROPPINGS TO THE EGYPTIAN SEMI- INTENSIVE AQUACULTURED TILAPIA NILOTICA FISH FEEDS

FATMA H. M. ALI *and M. A. IBRAHIM **

*Food hygiene department, Faculty of Vet. Med. Cairo University , Beni-suef.

**Hygiene, Management and Zoonosis department, Faculty of Vet. Med. Cairo University , Beni-suef

Received: 19. 5. 2003

Accepted: 14. 12. 2003

SUMMARY

A total of 125 sample were investigated in the following order: 25 each of fish samples were collected before and after adding of (organic material) poultry droppings to fish farm, 25 water samples of fish farm were collected before and after addition of poultry droppings as well as 25 poultry droppings samples. The collected samples were examined for APC at 35°C and 5°C, Enterobacteriaceae and Pseudomonas / Aeromonas counts. The counts in fish muscle were $5 \times 10^3 \pm 1.5 \times 10^2$, $9.4 \times 10^2 \pm 1.23 \times 10^2$, $7 \times 10^2 \pm 2.3 \times 10^2$ and $2 \times 10^2 \pm 4.3 \times 10$ before addition of poultry droppings respectively. Such counts were significantly increased at $p < 0.05$ each constituting, after addition of the droppings $9 \times 10^4 \pm 3.64 \times 10^3$, $9.5 \times 10^3 \pm 2.2 \times 10^3$, $6.75 \times 10^3 \pm 2 \times 10^3$ and $1.3 \times 10^3 \pm 1.4 \times 10^2$ organisms/gm fish muscle respectively. Water samples showed that the counts were $8.6 \times 10^3 \pm 2 \times 10^3$, $4 \times 10^3 \pm 9 \times 10^2$

, $2.1 \times 10^2 \pm 5 \times 10$ and $4.0 \times 10^2 \pm 8.0 \times 10$ organisms/ml before adding of poultry droppings respectively ;while such counts were significantly increased at $p < 0.05$ to $9 \times 10^6 \pm 2 \times 10^6$, $6 \times 10^5 \pm 1.5 \times 10^5$, $4.3 \times 10^5 \pm 1.4 \times 10^3$ and $7.2 \times 10^4 \pm 1.3 \times 10^3$ organisms/ml respectively after addition of poultry droppings to fish farm water. While the counts in poultry droppings were $4.2 \times 10^9 \pm 1 \times 10^9$, $6.0 \times 10^6 \pm 2 \times 10^6$, $1.3 \times 10^6 \pm 5.0 \times 10^5$ and $8 \times 10^4 \pm 2 \times 10^4$ organisms/gm respectively. Salmonellae, Yersinia enterocolitica, Pseudomonas aeruginosa, Aeromonas hydrophila and Campylobacter jejuni could be isolated from fish after add of poultry droppings with percentages 28, 36, 40,16 and 28 respectively. The public health risks of the isolated organisms were also discussed.

INTRODUCTION

The quality of fresh fish is of great concern to the

fish producers as well as the consumers. Because of the consideration that fish is extremely perishable food commodities which mainly occurs as a result of bacterial activities ended by reduction of the fish quality and consequently leads to rapid spoilage and/ or cause human illness. In addition, it was reported that faulty rearing, catching and processing can lead also to contamination of the fish with pathogenic micro-organisms ICMSF (1978). Jay (1986) and Hays (1992) mentioned that fish caught from polluted sources was highly contaminated with different bacterial types than those collected from non polluted ones. They added that the flesh of freshly caught fish was nearly sterile shortly after capture except slime coat on the skin, gills and the intestinal tract. In respect to food requirements; fish may reared within flooded rice paddy fields or ducks which may reared in association with fish ponds. There are many system for fish farming as very intensive, high intensive and in between them there is the standing- water ponds which constitute the majority of fish rearing systems. In the last one; fertilizer are added together with some supplementary feeding, in order to enhance natural productivity within the ponds resulting in high yields of species such as carp, either alone or more commonly with other species in polyculture systems Shepherd and Bromage (1992). The use of organic materials for fertilization as fish meal, cottonseed meal, grasses, leaves, hay and the various kinds of manure. It was found that manure of chickens or hogs which were fed on grains was much better than that of

another animals which fed on hay. The added fertilizers to water will develop microscopic plant organisms (phytoplankton) which serve as food for fish either directly or indirectly. The small plants may be eaten by microscopic animals (zooplankton) and the zooplankton eaten by the minnows Brown and Gratzek (1980).

In Egypt; organic matters (poultry droppings) may added to the fish feeds of semi- intensive system. Therefore the added poultry droppings will contaminate the fish farms water with various types of bacteria and; subsequently contaminate the fish which will carry great risk to the human consumer.

Fish bacterial diseases such as vibriosis, haemorrhagic septicaemia, gill disease of bacterial cause and disease associated with skin lesions as discoloration, ulcers, haemorrhagic patches and furunculosis are considered of great importance for both of public health and the shelf life of the fish Inglis et al., (1993).

Huss (1995) mentioned that primarily aquatic organisms and readily isolated from freshwater and estuarine finfish were genus *Aeromonas*. On the other hand ICMSF (1998) published that *aeromonas* well establish as a component of spoilage microflora of reared and wild freshwater fish when stored in air at refrigeration temperatures. Cesar et al., (2001) could isolate *Aeromonas hydrophila* from 29% of the aquacultured fresh water fish.

Pin et al. (1994) reported that *Aeromonas hydrophilla* was isolated from a variety of foods including fishes. Amal and Bastawrows (1999) could isolate *Campylobacter* organisms from *Tilapia nilotica* fishes collected from polluted water; they mentioned that the fish carried the organism mechanically only without suffering from the disease. They reported that *Campylobacter* organisms were isolated from 7 out of 115 *Tilapia nilotica* fish samples (6.8%) and the isolated organisms from the 7 positive *Tilapia nilotica* fish samples were identified as 4 and 3 *Campylobacter jejuni* and *Campylobacter coli* respectively. Skirrow, (1990) and Fang et al., (1991) mentioned that raw meats including fish and shellfish, have been implicated as a source of *Campylobacter enteritis*. They added that the infectious dose can be as low as a few hundreds of bacteria and they considered that fish is one of the important sources of human infection. Gibson (1993) isolated *Salmonellae* from fish and shellfish collected from sewage polluted water. Fish constitute about 5 % from the causes of human salmonellosis. On the same direction Pattic et al., (2001) isolated *Listeria monocytogenes*, *Salmonella*, *Yersinia enterocolitica* from aquacultured finfish.

The aim of this study is to direct the lights to the great risks resulted from addition of poultry droppings to the feeds of cultured *Tilapia nilotica* fish in Egypt; as it may constitute a major source of

pathogenic bacteria to fish, and consequently to the consumer. Therefore the potential hazard from the consumption of such fishes may be claimed to the use of poultry droppings as an ingredient of the cultured fish feed.

MATERIALS AND METHODS

Collection of samples:

1- Fish samples: From fresh *Tilapia nilotica* fishes in semi-intensive farm at Beni-suef city; 25 samples each were collected 3 hour before and after addition of dried poultry droppings mixed with hay to farm. They add dried poultry droppings mixed with hay to the fish feed by a ratio 1- 3 once/ day. Samples weight ranged from 200 ñ 450 gm of each one. The collected samples were rapidly transferred to the laboratory in ice box for further examination.

2- Poultry droppings: From dried poultry droppings mixed with hay (collected from broiler chicken farms), 25 samples each is one kgm were collected in sterile polyethylene bags, and transferred directly to the laboratory for further examination.

3- Water samples: From the fish farm, 25 samples each of 500 ml were collected two times before and after addition of dried poultry droppings mixed with hay to the fish feeds in the fish farm.

Collected samples were transferred in clean sterile glass bottles (500 ml).

Preparation of samples:

1- Fish samples: According to the technique recommended by AOAC (1990); 10 grams of each fish muscle samples were taken under complete aseptic condition after flaming of the surface and homogenized with 90 ml sterile peptone water 0.1% in sterile container. Then 10 folds of serial dilution up to 10^6 was prepared from the obtained homogenate.

2- Poultry dropping mixed with hay samples:

Ten grams of each sample were added to 90 ml sterile peptone water 0.1% and homogenized in sterile container from which 10 folds of serial dilutions up to 10^6 were prepared AOAC (1990).

3- Water samples: were examined according to the technique recommended by APHA (1989).

Bacteriological examination:

1-Determination of total colony count in muscle sample (APC at 35°C and 5°C).

The pouring plate technique recommended by AOAC (1990) was applied. One ml from each dilution was pipetted separately in a sterile petri-dishes. Fifteen ml of melted standard plate count agar at 42- 45 5°C were poured; thoroughly mixed and then left to solidify. The inoculated plates were incubated at 35°C for 48 ±2hrs in

(mesophiles) .In case of (psychrophiles), plates were incubated at 5°C for 7-10 days.

Aerobic plate count /gram = No. of colonies x dilution

3- Determination of Enterobacteriaceae count:

The same technique of the pouring method recommended by AOAC (1990) was applied using violet red bile glucose agar Gork (1976). The plates were incubated at 37°C for 24 hrs. All purple colonies were then counted.

Enterobacteriaceae count/gram = No. of colonies x dilution

4-Determination of Pseudomonas/aeromonas count:

The applied technique recommended by Pierson et al.(1970) was applied by using GSP medium. One hundred microlitres from each dilutions were transferred aseptically to the surface of GSP medium (GSP; Merck; Art 10230) and then spread by a sterile glass spreader. Inoculated plates were incubated at 25°C for 3days. All pink colonies were counted as Pseudomonas while yellow colonies were counted as Aeromonas.

Pseudomonas count/gm = No. of colonies x dilution

Aeromonas count/gm = No. of colonies x dilution

5- Isolation of Pseudomonas Aeruginosa & Aeromonas hydrophila.

The suspected colonies were identified according to the technique recommended by APHA (1992).

6- Isolation of Salmonellae

The modified techniques recommended by Rappaport et al. (1956) and Harvey and Price (1981) was followed. Ten grams of fish muscle samples were homogenized separately with 90 ml buffered peptone water under aseptic conditions by the using a homogenizer (Universal Laboratory Aid made in Poland) for 1 minute. The homogenates were incubated at 37°C for 18-24 hrs. One ml of pre-enriched culture was transferred to 10 ml Rappaport's vassiliadis enrichment broth (Oxoid) and then incubated at 43°C for 24 hours. A loopful of the selective enrichment broth was streaked on the surface of XLD (Oxoid; CM 469) plates, then incubated at 37°C for 24 hrs, then identified.

7-Isolation of Yersinia enterocolitica

The applied technique recommended by APHA (1992) was carried out.

Ten grams of fish muscle was homogenized with 90 ml of a modified Rappaport (Oxoid). The homogenate was incubated at 25°C for 3 days. A loopful of enriched broth was streaked onto plates of Yersinia Selective Agar Base (Oxoid; CM 653) to which Yersinia Selective Supplements (Oxoid, SR 109) was added. The inoculated plates were incubated at 32°C for 18hrs. Suspected colonies were identified by APHA (1992).

8-Isolation of thermophilic Campylobacter :

The technique recommended by Nachamkin

(1995) was applied. A 20 grams quantity of each sample (flesh including skin, liver and kidney) were added to 50 ml of Preston campylobacter enrichment broth comprised brucella broth plus 5% lysed horse blood, preston campylobacter selective supplement and campylobacter growth supplement. The mixture was shaken and inoculated broth was incubated at 42°C for 48 hrs in a gas - pak jar containing a gas generating kit (Oxoid, BR56) for campylobacters, which produce approximately 85% Nitrogen, 10% Co₂ and 5% O₂ Bolton and Robertson, (1982). A loopful was taken from each tube and placed onto a clean dry slide, covered and examined by dark field microscopy for motile bacteria Skirrow, (1977). Preston broth containing motile bacteria (campylobacter) were cultured onto Preston agar (brucella agar base supplemented with Preston's selective agents). The plates were incubated at 42°C for 48 hrs under microaerophilic conditions. The plates were examined for growth and characteristics of campylobacter colonies, further identification was carried out following the techniques by APHA (1992).

Statistical analysis:

Statistical analysis was done according to Knapp and Miller (1992) and Ingelfinger et al.(1994).

The analytical test used is unpaired student T-test for comparing means of the two groups at a significance level 0.05.

Table (1) Bacteriological counts obtained from the muscles of tilapia nilotica fishes samples collected from the fish farm before and after addition of poultry droppings mixed with hay to the used feeds.

Micro-organisms counts	Bacterial counts of samples collected before addition of p.d*	Bacterial counts of samples collected after addition of p.d*
Aerobic plate count at 35°C	$5 \times 10^3 \pm 1.5 \times 10^2$	$9 \times 10^4 \pm 3.64 \times 10^3$
Aerobic plate count at 5°C	$9.4 \times 10^2 \pm 1.23 \times 10^2$	$9.5 \times 10^3 \pm 2.2 \times 10^3$
Enterobacteriaceae count	$7 \times 10^2 \pm 2.3 \times 10^2$	$6.75 \times 10^3 \pm 2 \times 10^3$
Pseudomonas/Aeromonas count	$2 \times 10^2 \pm 4.3 \times 10$	$1.3 \times 10^3 \pm 4.1 \times 10^2$

*p.d = poultry droppings mixed with hay

Table (2) Isolated organisms from the muscles of Tilapia nilotica fishes samples collected from the fish farm before and after addition of poultry droppings mixed with hay to the used feeds

Isolated organisms	Samples collected before addition of p.d*		Samples collected after addition of p.d*	
	Number	Percent	Number	Percent
Salmonellae	0	0	7	28
Yersinia enterocolitica	0	0	9	36
Pseudomonas aeruginosa	1	4	10	40
Aeromonas hydrophila	0	0	4	16
Campylobacter species	0	0	7	28

p.d*= poultry droppings mixed with hay

Table (3) Serotyping of isolated organisms from the muscle samples

Salmonella species	No. of positive samples	Campylobacter species	No. of positive samples
<i>S. typhimurium</i>	4	<i>C. Jejuni</i>	5
<i>S. tallahase</i>	3	<i>C. Coli</i>	2

*p.d = poultry droppings mixed with hay

Table (4) Bacteriological counts obtained from poultry droppings mixed with hay (mean value).

Microorganisms counts	Counts (mean value)
Aerobic plate count at 35°C	$4.2 \times 10^9 \pm 1 \times 10^9$
Aerobic plate count at 5°C	$6.0 \times 10^7 \pm 2 \times 10^6$
Enterobacteriaceae count	$1.3 \times 10^7 \pm 5.0 \times 10^6$
Pseudomonas/Aeromonas count	$8.0 \times 10^4 \pm 2.0 \times 10^4$

Table (5) Isolated organisms from poultry droppings mixed with hay which added to the cultured fish feeds.

Isolated organisms	No. of positive samples	Percent
Salmonellae	15	60
<i>Yersinia enterocolitica</i>	10	40
<i>Pseudomonas aeruginosa</i>	13	52
<i>Aeromonas hydrophila</i>	5.0	20
Campylobacter species	14	56

Table (6) Serotyping of isolated organisms from poultry droppings mixed with hay which added to the cultured fish feeds

Salmonella species	No. of positive samples	Campylobacter species	No. of positive samples
S. typhimurium	9	C. Jejuni	10
S. paratyphi	2	C. Coli	4
S. tallahase	4		

Table (7) Bacteriological counts obtained from fish farm water samples before and after addition of poultry droppings mixed with hay

Micro-organisms counts	Samples collected from water before addition of p.d*	Samples collected from water after addition of p.d*
Aerobic plate count at 35°C	$8.6 \times 10^3 \pm 2.0 \times 10^3$	$9 \times 10^6 \pm 2.0 \times 10^6$
Aerobic plate count at 5°C	$4.0 \times 10^3 \pm 9.0 \times 10^2$	$6.0 \times 10^5 \pm 1.5 \times 10^5$
Enterobacteriaceae count	$2.1 \times 10^2 \pm 5.0 \times 10$	$4.3 \times 10^5 \pm 1.4 \times 10^3$
Pseudomonas/Aeromonas count	$4.0 \times 10^2 \pm 8.0 \times 10$	$7.2 \times 10^4 \pm 1.3 \times 10^3$

p.d*= poultry droppings mixed with hay

Table (8) Organisms isolated from fish farm water samples collected before and after addition of poultry droppings mixed with hay

Type of Micro-organisms	Water samples collected before addition of p.d*		Water samples collected after addition of p.d*	
	Number	Percent	Number	Percent
Salmonellae	0	0	10	40
Yersinia enterocolitica	1	4	10	40
Pseudomonas aeruginosa	2	8	12	48
Aeromonas hydrophila	0	0	4	16
Campylobacter species	0	0	8	23

p.d*= poultry droppings mixed with hay

Table (9) Serotyping of organisms isolated from water samples

Salmonella species	No. of positive samples	Campylobacter species	No. of positive samples
S. typhimurium	6	C. Jejuni	5
S. tallahase	4	C. Coli	3

DISCUSSION

The data recorded in table (1) revealed that the mean value of Aerobic plate count at 35°C and 5°C, Enterobacteriaceae count and Pseudomonas/Aeromonas count of fish muscle were $5 \times 10^3 \pm 1.5 \times 10^2$, $9.4 \times 10^2 \pm 1.23 \times 10^2$, $7 \times 10^2 \pm 2.3 \times 10^2$ and $2 \times 10^2 \pm 4.3 \times 10$ organisms/gm fish muscle before addition of poultry droppings to fish farm respectively, while after addition of poultry droppings it was significantly increased at $p < 0.05$ to $9 \times 10^4 \pm 3.64 \times 10^3$, $9.5 \times 10^3 \pm 2.2 \times 10^3$, $6.75 \times 10^3 \pm 2 \times 10^3$ and $1.3 \times 10^3 \pm 1.4 \times 10^2$ organisms / gm fish muscle .

The obtained data revealed that the bacterial counts of cultured *Tilapia nilotica* fish muscle before addition of poultry droppings to fish farm mixed with its feed were lower than after addition of these droppings. The addition of poultry droppings contaminate water of fish farm, fish eat these droppings and contaminate fish itself.

Shewan (1971) reported that the counts of bacteria in fish muscle ranged from Zero to 10^3 de-

pending on the condition of the environment in which the fish live, method of catching , degree of exhaustion, stress of fish during catching, the type of fish, the type of muscle and healthy condition of fish.

Mesophiles (APC at 35°C) are considered as a disease problem in fish while * Psychrophiles (APC at 5°C) and Pseudomonas/Aeromonas counts are considered spoilage agents. Whereas Enterobacteriaceae indicate faecal contamination of fish which may lead to healthy problem to consumers Machee et al., (1995). The microbiological safety of fish, shellfish and their products depends on the interactions of the normal spoilage flora with pathogenic agents. The spoilage flora arises from the environment the fish have been taken from and exposed to post-mortem Cahill, (1990).

Table (2) contain the isolated organisms from the muscles of *Tilapia nilotica* fishes samples collected from the fish farm before and after addition of dried poultry droppings mixed with hay to the used feeds. It was clear that all samples collected

used feeds. It was clear that all samples collected before addition of poultry droppings were free from *Salmonellae*, *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Campylobacter* species; only one sample was contaminated with *Pseudomonas aeruginosa* which may result from normal sources of the organism before addition of dried poultry droppings mixed with hay or may be due to previous addition. On the other hand the collected samples after addition of poultry droppings were contaminated with *Salmonellae*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Campylobacter* species in percentages 28,36, 40,16 and 28 respectively. Such results were going with those mentioned by Skirrow (1990), Fang et al. (1991), Gibson (1993), Pin et al. (1994), ICMSF (1998), Amal and Bastawrous (1999), Cesar et al. (2001), and Pattic et al. (2001).

Concerning the public health importance of the isolated organisms, it was found that *Salmonellae* are the main causative agent of food poisoning to human. While some strains of *A. hydrophila* are capable of causing illness in fish as well as humans who may acquire infections through open wounds or by ingestion of sufficient numbers of organisms in food and water (Mathewson & Dupont 1992). Bacteraemia is the most common pathogenic manifestation of *aeromonas* manifestation in humans. Reports of *aeromonas* gastroenteritis and wound infections in humans have been

recorded (Haburchak, 1996). *A. hydrophila* have been implicated as a causative agents of human gastroenteritis, meningitis, endocarditis and osteomyelitis (Yadav and Kumar, 2000).

Y. enterocolitica at the same time is associated with a spectrum of clinical syndromes in man such as acute gastroenteritis, mesenteric lymphadenitis, arthritis and eye infection (Walker, 1989). While *Campylobacter* can establish a temporary asymptomatic carrier state, as well as illness in humans. This is especially prevalent in developing countries. Park and Sanders (1992); Stern and Bolton (1994), Nachamkin et al., (1995) and Abeyta (1998). Adak et al., (1995) reported that fishes have been implicated as a source of *campylobacter* enteritis in humans. In this respect Allos (2001) reported that infection with *campylobacter jejuni* is one of the most common causes of gastroenteritis world wide. A typical case is characterized by diarrhoea, fever and abdominal cramps.

Table (3) showed the serotyping of isolated organisms from the muscle samples. It was found that serotyping revealed that *S. typhimurium* and *S. tallahassee* were isolated from 4 and 3 samples respectively. While *Campylobacter jejuni* and *Campylobacter coli* were isolated from 5 and 2 samples respectively. Such results were going with those obtained by Skirrow (1990) Fang et al. (1991) Gibson (1993), Amal and Bastawrous (1999), and Pattic et al. (2001).

Table (4) contained the bacterial counts obtained from the dried poultry droppings mixed with hay, which were Aerobic plate count at 35°C and at 5°C, Enterobacteriaceae and Pseudomonas/Aeromonas counts $4.2 \times 10^9 \pm 1 \times 10^9$, $6.0 \times 10^7 \pm 2 \times 10^6$, $1.3 \times 10^7 \pm 5.0 \times 10^6$ and $8 \times 10^4 \pm 2 \times 10^4$ organisms/gm respectively.

The results achieved in table (5) showed that the isolated organisms from poultry droppings mixed with hay which added to the cultured fish feeds were Salmonellae, Yersinia enterocolitica, Pseudomonas aeruginosa, Aeromonas hydrophila and Campylobacter species in percentages 60, 40, 52, 20 and 56 respectively. Such results explained the possibility of contamination of fish muscles with the isolated organisms which consequently affect the consumer with the specific diseases. Jay (1986) and Hays (1992) supported these results through their studies. The serotyping of isolated organisms from poultry droppings mixed with hay which added to the cultured fish feeds which included in table (6) was *S. typhimurium*, *S. paratyphi* and *S. tallahase*, *Campylobacter jejuni*, *Campylobacter coli*. The serotyping were isolated from the positive samples were 9, 2, 4, 10 and 4 respectively. Such serotyping was going with recorded by Gibson (1993) and Pattic et al., (2001).

In table (7) the bacteriological counts obtained from fish farm water samples collected before and after addition of poultry droppings mixed with

hay were recorded as Aerobic plate count at 35°C, Aerobic plate count at 5°C, Enterobacteriaceae count and Pseudomonas/Aeromonas count which were $8.6 \times 10^3 \pm 2.0 \times 10^3$, $4.0 \times 10^3 \pm 9.0 \times 10^2$, $2.1 \times 10^2 \pm 5.0 \times 10$ and $4.0 \times 10^2 \pm 8.0 \times 10$ organisms/ml before adding the mixture. Such counts were significantly increased at $p < 0.05$ to $9 \times 10^6 \pm 2.0 \times 10^6$, $6.0 \times 10^5 \pm 1.5 \times 10^5$, $4.3 \times 10^5 \pm 1.4 \times 10^3$ and $7.2 \times 10^4 \pm 1.3 \times 10^3$ organisms/ml after addition of the mixture respectively

It was clear that the counts obtained from the samples collected after addition of the mixture were much higher than those obtained before addition of the mixture.

Table (8) contained the organisms isolated from the fish farm water samples collected before and after addition of poultry droppings mixed with hay. These organisms were Salmonellae, Yersinia enterocolitica, Pseudomonas aeruginosa, Aeromonas hydrophila and Campylobacter species in percentages 0, 4, 8, 0 and 0 in collected samples before addition of the mixture respectively. Such results mean the presence of Yersinia enterocolitica and Pseudomonas aeruginosa in the farm water before addition of the mixture. This may be old contamination from the previous times of addition of the mixture. While the organisms isolated from the samples collected after addition of the mixture were Salmonellae, Yersinia enterocolitica, Pseu-

domonas aeruginosa, Aeromonas hydrophila and Campylobacter species in percentages 40, 40, 48, 16 and 23 respectively. Such results show the great difference between the number of the positive samples of water before and after addition of the mixture. These results were going with those obtained by Skirrow (1990), Fang et al., (1991), Gibson (1993), Pin et al., (1994), ICMSF (1998), Amal and Bastawrous (1999), Cesar et al., (2001), and Pattic et al., (2001), Finally table (9) clarified the serotyping of organisms isolated from water samples which were *S. typhimurium* from 6 samples, *S. tallahase* from the 4 samples, *Campylobacter jejuni* from 5 samples and *Campylobacter coli* from 3 samples. These results are similar to those obtained by Gibson (1993) and Pattic et al., (2001).

Recommendations

As concluded from the results achieved in this study it will be sure that addition of poultry droppings to the farm fish feeds constitute great hazard to the fish consumer as these organic materials are highly contaminated with some pathogenic organisms. Therefore it is preferable to avoid the use of poultry droppings as fish feed additive.

Acknowledgement.

Grateful acknowledgement to Dr. Mohamed Abdel aziz, Fac. of Vet. Med., Cairo University for his supplying with samples.

REFERENCES

- Abeysa, C.(1998): Molluscan shellfish; In Microbiology Hand book No.3 Fish and seafood. R.A. Lawley and Gibbs (Ed).p.67-99.
- Adak,G.K.; Cowden, J.M.; Nicholas, S. and Evans, H.S. (1995): The public health laboratory service national case- control study of primary indigenous sporadic cases of campylobacter infection .J.Epidemiol. Infect., 115 (1):15-22.
- Allos,B.M.(2001): Campylobacter jejuni infections: Update on Emerging issues and trends. Clin. Infect. Dis. 15, 32 (8) 1201.
- Amal, A.Mohamed and Bastawrows ,A. F. (1999): Studies on campylobacter organisms as food-poisoning organisms in fresh water and salted fishes "Moloha" in Assiut Governorate. J. Assiut Vet. Med.,40: 210- 219.
- American Public Health Association, APHA(1989): Standard Methods for the Examination of Water and Waste Water.17th ed ,American Public Health Association ,Washington.
- American Public Health Association, APHA(1992): Compendium of Methods for the Microbiological Examination of Foods; 3rd ed, Edwards Brothers, Washington.
- Andrews,W.H.and Messer,J. (AOAC1990): Microbiological Methods,P.425.Official Methods of Analysis of the Association of Official Analytical Chemists(Helrich,K. Editor)15th ed. Vol. I. The Association of Official Analytical Chemists, Inc.,USA.
- Bolton, F. J. and Robertson, L. (1982): A selective medium for isolating Campylobacter jejuni/coli. J.Clini. Path.,35

:462-467.

Brown, E. E. and Gratzek, J. B. (1980): Fish Farming Handbook. chapter (4),63-65. Avi Publishing Comp., Inc. USA.

Cahill, M.M. (1990): Bacterial flora of fishes: a review. Microbial Ecology 19, 21-41.

Cesar, J.; Santos, A.; Garcia-Lopez, M.; Gonzalez, N. and Otero, A. (2001): Mesophilic Aeromonads in wild and aquacultured freshwater fish. J. Food Prot. 64, 687-691.

Fang, G.; Araujo, V. and Guerrant, R.L. (1991): Enteric infection associated with exposure to animals or animal products. Infect. Dis. Clin. North. Am., 5, 681-701.

Gibson, D.M. (1993): Hygiene and Safety of Seafood: Chapter (8), Ruitter: Fish and Fishery products; composition, nutritive properties and stability. CAB international.

Gork, F. P. (1976): Über die Ursachen von Qualitätsmängeln bei tiefgefrorenen Fertiggerichten auf Fleischbasis in der Fluggast-Verpflegung. Doktor Ingenieur Diss., Berlin

Haburchak, D.R. (1996): Aeromonas hydrophila: An underappreciated Danger to Fisherman. Infect in Med. 13 (10): 893-896.

Harvey, R.W. and Price, T.H. (1981): Comparison of selenite. Foreman, Muller Kauffmann tetrathionate and Rappaport's medium for salmonella isolation from chicken giblets and after pre-enrichment in buffered peptone water. J. Hyg. Camb. 87, 219.

Hayes, P.R. (1992): Food Microbiology and Hygiene. (Book) 2nd ed., P. 35. Elsevier Science Publishers (LTD).

Huss, H. H. (1995): Quality and quality changes in fresh fish. Food and Agriculture Organization, Rome, Italy.

ICMSF (1978): Microorganisms in foods. Vol. II Univ. of

Toronto, Press, Toronto Canada. 213 p. International Commission on Microbiological Specification for Foods.

ICMSF (1998): Microorganisms in foods, 6: microbial ecology of food commodities. Blackie Academic & Professional, London. International Commission on Microbiological Specification for Foods.

Ingelfinger, J. A., Mosteller, F. M., Thibodeau, L. A. and Watre, J. H. (1994): Biostatistics in Clinical Medicine. Mc Graw-Hill Inc., New York.

Inglis, V.; Ronald, J.R. and Niall, R. (1993): Bacterial disease of fish, Black-Well Scientific Publication Osny Mead Oxford- OX2 OEL. 3; 221.

Jay, M.J. (1986): Modern Food Microbiology 3rd ed. CBS Publishers and distribution (Indian).

Knapp, R. and Miller, M. C. (1992): Clinical Epidemiology and Biostatistics. Harwal Publishing Pennsylvania.

Machee, D.D.; Ostland, V.E.; Lumsden, J.S.; Derksen, J. and Ferguson, H.L.D. (1995): Influence of feeding on the development of bacterial gill disease in rainbow trout on-crophynchus mykiss. Diseases of Aquatic organisms, 21:3, 163-178.

Mathewson, J. and Dupont, H.L. (1992). Aeromonas species: role as human pathogens. In: Remington, J.S., Swartz, M.N. (eds.), Current Clinical Topics in Infectious Diseases. 12, Camb.: Blackwell Sci. 26-36.

Nachamkin, I. (1995): Campylobacter and Aerobacter. Manual of Clinical Microbiology. 6th ed. Eds Murray, P.R.; Baron, E.J. et al., ASM press, Washington D.C. 483-491.

Park, C.E. and Sanders J.W. (1992): Occurrence of thermo-tolerant campylobacters in fresh vegetables sold at farm-

- ers, outdoor markets and supermarkets. *Can. J. Microbiol.* 38,313-316.
- Pattic ,C.; Owens,S.; Cohen,E.and Westhoff,C.(2001): Effect of sampling method on representative recovery of microorganisms from the surfaes of aquacultured fin-fish.*J.Food Prot.*64,1515-1520.
- Pierson,M.D.,Thompson,D.L.C. and Ordal,Z.J.(1970): Microbiological, sensory and pigment changes of aerobically and anaerobically packed beef. *J.Food Technol.* 24,1171.
- Pin,C.; Marin, M.L.; Garcia,M.L.;Tormo,J.;Selgas, M.D. and Casas,C.(1994): Incidence of motile *Aeromonas* spp. in foods. *Microbiologica Sep.*,16, 257-262.
- Rappaport, F.;Konforti, N. and Nawon ,B.(1956): New enrichment medium for certain *Salmonellae*. *J. Clin. Pathol.* 9, 261.
- Shepherd,J.C. and Bromage, N.R. (1992): *Intensive Fish Farming*.Oxford, Blackwell Scientific Publications.2-6.
- Shewan, J.M.(1971): Bacteriological standard for fish and fishery products. *Chemy.Ind.Feb.*7: 193-199.
- Skirrow, M.B.(1977): *Campylobacter enteritis : A new disease*. *J. Brith. Med.*,2: 9-11.
- Skirrow, M.B.(1990): Foodborne illness i*Campylobacter* i the *Lancet*, 336:921-923.
- Stern,N. and Bolton, E. (1994): improved enrichment recovery of *campylobacter* spp.from broiler chicken carcasses. LAMFIS,8 ist annual meeting. Abstract 29.
- Walker, S.J. (1989): *Yersinia* in *Advanced Food Microbiology Course Surrey Univ. Micro. Dep. Campden Food and Drink Research Association. Chipping Campden, Glos.GL, 556 LD.*
- Yadav, A.S. and Kumar, A. (2000): Prevalence of enterotoxigenic motile aeromonads in children, fish, milk and ice-cream and their public health significance. *Southeast Asian J. Trop. Med. Public Health.* 3, 153- 156.