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PRELIMINARY STUDIES ON THE EFFECT OF LIVESTOCK MANURE APPLICATION ON BACTERIAL FISH DISEASE AND HUMAN HYGIENE

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ABSTRACT

An investigation on the effect of application of livestock manure, the biomass of aerobic heterotrophic bacteria and Colicin population and distribution of Aeromonas, Pseudomonas and pathogenic bacteria of the human digestive tract which are also present in the body mucus of black carp, grass carp, silver carp and bighead carp as well as in pond water (on the application of manure of chicken, duck, cow and pig) was conducted in the fish farming areas in Wuxi, China. The results show less frequency of fish pathogenic bacteria in the four kinds of manure. Aeromonas and Pseudomonas population were, however, higher in the manured pond water than in the non-manured pond. Although there was less amount of fish pathogenic bacteria in the animal manure, body mucus of black carp and grass carp which well in the pond bottom and mid-level can cause the occurrence of Pseudomonas, is one of the pathogens of Stigamatosis, due to the decomposition manure in the pond bottom. With the exception of chicken manure in

which there was Group E Salmonella, no other pathogenic bacteria was found in the manure of duck, cow and pig. None was found either on the fish body nor in the fish viscera in the manure-applied fish pond. Bacteria were pathogenic. The fish cultured in the manure-applied pond were descaled and washed, the quantity of Colicin was reduced by 100–1000 times, the concentration of which was almost the same as that in the manure-free pond. MPN was less or equal to 40***(*** MPN.--- The most Probable number of Colicin within sq. sm of fish body. Results indicated that the fish cultured in manure-applied ponds after treatment by washing are hygienic as human food and not harmful to human health.

INTRODUCTION

Application of livestock manure in fish farming has been practised in China for thousands of years, but little attention has been given to the environmental effects. The pond water becomes fertile upon the application of manure, resulting in more food organisms, thus a high fish production. It was only recently that much concern has been shown on fish and human health as a result of application of manure in pond water. An understanding of the white-skin, Haemorrhagical septicaemia, Furunculosis, Stigmatosis, Lepidorthosis which are all caused by the bacterial infections in China's fish farms, has been gaine from recent studies. The pathogens of these disease are Aeromonas and Pseudomonas which are Grains gram-Negative, non-bud Bacillus. However, there are other organisms that have not been well studied. These include Salmonella, Shigalla, Camylobacter jejuni, Yersinia enterocolitica which are some of the main causes of acute human enteritis. The present study investigated the distribution of the above-mentioned pathogenic bacteria in the manure of chicken, duck, cow and pigs, pond water, and mucus of black carp, grass carp, silver carp and bighead. A comparison was made of the amount of Colicin in fish body after descaling and washing an specimens obtained from manured and non-manured ponds.

MATERIALS AND METHODS

The trials were made at Holei Fish Farm, Holei, No. 1 fish farm, Wuxi and the farm at the Centre. Test ponds were applied with independent kind of manure, while the control ponds had none. The area of individual ponds used for the research on bacterial fish disease in 1985–1986 respectively were 0.34, 2.15, 2.15, 0.52 and 0.048 ha. For hygiene of fish as human food individual ponds of 0.13, 2.15, 2.15, 0.13, 0.13 ha in extend were used in 1987. Average water depth was 2–2.5 m. No feed supplement was used in both control and test ponds. The species stocked were black carp, grass carp, silver carp, bighead, common carp, Japanese koi and blunt snout. The rate of application of chicken, duck, cow and pig manure respectively were 3675 kg/ha, 628 kg/ha, 25,580 kg/ha, 23,557 kg/ha, respectively. The ponds received conventional feeding and management.

Duck manure and cow dung collected from the duck pens and dairy farms of Holei Fish Farm were used in 1985–1986. Chicken and pig manure was collected from poultry sheds of the Centre and pig farms of Holei No. 1 Fish Farm, respectively. In 1987 they were obtained from the poultry farm of Wuxi Country and the Centre's pig farm. The manure of chicken duck, cow and pig was fermented. Part of duck manure was in fresh state.

1. The examination of bacterial fish diseases

Sampling. From April 1985 to Sept. 1986, 7 samples were collected from manure of chicken, duck, cow and pig and from pond water. Samples were regularly collected three times from black carp, grass carp, silver carp and bighead.

1.1 Wet manure 15–20 mg was placed in an sterilized brown bottle form which 0.5 mg was prepared.

1.2 5 ml. of pond water was obtained using a 250 ml-bottle.

1.3 Body mucus of black carp, grass carp, silver carp and bighead (3 fish of each species) scraped and 0.5 mg of mixture was obtained. At the same time, mechanical gauze was used to clean up 30 sq. cm of the fish body before weighing the fish and obtaining the bacteria count on 1 sq. cm.

Culture and classification. The manure, water and fish body mucus were diluted and cultured in broth. They were separated on the flat plate according to the characteristics of the Aeromonas and Pseudomonas. Thereafter gram-staining, Flagellum staining and morphological observations of bacteria were carried out. Cell pigment oxide enzyme, glycogen fermentation, oxidation, liquified gelatin, alcohol oxidation were used to classify the Aeromonas and Pseudomonas.

Counting. The diluted manure, water and mucus samples were cultured in broth at 27°C for 48 hours. Number of aerobic heterotrophic bacteria from different samplings were counted using a Gallen kamp counter.

2. The hygienic examination of fish as human food

Sampling of fish body mucus. Black carp, grass carp, silver carp and bighead mucus samples were obtained by scraping from 22.5 sq.cm area of both sides of the back of specimens. Samples were placed in 225 ml sterilized of normal saline water mixed up by constant stirring. Further dilutions were made with a view to examine the intestinal bacteria.

Sampling of fish skin. After descaling, fish were thoroughly washed with the tap water and 22.59 cm of skin from both back sides was taken off with scissors. The skin was cut into pieces before putting in the 225 ml of normal saline water. Subsequent steps were the same as above.

Sampling of fish viscera. Medical gauze treated with 75% alcohol was used to disinfect the fish body after washing. The abdomen was cut open with a disinfected knife to get the fish viscera from which the liver, gallbladder, and fat were isolated. After pounding, 10 g were weighted and placed in 90 ml of sterilized normal saline water for uniform mixing. Further dilutions were needed for the examination of Colicin.

The determination method on the Colicin population, Salmonella, Shigalla, Camylobacter Jejuni, and Yersinia enterocolitica from manure, fish body mucus and fish viscera followed standard methods adopted for determination of Food Hygiene in Micro-organisms by the Health Ministry of People's Republic of China and Hygienic Epidermic Prevention Code published in 1979 in Shanghai.

The above-mentioned procedures were all adopted under sterilized conditions.

RESULTS

1. Bacterial Diseases

1.1 The numerical comparison of aerobic heterotrophic bacteria in the livestock manures, pond water and fish body mucus.

Table 1 indicates that the number of aerobic heterotrophic bacteria ranges between $(1.2+0.2) \times 10^8$ – $(2.8+2.2) \times 10^8$ in the manure of chicken, duck, cow and pig. The number of aerobic heterotrophic bacteria in the pond water rapidly increased with the application of manure. This type of bacteria in the test pond water ranged between $(2.6+0.53) \times 10^3$ – $(7.3+-3.4) \times 10$ while in the control pond, it was $(1.2+0.5) \times 10^3$. The number in the test pond was significantly higher ($P<0.05$) than in the control.

Table 1. Aerobic heterotrophic bacteria in the animal manure and pond water.

Types of ponds	Animal manure (No./g)	Pond water (No./ml)
Chicken manure	$(2.8\pm2.2) \times 10^8$	$(2.6\pm0.5) \times 10^3$
Duck manure	$(2.6\pm1.4) \times 10^8$	$(3.2\pm1.8) \times 10^3$
Cow manure	$(1.2\pm0.2) \times 10^8$	$(5.1\pm2.7) \times 10^3$
Pig manure	$(1.9\pm1.2) \times 10^8$	$(7.3\pm3.4) \times 10^3$
Control	-	$(1.2\pm0.5) \times 10^3$

The quantity of mucus obtained from 30 sq.cm of skin of black carp, grass carp, silver carp and bighead, respectively, were 0.17, 0.14, 0.10 and 0.18. Details are shown in Table 2.

Table 2. Aerobic heterotrophic bacteria in fish body mucus

Type of ponds	Silver carp	Bighead carp	Grass carp	Black carp
Chicken	5×10^2	1×10^3	9×10^3	1×10^4

manure				
Duck manure	1×10^3	3×10^3	5×10^3	1×10^4
Cow manure	5×10^3	3×10^4	4×10^5	5×10^5
Pig manure	2×10^4	3×10^4	1×10^5	1×10^5
Control pond	3×10^4	7×10^2	5×10^3	-

a. The number of aerobic heterotrophic bacteria observed in fish body mucus was related to the amount of manure applied. It ranged between 10^3 – 10^5/sq.cm of fish body in the fish specimens obtained from cow- and pig-manured ponds and 10^2 – 10^4 in both chicken- and duck-manured ponds. Application of large quantities of cow and pig manures resulted in greater bacterial biomass.
b. The number of bacteria observed in black carp and grass carp, and silver carp and bighead carp were 10^4 – 10^5, 10^3 – 10^5, respectively, and in silver carp and bighead carp 10^2 – 10^4.
c. The number of aerobic heterotrophic bacteria biomass in water is directly propositional to that of the fish body mucus of silver carp and bighead carp as these fish dwells in the water which is directly in contact with the fish body.

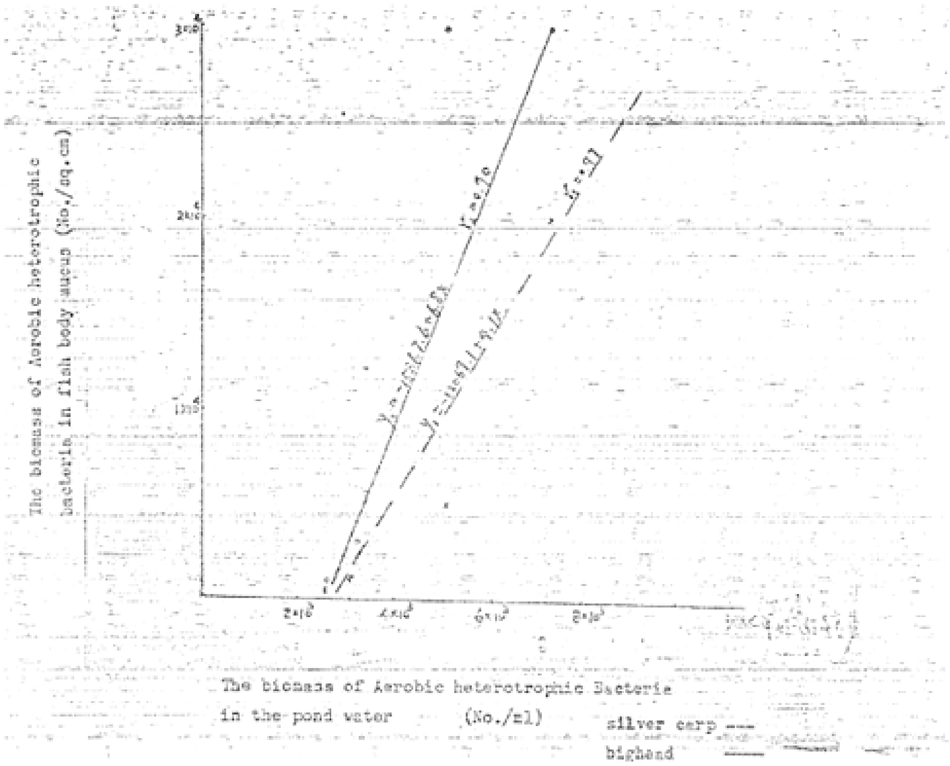

Fig. 1. The relationship between pond water and fish mucus in terms of aerobic heterotrophic bacteria.

where X : aerobic heterotrophic bacteria No./ml in pond water
Y1: aerobic heterotrophic bacteria No./ml in bighead carp mucus
Y2: aerobic heterotrophic bacteria No./ml in silver carp mucus

the linear regression equation is as:

$$Y1 = -15367.6 + 6.8x \qquad r1 = 0.90$$

$$Y2 = -12069.1 + 4.1x \qquad r2 = 0.97$$

2.2 The distribution of Aeromonas and Pseudomonas in the animal manure pond water and fish body mucus

Some 283 types bacteria were isolated from animal manures, pond water and fish body mucus. After a careful examination, 22 of them were identified as Aeromonas and 28 were Pseudomonas. The two types of bacteria existed in almost all the samples collected from manure and pond water. The frequency of occurrence of Aeromonas in the four types of manure was less while it had a higher frequency of occurrence in the ponds treated with manure. The number of Aeromonas was highest in the duck-manure followed by chicken manured ponds, and by pig- and cow-manured ponds. Pseudomonas was less in number in both manure treated with manure and pond water. However, Aeromonas and Pseudomonas were higher in number in the test ponds than in control ponds (Table 3).

Table 3. Frequency of occurrence of Aeromonas and Pseudomonas in the animal manure and pond water.

Type of bacteria	animal manure				pond water treated with manure				
	chicken	duck	cow	pig	chicken	duck	cow	pig	control
Aeromonas spp.	1	1	0	1	4	5	3	3	2
Pseudomonas spp.	2	2	2	1	1	2	2	2	1
Total	3	3	2	2	5	7	5	5	3

Table 4. Frequency of occurrence of Aeromonas and Pseudomanas in the fish body mucus.

Types of bacteria	Chicken manure ponds				Duck manure ponds				Cow manure ponds				Pig manure ponds				Control ponds			
	BC	GC	SC	BH	BC	GC	SC	BH	BC	GC	SC	BH	BC	GC	SC	BH	BC	GC	SC	BH
Aeromonas spp.				1		1														
Pseudomonas spp.	1				1	2	1			2			2		1		2		1	

*** BC, GC, SC, BH indicate black carp, grass carp, silver carp and bighead

It is evident from the Table 4 that the bodies of black carp and grass carp were attacked by Psuedomonas in both test and control ponds, which may be due to the dwelling strata in the water and large number of the aerobic heterotrophic bacteria present on the body mucus.

2. Hygiene of fish as human food

2.1 The population of Colicin and human intestine pathogenic bacteria in the Animal manure.

Although common bacteria were observed in the animal manure, Slmonella group E, with an exception of other pathogenic bacteria, was observed only in the duck manure (Table 5).

Table 5. Colicin population and pathogenic bacteria in the animal manure.

Types of manure	Colicin population	Salmonella	Shigalla	Camylobactor jejuni	Yersinia enterocolitica
Chicken manure	10^{-6}	-	-	-	-
Duck manure	10^{-8}	Group E	-	-	-
Cow manure	10^{-8}	-	-	-	-
Pig manure	10^{-8}	-	-	-	-

II. Number of Colicin and human intestine pathogenic bacteria in the fish body mucus and viscera.

The water layer in which the fish dwells is a main source of Colicin population in the fish body mucus in the ponds treated with manure (Table 6). The MPN ranges between 930–4600 in silver carp, 4600–2400 in bighead and 1500-2400 in black and grass carp, while in the control ponds, with an exceptionof slightly higher figure in grass carp. The MPN of others as silver carp, bighead carp and black carp were all <30. It is evident that the Colicin population in the body mucus of the upper dwellers is apparently less, while, on the contrary, the mid and bottom dwellers were heavily attacked. Tkh control pond received no manure at all. MPN of black carp body mucus was <30.

The human pathogenic bacteria were not observed in the fish mucus. Table 7 indicate the Colicin population in fish viscera. The population was higher in the manured ponds than that in the control ponds and increased with the quantity if manure applied. (Fig. 2). The Colicin population increased with the application of cow manure reaching the maximum of about $10^{-6} – 10^{-9}$. The values for the ponds treated with pig manure, chicken and duck manure wre $10^{-2} – 10^{-5}$, $10^{-2} – 10^{-3}$ and 10^{-2} respectively.

Table 6. The Colicin population and Pathogenic bacteria of human intestines in the fish body pre or post treatment.

ponds	fish species	MPN(No./100cm^2)		Salmonella		Shigalla		C.Jejuni		Y. enterocolitica	
		pre	post	pre	post	pre	post	pre	post	pre	post
chicken manured	SC	4600	30	-	-	-	-	-	-	-	-
	BH	≥ 24000	30	-	-	-	-	-	-	-	-
	GC	≥ 24000	30	-	-	-	-	-	-	-	-
	BC	≥ 24000	30	-	-	-	-	-	-	-	-
duck manured	SC	930	30	-	-	-	-	-	-	-	-
	BH	4600	30	-	-	-	-	-	-	-	-
	GC	15000	30	-	-	-	-	-	-	-	-
	BC	≥ 24000	30	-	-	-	-	-	-	-	-
cow manured	SC	4600	30	-	-	-	-	-	-	-	-
	BH	≥ 24000	30	-	-	-	-	-	-	-	-
	GC	≥ 24000	30	-	-	-	-	-	-	-	-
	BC	≥ 24000	30	-	-	-	-	-	-	-	-
pig manured	SC	4600	40	-	-	-	-	-	-	-	-
	BH	≥ 24000	30	-	-	-	-	-	-	-	-
	GC	≥ 24000	30	-	-	-	-	-	-	-	-
	BC	≥ 24000	30	-	-	-	-	-	-	-	-
control	SC	< 30	30	-	-	-	-	-	-	-	-
	BH	< 30	30	-	-	-	-	-	-	-	-

	GC	250	40	-	-	-	-	-	-	-	-
	BC	< 30	30	-	-	-	-	-	-	-	-

Table 7. The Colicin population and pathogenic bacteria in fish viscera.

ponds	species of fish	No. of bacteria per g.	the max. dilution	Salmonella	C.jejuni	Y.enterocolitica
chicken manure	SC	4.0×10^5	10^{-3}	-	-	-
	BH	1.0×10^5	10^{-3}	-	-	-
	GC	1.0×10^5	10^{-3}	-	-	-
	BC	1.0×10^4	10^{-2}	-	-	-
duck manure	SC	4.7×10^3	10^{-2}	-	-	-
	BH	9.8×10^3	10^{-2}	-	-	-
	GC	6.9×10^3	10^{-2}	-	-	-
	BC					
cow manure	SC	1.5×10^8	10^{-9}	-	-	-
	BH	2.1×10^9	10^{-8}	-	-	-
	GC	5.2×10^6	10^{-6}	-	-	-
	BC					
pig manure	SC	2.7×10^7	10^{-5}	-	-	-
	BH	2.0×10^7	10^{-5}	-	-	-
	GC	6.1×10^6	10^{-4}	-	-	-
	BC	1.0×10^5	10^{-2}	-	-	-
control	SC	1.0×10^4	10^{-1}	-	-	-
	BH	1.0×10^3	10^{-1}	-	-	-
	GC	2.8×10^3	10^{-1}	-	-	-
	BC					

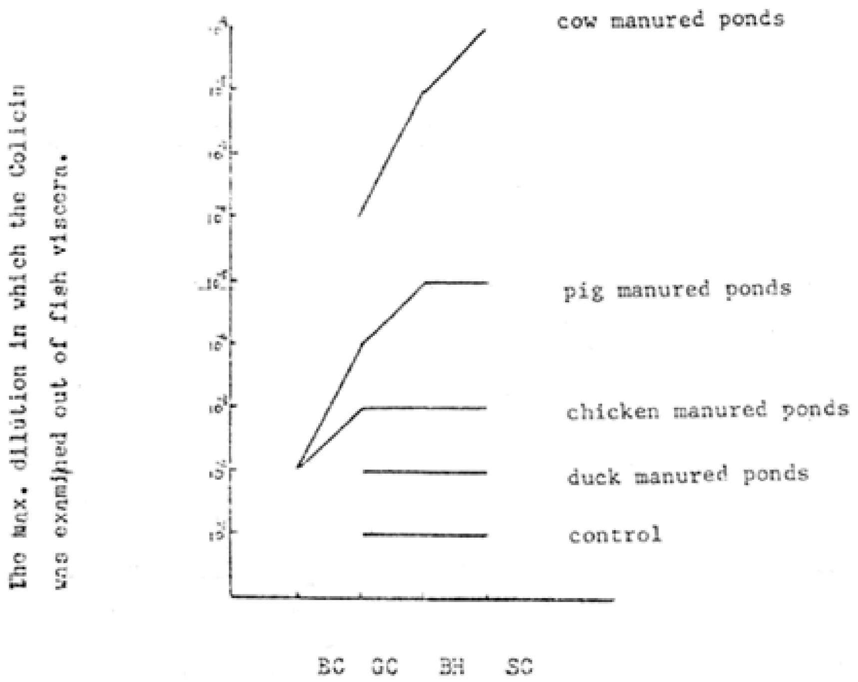

Fig. 2. The relationship between the Colicin population of fish viscera and quantity of animal manure applied in the fish ponds (data from 1985)

The value for the control pond was 10^{-1}. The Colicin population of viscera of silver carp and bighead carp in ponds treated with cow manure was 10^{-8} – 10^{-9} in dilution, and 10^{-5} in the ponds treated with pig manure. This may be associated with the filter-feeding habit of silver and bighead carp. In addition to the plankton, these species eat part of the manure and detritus Colicin in the viscera of black carp and grass carp were found less compared to that in silver carp and bighead. It indicates that the Colicin population of fish body and the viscera is invariably changed with water stratification.

2.3 The Colicin population of fish body before and after the treatment.

Before being as food consumed, fish needs descaling and washing. The Colicin population of manure-fed fish was reduced by 100–1,000 times after the treatment (Table 6). The Colicin population per 100 sq.cm of fish body after washing was < 40, which was almost the same as in the fish in ponds that received no manure application.

DISCUSSION

<u>Influence of manure application on bacterial fish diseases.</u>

The use of livestock manure and agricultural waste products in fish farming not solves the problem of fish feed and manure shortage and reduces costs. It also effectively increases fish production. In China, integrated fish farming has enabled a fish yield of 5,000–10,000 kg/ha.

The experiment shows that the aerobic heterotrophic bacteria existing in each gram of manure of chicken, duck, cow and pig respectively ranges between (1.2+0.2) × 10^8 – (2.8+2.2) × 10^8 (Table 1). According to the data collected each gram of manure consists of Colicin population at the rate of 10^6 – 10^7, but a limited number of Aeromonas and Pseudomonas. Pseudomonas, in particular, is the least in number. It is evident that Aeromonas and Pseudomonas with manure as a carrier are not abundant in the fish pond. The manure itself does not play a direct role in disseminating the bacterial fish diseases.

Aquatic bacteria largely increase in number along with the application of animal manure thus increasing the number of Aeromonas and Pseudomonas, too. Aeromonas and Pseudomonas were greater in number in the test than in the control ponds (Table 3), Aeromonas being more evident. The organic matter in the water increases due to manure application and the fish pathogenic bacteria, to some extent, are multiplied.

After the application of manure the aquatic bacteria increase in number; so do the aerobic heterotrophic bacteria in fish body mucus (Fig. 1). Because of the sedimentation of applied manure and pond mud in both manure applied ponds and controlled ponds, the amount of bacteria in the water column is decreasing from pond bottom to the surface layer of water with the continuous release of micro-organisms from the sediments. The bottom and, surface dwellers differ in the number of aerobic heterotrophic bacteria in fish mucus. The population of bacteria in black carp and grass carp was 10^3 – 10^5, while in silver carp and bighead it was between 10^2 – 10^4 (Table 2). In the test and control ponds, the Pseudomonas in the mucus of black carp and grass carp is enhanced (Table 4) which may be one of the main causes of Stigmatosis for black carp and grass carp. Over application of manure or feeds results in increased acidity and oxygen depletion in water. Furthermore, if the fish is accidentally injured it lowers its resistance against parasitic invasions, including pathogenic bacteria. In addition, fish immunity are important factor. The epidemic diseases of fish, due to their complexity, needs more systematic research. In China, sufficient attention is paid to the routine management of ponds with adequate manure application and proper control of physico-chemical conditions of water thus the bacterial fish diseases to great extent are under effective control.

Influence of manure application on the use of fish as human food

In China, cow, pig and chicken manure is normally fermented as compost before applying into fish ponds. This may be one of the reasons that no pathogenic bacteria were found in this test. Duck manure consists of Salmonella, but the quantity applied is largely diluted. Hence, human pathogenic bacteria are rare on the fish body. MPN values of both fish body and fish viscera indicates that the Colicin population in manured ponds is higher than that in non-manured ponds. However, with complete washing, the Colicin population is reduced to the value of 40 or less which is almost the same value in the control ponds. It is evident that washing of fish decreases the amount of human pathogenic bacteria to a minimum limit and that the hygiene of fish as human food is not affected. On the other hand, if the fish is not fresh enough and contaminated in the storage process, it must be thoroughtly washed and cooked at high temperature.

Recent studies have shown that the contagious diseases caused by the pathogenic bacteria in animal manure are controlled during the anaerobic fermentation process. Zhao Xi-hui (1985) reported that a temperature of 8– 25° for 44 days completely killed Bacillus typhi and Bacillus paratyphi; only 30 days, Bacillus dysenteriae was completely eradicated. The Colicin population increased from $10^{-5} – 10^{-7}$ to $10^{-4} – 10^{-5}$ during the period of 40–60 days. Digesters keep the manure for 30 days and within this period most of the pathogenic bacteria die off. Pig manure is fermented for biogas production the slurry of which is used in fish farming. After the anaerobic fermentation of animal manure the organic matters are roughly decomposed, which is beneficial for both pond sanitation and plankton multiplication. Fermentation of animal manure sharply reduces the chances of fish disease outbreak and improves the hygiene of fish as human food.

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