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Microbiological Quality of Catfish (*Clarias Gariepinus*) and Tilapia (*Tilapia Mossambica*) Obtained from Wet Markets and Ponds in Malaysia

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Abstract

The aim of this study was to determine the microbiological quality in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. A total of 108 samples (32 catfish, 32 tilapia, and 44 water samples) were obtained from nine wet markets and eight ponds in Penang, Malaysia. The feed in fish ponds were chicken offal, spoiled eggs and commercial fish feed. Using standard procedures, aerobic plate counts (APC), coliform, fecal coliform including *E. coli* were performed. A total 31/32 of catfish and 31/32 of tilapia exceeded the recommended microbiological standard for the APC. *E. coli* was less than 3 MPN/g for all catfish and tilapia samples. Temperature and pH of water ponds ranged from 26 to 27.5ºC and 6 to 6.8, respectively. Home-made feed using chicken offal and spoiled egg may contribute to the microbiological quality in fish. This highlights the importance of feed in aquaculture system.

Keywords: Catfish; Microbiological quality; Pond; Tilapia; Wet market

Introduction

Aquaculture, an important sector, has significant economic impact in many countries including Malaysia. Based on the recent data, freshwater fish is cultured using pond culture, ex-mining pool, freshwater cage, cement tank, canvas tank, and freshwater pen culture systems [1]. The highest freshwater fish production in Malaysia was catfish reared in pond culture (64.9%) followed by tilapia reared in ex-mining pools (18.2%) [1]. FAO [2] reported that aquaculture production in Malaysia is marketed locally for domestic consumption. Besides quantity of aquaculture production, the application of hygiene and food safety procedures in fish production should be taken into account [3]. The inappropriate aquaculture practice may become a concern for food safety issue [4]. Yet, intensification of fish production is still raising the risk of disease due to high density stocking of fish, antibiotics, poor water control and other factors [3]. Feed, an important diet for the growth of fish [5,6], may promoted the risk of disease for fish and ultimately for human [7]. In Asia-Pacific region, commercial feed and home-made feed have been used to feed the fish in freshwater aquaculture production. The home-made feed was chicken offals, spoiled eggs or waste food [8] which may affect to the quality of fish.

However, there is still limited data regarding the microbiological quality of fish distributed in Malaysia which were fed using home-made feed (chicken offal or spoiled eggs) and commercial feed. The microbiological quality of fish can be measured by using aerobic plate counts, coliform counts, fecal coliform counts and *E. coli* counts. Thus, this study was carried out to fill in this gap. The aim of this study was to determine the microbiological quality of catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds (fed with chicken offals, spoiled eggs or commercial fish feed) in Malaysia.

Materials and Methods

Samples

Catfish and water samples were collected from local wet markets (A,B,C,D, and E), pond A1, Pond A2, Pond B1 and Pond B2. Tilapia and water samples were collected from local wet markets (E,F,G, and H), pond C1, Pond C2, Pond D1 and Pond D2. Alive catfish and tilapia was placed in sterile plastic bag that was filled with water. Further, the plastic bag was filled with oxygen and banned with rubber. Dead tilapia obtained from wet market was placed in sterile plastic bag and kept in the box with temperature approximately 4°C during transportation to the laboratory. Water samples were collected using sterile test tubes, and then transferred to the laboratory in ice box with temperature approximately 4°C. The samples were proceeded in the laboratory within 6 hours. Ponds A1 and Pond A2 used chicken offal to fed catfish. Pond C1 and C2 used spoiled egg to fed tilapia. Pond B1, B2, C1 and C2 used commercial fish feed.

Determination of aerobic plate count (APC)

Twenty five grams of catfish or tilapia was mixed with 225 mL of 0.1% Pepton Water (PW, Oxoid, Baringstoke, Hampshire, United Kingdom) and homogenized by using stomacher (Interscience, France) for 120 sec. The dilution was prepared by pipeting 1 mL of aliquot and mixed with 9 mL of 0.1% PW. The dilution was made from 10^{-1} to 10^{-6}. About 100 mL of aliquot was spread on Plate Count Agar (PCA, Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 24-48 h. Total number of colonies were counted and calculated as BAM Manual Protocol [9]. Total aerobic count was expressed as log cfu g^{-1}. Twenty five millilitres of water sample was mixed with 225 mL of 0.1%

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PW and homogenized by using stomacher for 120 sec. The dilution was prepared from 10^{-1} until 10^{-6} and plated on PCA as BAM Manual Protocol. Aerobic Plate Count (APC) was determined by using spread method and incubated at 37°C for 24-48 h [9]. This was expressed as log cfu mL^{-1}.

Determination fecal coliform count

Fecal coliform counts were determined using MPN method [10]. The equipment and solutions were sterilized in an autoclave before each use. The microbial quality of the catfish and tilapia were assessed by sampling 25 g of catfish and tilapia. One mL of each dilution (10^{-1} until 10^{-5}) was transferred into three tubes of Lauryl Sulfate Tryptose Broth (LST, Oxoid, Baringstoke, Hampshire, United Kingdom) and incubated at 37°C for 24 hours. Approximately, 10 µL of LST broth from positive tubes were transferred into 10 mL of Brilliant Green Lactose Bile Broth (BGLB, Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 24 h. Turbid tubes with gas were considered as positive and coliform counts were expressed as MPN g^{-1} or MPN mL^{-1}.

Fecal coliform bacteria are used as the fecal indicator in guidelines for wastewater reuse in irrigation and aquaculture [11]. Therefore, the water in the fish ponds was subjected to microbiological investigation using fecal coliforms as indicators of fecal pollution. Fecal coliform count was determined by transferring 10 µL of LST broth from positive tubes into three tube of *Escherichia coli* Broth (EC, Merck KGaA, Darmstadt, Germany) and was incubated at 44-45°C for 24 h. Tubes showing gas and turbidity were considered positive for the presence of fecal coliform and these were expressed as MPN g^{-1} or MPN mL^{-1}. A loopful of broth in gasing tube was streaked on L-EMB agar (Merck, Germany) and incubated at 35°C for 18-24 h. Presumptive E. coli grew as distinctive metallic green sheen colony on L-EMB Agar. Biochemical tests such as gram staining, catalase, cytochrome oxidase, microscopic observation, indole production, Voges-Proskauer reaction, methyl read reaction, citrate production and re-inoculated back into LST to confirm gas production were carried out as the Bacteriological Analytical manual [10]. *E. coli* culture (Food Microbiology Laboratory, School of Industrial Technology, USM) was used as control.

Temperature and pH of water analyses

Temperature and pH of water ponds were measured using Portable Digital pH Meter (Hanna, Model HI 8424, Romania). The sampling was measured every sampling day between 09.00 and 10.00 a.m.

Statistical Analysis

The statistical analysis of APC, coliform, faecal coliform and E. Coli in catfish obtained from different wet market and ponds were determined by using one-way ANOVA (SPSS software for Windows Version 13) at the significance level ($P<0.05$). The same statistical analysis was also applied for APC, coliform, faecal coliform and *E. coli* in tilapia obtained from different wet market and ponds. In similar, one way ANOVA was applied to determine APC, coliform, faecal coliform, *E. coli*, temperature and pH of water obtained from different wet market and ponds.

Results

Aerobic plate counts

Aerobic plate counts (APC) are a widely accepted measure of the general degree of microbial contamination [12]. In this study, the mean APC values were ranged from 5.30 to 6.84 log 10 cfu g^{-1} for catfish and 5.77 to 9.12 log 10 cfu g^{-1} for tilapia (Table 1). There was no significant different ($P>0.05$) among total aerobic bacteria in the catfish obtained from the ponds. Similarly, there was also found in tilapia obtained from all of the ponds (Table 1). However, there was significant different ($P<0.05$) between total bacteria in catfish obtained from wet market D (Gelugor wet market) and other markets. The significant different ($P<0.05$) was also observed between total bacteria in tilapia obtained from two markets (F,G) and other markets (E,H).

This present study found that 31/32 of catfish and 31/32 of tilapia

Location	Total aerobic counts (log CFU g^{-1} or log CFU mL^{-1})			Coliform counts (Log MPN g^{-1} or Log MPN mL^{-1})			Fecal coliform counts (Log MPN g^{-1} or Log MPN mL^{-1})		
	Catfish	Tilapia	Water	Catfish	Tilapia	Water	Catfish	Tilapia	Water
Wet market									
A (Bukit Mertajam)	6.84 ± 0.04^{e}	NA	7.12 ± 0.82	2.81 ± 1.09^{cd}	NA	3.7 ± 0.43^{de}	1.11 ± 0.44^{c}	NA	1.12 ± 0.48
B (Bagan Ajam)	6.76 ± 0.02^{de}	NA	7.07 ± 0.41	3.56 ± 0.34^{cd}	NA	3.96 ± 0.07^{de}	1.04 ± 0.49^{c}	NA	1.01 ± 0.37
C (Nibong Tebal)	5.86 ± 0.04^{cd}	NA	7.25 ± 0.39	3.75 ± 0.26^{cd}	NA	3.77 ± 0.23^{cde}	0.97 ± 0.33^{c}	NA	1 ± 0.43
D (Gelugor)	5.30 ± 0.02^{c}	NA	6.27 ± 0.05	2.52 ± 1.07^{c}	NA	3.17 ± 0.47^{cd}	0.95 ± 0.34^{c}	NA	1.38 ± 0.70
E (Bayan Baru)	6.55 ± 0.06^{de}	7.85 ± 0.55^{d}	$^{b}6.35 \pm 0.06$	3.73 ± 0.27^{cd}	3.63 ± 0.30^{de}	$^{b}3.86 \pm 0.18^{cde}$	0.94 ± 0.41^{c}	1.52 ± 0.19^{d}	$^{b}1.41 \pm 0.75$
F (Hypermarket S1)	^{a}NA	8.49 ± 0.13^{e}	NA	NA	3.93 ± 0.09^{e}	NA	^{a}NA	1.44 ± 0.27^{d}	NA
G (Hypermarket S2)	NA	9.12 ± 0.46^{e}	NA	NA	3.96 ± 0.06^{e}	NA	NA	1.57 ± 0.19^{d}	NA
H (Chowrasta)	NA	8.95 ± 0.21^{de}	NA	NA	3.55 ± 0.32^{de}	NA	NA	1.35 ± 0.51^{d}	NA
Ponds									
A1	6.29 ± 0.49^{de}	NA	6.56 ± 0.53	4.1 ± 0.07^{d}	NA	3.8 ± 0.42^{cde}	0.99 ± 0.5^{c}	NA	1.63 ± 0.28
A2	6.48 ± 1.07^{de}	NA	6.93 ± 0.40	4.16 ± 0.18^{d}	NA	4.19 ± 0.16^{e}	1.2 ± 0.63^{c}	NA	1.37 ± 0.79
B1	6.23 ± 0.39^{de}	NA	6.55 ± 0.03	3.32 ± 0.48^{cd}	NA	3.09 ± 0.08^{cd}	0.79 ± 0.29^{c}	NA	0.67 ± 0.33
B2	6.69 ± 0.01^{de}	NA	6.45 ± 0.03	2.46 ± 0.09^{c}	NA	2.90 ± 0.84^{c}	0.90 ± 0.13^{c}	NA	0.96 ± 0.09
C1	NA	6.28 ± 0.45^{c}	5.78 ± 0.58	NA	3.38 ± 0.58^{de}	3.38 ± 0.58	NA	0.6 ± 0.02^{c}	0.6 ± 0.22
C2	NA	6.62 ± 0.25^{c}	6.35 ± 0.47	NA	3.84 ± 0.34^{e}	3.92 ± 0.22	NA	0.5 ± 0.05^{c}	0.58 ± 0.18
D1	NA	5.77 ± 0.39^{c}	5.80 ± 0.65	NA	3.09 ± 0.03^{d}	2.39 ± 0.60	NA	0.5 ± 0.05^{c}	0.5 ± 0.05
D2	NA	5.98 ± 0.58^{c}	5.76 ± 0.40	NA	1.35 ± 0.44^{c}	2.28 ± 0.27	NA	0.48 ± 0.0^{c}	0.48 ± 0.0

a=not available; b=water obtained from catfish tank; c,d,e =in the same column with different superscript letters are different ($P<0.05$); A1 and A2=catfish pond use chicken offal; B1 and B2=catfish pond use commercial fish feed; C1 and C2=tilapia pond use spoiled egg; D1 and D2=tilapia pond use commercial fish feed; Total aerobic and fecal coliform counts in water samples obtained from all sources were not significant different ($P>0.05$). *E Coli* was counted less than 3 MPN/gr (0.47 log MPN/gr) for all catfish and tilapia samples and less than 3 MPN/mL (0.47 log MPN/L) for all water samples.

Table 1: Total aerobic, coliform and fecal coliform counts in catfish, tilapia and water obtained from ponds and wet markets in Malaysia.

Ponds	Temperature (°C)	pH
A1	26.5 ± 0.5	6.2 ± 0.17
A2	26.83 ± 0.76	6.37 ± 0.38
B1	26.8 ± 0.72	6.4 ± 0.36
B2	26.93 ± 0.12	6.3 ± 0.21
C1	26.47 ± 0.06	6.3 ± 0.09
C2	26.85 ± 0.21	6.2 ± 0.06
D1	26.7 ± 0.34	6.42 ± 0.27
D2	26.67 ± 0.40	6.49 ± 0.28

A1 and A2=catfish pond use chicken offal; B1 and B2=catfish pond use commercial fish feed; C1 and C2=tilapia pond use spoiled egg; D1 and D2=tilapia pond use commercial fish feed.

Table 2: Temperature and pH of water obtained from ponds in Malaysia.

samples exceeded the recommended microbiological standard. International Commission of Microbiological Specific for Foods [13] stated that the total aerobic bacteria in fresh and frozen fish should be less than 5.7 log g^{-1} to meet Good Manufacturing Practise Criteria. However, 1/32 of catfish and 20/32 of tilapia samples were observed to be more than 7 log cfu g^{-1} which exceeded the safety or quality limit standard. International Commission of Microbiological Specific for Foods [13] revealed that the total aerobic bacteria in fresh and frozen fish should be less than 7 log g^{-1} to meet safety or quality standard.

Coliform, fecal coliform and *E. coli*

The coliform count ranged from 1.46 to 4.18 log MPN gr^{-1} for catfish, 1.6 to 4.04 log MPN gr^{-1} for tilapia, and 2.04 to 4.36 log MPN mL^{-1} for water. The coliform count was significant different ($P<0.05$) among catfish obtained from pond A1-A2 (chicken offals feed) and pond B2 (commercial fish feed). Similarly, there was the significant different ($P<0.05$) among tilapia obtained from pond C1-C2 (spoiled eggs feed) and pond D2 (commercial fish feed). Coliform count in water samples obtained from pond A2 (chicken offals feed) was also significant different ($P<0.05$) with those obtained from pond B1-B2 (commercial fish feed) (Table 1). However, coliform count in water samples obtained from pond C1-C2 (spoiled eggs feed) and pond D1-D2 (commercial fish feed) was not significant different ($P>0.05$). Similarly, the coliform count was not significant different ($P>0.05$) among water samples obtained from catfish tank at each wet market.

Fecal coliform ranged from 0.48 to 1.63 log MPN g^{-1} for catfish, 0.48. to 1.81 log MPN g^{-1} for tilapia, 0.48 to 1.97 log MPN mL^{-1} for water. There was not significant different ($P>0.05$) between fecal coliform in catfish obtained from wet market and pond. This was also not significant different between fecal coliform in tilapia obtained from wet market and pond. Similarly, fecal coliform was not significantly different ($P>0.05$) between water obtained from wet market and pond.

In this present study, *E. coli* was found for less than 3 MPN g^{-1} for catfish, tilapia and water. There was no significant different ($P>0.05$) between water obtained from wet market and pond. Similar statistics results were also observed in catfish and tilapia obtained from wet market and pond.

Temperature and pH

This present study observed temperature of water ponds ranged from 26 to 27.5°C (Table 2). Temperature of water in catfish tank obtained from different wet market was no significant difference ($P>0.05$). This was also observed in temperature of water ponds. There was no significant difference ($P>0.05$) between temperature of water ponds in Pond A1, A2, B1, and B2. This present study also observed that pH of water ponds ranged 6 to 6.8 (Table 2). There is no significant different ($P>0.05$) between pH of water obtained from wet market A, B, C, D and E. The similar statistical analysis result was also observed in wet market E, F, G and H. There is no significant different ($P>0.05$) between pH of water obtained from Pond A1, A2, B1 and B2. This was also observed in pH water obtained from Pond C1, C2, D1 and D2. However, pH of water pond obtained from Pond A1, A2, C1, and C2 was relatively higher compared to those obtained from Pond B1, B2, D1, and D2.

Discussion

The results showed variation in total aerobic count in water form ponds, and catfish and tilapia from wet markets and ponds. The highest total aerobic count was observed in tilapia obtained from wet market H. This result similar to Shinkafi and Ukwaja [14] whom reported the high load of bacteria in tilapia sold in the central market of Sokoto Nigeria. The present study found that tilapia was not delivered as live fish but fresh or chilled fish. This condition might alter the growth of bacteria in the fish due to the spoiled process and temperature of storage. Keller [15] revealed that bacteria might increase due to the temperature and time of storage. Other study reported that spoilage was evident after 13 h at room temperature (26-29°C) in fresh common carp (Cyprinus carpio L) [16].

This present study indicated that type of feed did not affect to the bacterial load in the fish. The nutrient of different feed was shown to be similar each other for the growth of bacteria. The present study also found that high bacterial load in fish occurred in the high temperature of water. Catfish and tilapia are cold-blood animals and have the same temperature as their surroundings [17]. Al-harbi [18] reported that temperature of water correlated with the load of bacteria in fish. In the present study, temperature of water ranged from 26 to 27.5°C. Adam and Moss [19] revealed that mesophiles and psychrotrops bacteria grew in the ranges of 15 to 47°C and -5 to 35°C, respectively. Boyd and Tanner [20] reported that coliform in catfish ponds at Auburn. Alabama was greater in summer and spring compared to other seasons when the temperature was going to decrease.

This present study observed that pH of water ranged from 6 to 6.8. However, pH of earthen pond water (3/12) and pH of ex-mining pools water (12/12) was exceeded from the recommended pH.

Chapman [21] revealed that the recommended pH of water pond ranged from 6.5 to 9 for catfish farming. Ross [22] stated that the recommended pH of water pond for tilapia farming ranged from 7 to 9. Accumulation of waste feed and fish faecal material results in changes in the sediment, characterized by high content of organic material and accumulation of nitrogenous and phosphorous compounds which may induce the benthic communities [23] and affect the pH of water ponds [24]. Fish will be stressed and die if the pH reach below 5 or above 10 [24]. In the present study, the depth of ponds was ranging from 4 to 7 m for catfish ponds and 18 to 50 m for tilapia ponds. Tilapia was farmed in ex-mining pools. Mente et al. [23] revealed that the growth of benthic algal bloom occurred in the depth ranging from 20 to 50 m. Thus, this may induce the pH changes in water ponds and promote the stress of the fish.

This study found that coliform in catfish and tilapia fed with chicken offals or spoiled eggs were relatively higher compared to those fed with commercial fish feed. These were observed also in water samples. Boyd and Tanner [20] reported that the high organic matter input in feed could increase the coliform in the catfish ponds. Other

study reported that chicken [25] and eggs [26] were potential agents for coliform. Thus, chicken offals and spoiled eggs introduced to the aquaculture system will increase coliform and reduce the hygiene level in the ponds.

This study indicated that the level of coliform correlated to the density of the ponds. The density of fish in the ponds ranged from 10-32 of catfish m^{-2} and 6-10 of tilapia m^{-2}. Coliform count was shown to be relatively higher in catfish compared to tilapia. These were also observed in water samples (Table 1). Previous study revealed that high density of fish related with coliform and other bacteria in water and fish [27].

Besides that, stream and hold water used in earthen ponds and ex-mining pools might be contaminated by coliform bacteria. Vŝetičková and Adámek [28] reported that the pattern of water quality changes after the flow through the pond was predominantly influenced by inlet water quality. Francy et al. [29] reported that total coliform in 136 stream and 143 ground water samples collected in five hydrology system of the United States were found in 99% and 20%, respectively. That study reported that the land use related to the density of coliform in stream water. Francy et al. [29] also reported that the presence of septic systems and well depth related with the density of coliform in ground water. Blogoslawski et al. [30] reported that pathogenic bacteria can introduce to the hatchery systems though a contaminated water source.

The coliform level was found relatively higher in fresh or chilled tilapia compared to live catfish in wet markets. The coliform level might promoted the deterioration in tilapia and increase the density of coliform. Gelman et al. [16] reported that spoilage was evident after 13 h at room temperature (26-29°C) in fresh common carp (Cyprinus carpio L).

This study found that the density of fecal coliform was observed to be significant different ($P<0.05$) in tilapia which were sold in wet market and reared in ponds. These might be occurred due to deterioration in tilapia. Keeping alive tilapia during distribution will make the high cost of transport, water and storage tanks. Thus, this fish was delivered as fresh or chilled tilapia in wet markets. Geldreich and Clarke [31] reported that the fate of fecal coliforms in the fish indicated that these organisms can probably survive and multiply when fish temperatures were 20°C or higher, but only when the organisms are retained in the gut for periods beyond 24 h. Moreover, the human health risk may rise when *E. coli* contaminate the fish. The present study found level of total aerobic bacteria, coliform and fecal coliform in catfish, tilapia and water increase and exceed the microbiological standard due to the use of chicken offal and spoiled egg as feed.

Conclusions

Chicken offals and spoiled eggs can be potential source for the bacterial contamination to water and fish. This evidence highlights the importance of feed quality in aquaculture system.

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