

The Bacteriological Quality of Chicken Offal and Spoiled Egg as Feed for Catfish and Tilapia Rearing in Penang, Malaysia

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Abstract

*Chicken offal, spoiled egg and commercial fish feed were used as feed in cultured ponds of catfish and tilapia in and around Penang, Malaysia. The total aerobic bacteria, coliform and fecal coliform was investigated on catfish (*Clarias gariepinus*), tilapia (*Tilapia mossambica*) and its water. A total of 48 samples (12 intestine of catfish, 12 intestine of tilapia, 24 water samples) were taken from 8 pond in and around Penang, Malaysia. All fish samples fed by chicken offal or spoiled egg were heavily contaminated by total aerobic bacteria (5 to 7.26 log₁₀ cfu/gr), coliform (2800 to 11000 MPN/gr) and fecal coliform (3 to 430 MPN/gr). Meanwhile, fish fed by commercial pellet were less contaminated by total aerobic bacteria (5.33 to 6.58 log₁₀ cfu/gr), coliform (7 to 11000 MPN/gr) and fecal coliform (3 to 110 MPN/gr). Chicken offal and spoiled egg polluted the water in aquaculture system more than commercial fish feed. The highest level of total aerobic bacteria, coliform, fecal coliform in pond water were 7.21 log₁₀ cfu/ml, 11000 MPN/ml and 2800 MPN/ml. The bacteria load of chicken offal and spoiled egg might contaminate catfish, tilapia and its water. These will be a concern for quality and safety of catfish and tilapia in term of nutrition and human health.*

Key words: chicken offal, spoiled egg, catfish, tilapia, bacteriological quality

Introduction

In Malaysia, catfish and tilapia were the important aquaculture products and marketed for domestic consumption (FAO, 2012). The prices of those fishes were more consistent compared to marine fish. Thus, the demand of catfish and tilapia

tended to increase in the last decade. However, some problems have been met. Those were the limited land, the rising of production costs, lack of skilled labor, the threat of diseases, the high food safety and quality requirements. These issues made the aquaculture development to be more difficult (FAO, 2012). The rising of production cost can be reduced by replacing the commercial pellet feed with cheaper feed such as food waste from restaurant, chicken offal, spoiled egg or others.

In Malaysia, chicken offal and spoiled egg have been found to be used as feed for catfish and tilapia. However, the study about the effect of those feed for microbiological aspect in catfish and tilapia has not yet been done. To filling this gap, the present study will investigate microbiological aspect in catfish and tilapia which fed by different type of feed. The objective of this study was to investigate the bacteriological quality of chicken offal and spoiled egg as feed for catfish and tilapia rearing in Penang, Malaysia. The bacteriological aspects studies were total aerobic count, *coliform* count and *fecalcoliform* count in the intestines of fish and water of ponds. Fapohunda et al. (1994) reported that the verification of the microbiological quality, such as *coliform* and *fecalcoliform*, could explain whether the harvest or production presents a health hazard or not to human. *Coliform* and *fecalcoliform* were reported to be the most heavily contaminate the intestinal tract of fish (Salle, 1964).

Materials and Methods

Catfish and tilapia were obtained from 8 ponds located in Penang -Malaysia in November 2008 – September 2009. Sampling was done 3 times visiting for each ponds. During each visit 5-6 live catfish and tilapia were purchased from ponds, placed in sterile plastic bags and transported in polystyrene box to the laboratory. On arrival at the laboratory, the intestines of fish samples were pooled and analyzed for Aerobic Plate Count (APC), *coliform* and *fecal coliform*. Water samples were also obtained from the ponds where both catfish and tilapia were reared. This sampled and kept in sterile jar at 4 °C during transportation to the laboratory. The ponds were studied based on the type of feed given to the fish. The feed types were chicken offal for catfish feed, spoiled egg for tilapia feed and commercial fish pellet feed for both. The chicken offal was steamed and grinded before feed to the fish. The spoiled egg was mixed with other ingredients and was formed to pellet.

The intestines of catfish and tilapia were taken aseptically and chopped by using sterile knife. Twenty five grams of intestines was mixed with 225 ml of 1.5% Pepton Water (Oxoid) and homogenized by using stomacher (Interscience) for 120 sec. The dilution was prepared by pipeting 1 ml of aliquot and mixed with 9 ml of 1.5% Pepton Water (Oxoid). The dilution was done from 10^{-1} until 10^{-6} . About 100 ml of aliquot was spread on Plate Count Agar (Merck) and incubated at 37°C for 24-48 hours. Total number of colonies were counted and calculated as BAM Manual

Protocol (Maturin *et al.*, 2001). Total aerobic count was expressed as log cfu/g.

Coliform and *fecalcoliform* count were determined by using MPN method (Feng *et al.*, 2002). One ml of 10^{-1} until 10^{-5} dilution of intestines and water samples were transferred into three tubes of Lauryl Sulfate Tryptose (LST) Broth (Oxoid) and incubated at 37°C for 24h. Approximately, 10 µl of broth from positive tubes were transferred into 10 ml of Brilliant Green Lactose Bile (BGLB) broth (Merck) and incubated at 37°C for 24 hours. Turbid tubes with gas were considered as positive and coliform count were expressed as MPN/g or MPN/ml. *Fecalcoliform* count was determined by transferring 10 µl of BLGB broth from positive tubes into three tube of EC broth (Merck) and was incubated at 44-45 °C for 24 hours. Tubes showing gas and turbidity were considered positive for the presence of *fecalcoliform* and these were expressed as MPN/g or MPN/ml. *E. coli* cultures (Food Microbiology Laboratory, School of Industrial Technology, USM) were used as control.

Statistical Analysis

The difference in APC, *coliform* and *fecal coliform* ponds was determined by using one-way ANOVA, SPSS software for Windows Version 13.

Results and Discussion

In present study the microbiological quality of catfish and tilapia fed by using chicken offal, spoiled egg and commercial feed were evaluated. Our results showed that total aerobic count on intestinal of catfish and tilapia fed by chicken offal and tilapia fed by spoiled egg were observed relatively higher compared to catfish and tilapia fed by commercial fish feed. The aerobic plate count ranged from 5.00 to 7.26 \log_{10} CFU/g catfish fed with chicken offal, 5.97 to 6.69 \log_{10} CFU/g catfish fed with commercial feed, 5.33 to 6.58 \log_{10} CFU/g tilapia fed spoiled egg, 5.11 to 6.11 \log_{10} CFU/g tilapia fed commercial fish feed. Later on, the fish which were not eviscerated properly, the bacteria might spread and contaminate through the apparatus of the fish intestinal such as intestinal wall and intestinal cavity. The proteolytic enzymes originated from intestines and/or the inside of intestinal canal might act for the spoilage process (Andreji *et al.*, 2006).

In water samples, chicken offal and spoiled egg showed to be relatively higher on total aerobic count compared to commercial fish feed. There were no significant differences between type of feed on catfish, tilapia and water ($P > 0.05$). The aerobic plate count in water samples ranged from 6.00 to 7.21 \log_{10} CFU/g for water obtained from the pond use chicken offal, 6.00 to 6.9 \log_{10} CFU/g for pond use commercial feed, 5.32 to 6.58 \log_{10} CFU/g for water obtained from the pond use spoiled egg, 5.05 to 6.18 \log_{10} CFU/g for pond use commercial feed.

Chicken offal was more contaminate the water in pond compare to spoiled egg. Other study reported that chicken loaded by aerobic bacteria (Cohen *et al.*,

2007). Spoiled egg was formed as pellet to feed tilapia. The pellet form could feed tilapia efficiently and reduced the remained feed in water. Thus, the contamination in water was observed less than chicken offal which was not formed in pellet. The form of pellet in fish feed can significantly reduce pollution caused by fish feeding and improve both the feed efficiency as well as fish health (Agriculture Fisheries and Conservation Technology, 2004).

The detected values of *coliform* in intestinal of catfish and tilapia fed by using chicken offal and spoiled egg showed to be relatively higher compared to commercial fish feed. These were observed also in water samples. Thus, chicken offal and spoiled egg might be the source of coliform. There were significant different between intestines of catfish fed chicken offal and catfish fed commercial fish feed ($P < 0.01$). These were observed in tilapia and water samples also.

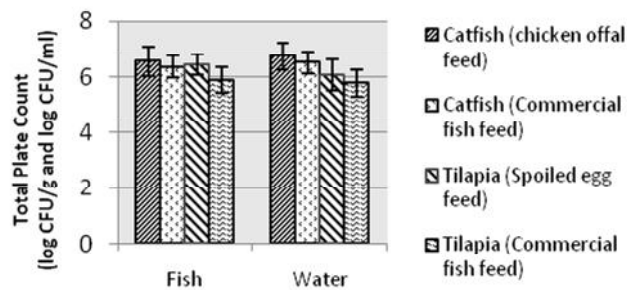


Figure 1. Distribution of Aerobic Plate Count in catfish, tilapia and water obtained from pond in Penang

Coliform count was 1100 MPN/g in the intestines of catfish fed with chicken offal, from 240 to 750 MPN/g in catfish fed with chicken offal, from 2800 to 11000 log MPN/g tilapia fed spoiled egg, from 7 to 11000 MPN/g tilapia fed commercial fish feed. Other study found that chicken and spoiled egg loaded by *coliform* (Cohen *et al.*, 2007; Theronet *et al.*, 2003). The composition of the intestinal flora is related in varying degree to the level of contamination of water and food in the environment (Geldreich *et al.*, 1966).

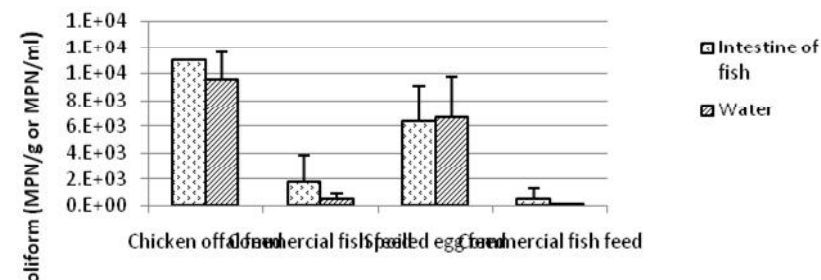


Figure 2. *Coliform* count in catfish, tilapia and water obtained from pond in Penang

Coliform count ranged from 2100 to 11000 log CFU/g in water samples from pond use chicken offal, 110 to 1100 log CFU/g from catfish pond fed with chicken offal, 40 to 1100 log CFU/g from tilapia pond fed spoiled egg, 70 to 110 log CFU/g from tilapia pond fed commercial fish feed. Chicken offal and spoiled egg used as feed might cause the increase of *coliform* in water. Chicken and spoiled egg which contained with *coliform* (Cohen et al., 2007; Theron et al., 2003) can transmit to the water (Pearson et al., 1987).

Intestines of catfish were highly contaminated with *fecal coliform* (Figure 3) with the mean level of 20 MPN/g, followed by intestines of tilapia with the mean level of 3.67 MPN/g. Other studies found that *fecal coliform* was observed in catfish and tilapia from the pond (Saber et al., 2004; Leung et al., 1992).

The mean for *fecal coliform* count in catfish fed with chicken offal (20 MPN/gr) was relatively higher compare to *fecal coliform* in catfish fed with commercial fish feed (9 MPN/g). In water samples from those sites, the significant difference was observed. The mean of *fecal coliform* in pond water (fed with chicken offal) was 48 MPN/g which was higher than those fed with commercial fish feed (8.3 MPN/g). Chicken and egg could be the sources for the presence of *fecal coliform* (Schwaiger et al., 2008; Cohen et al., 2007).

In tilapia, *fecal coliform* were no significant different between tilapia fed by using spoiled egg and commercial fish feed. This was also observed in water samples. The mean values were 3.67 MPN/g (tilapia fed spoiled egg), 3 MPN/g (tilapia fed commercial feed), 3 MPN/g (water of pond use spoiled egg) and 3 MPN/g (water of pond use commercial feed).

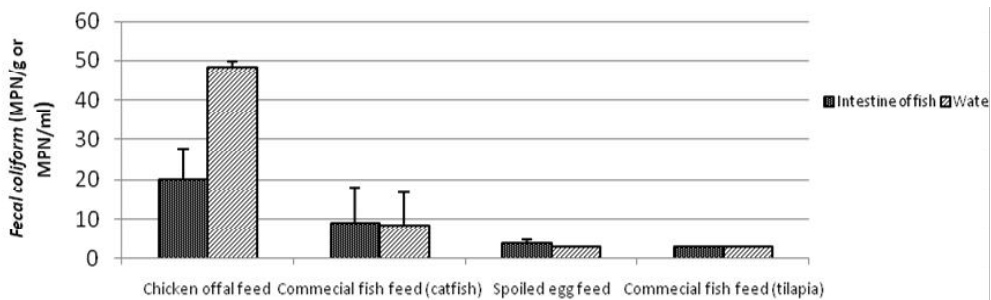


Figure 3. *Fecal coliform* count in catfish, tilapia and water obtained from pond in Penang

Conclusions

The results of this study showed that using chicken offal and spoiled egg as feed to catfish and tilapia cause heavily contamination by total aerobic bacteria (5 to 7.26 log cfu/g), *coliform* (2800 to 11000 MPN/g) and *fecal coliform* (3 to 43 MPN/g). These were relatively higher compared to fish fed by commercial pellet which had total aerobic bacteria (5.33 to 6.69 log cfu/g), *coliform* (7 to 11000 MPN/g) and

fecalcoliform (3 to 11 MPN/g). Chicken offal and spoiled egg polluted the water in aquaculture system more than commercial fish feed.

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References

- Andreji, J., Stranal, I., Kacanlova, M., Massanyi, P., Valent, M., 2006. Heavy metals content and microbiological quality of carp (*Cyprinus carpio*) muscle from two southwestern Slovak fish farm. In: J. Environ. Sci. Health, Part A, vol. A 41(6), 1071-1088.
- Agriculture Fisheries and Conservation Technology. 2004. Technological development. http://www.afcd.gov.hk/english/fisheries/fish_aqu/fish_aqu_techdev/fish_aqu_techdev.html download on 3 February 2012.
- Cohen, N., Ennaji, H., Bouchrif, B., Hassar, M., Karib., H. 2007. Comparative Study of Microbiological Quality of Raw Poultry Meat at Various Seasons and for Different Slaughtering Processes in Casablanca (Morocco). The Journal of Applied Poultry Research 16(4), 502-508.
- FAO, 2012. Food and Agriculture Organization of the United Nations for a world without hunger. http://www.fao.org/fishery/countrysector/naso_malaysia/en download on 12 March 2012.
- Fapohunda, A.O., MacMillan, K.W., Marshall, D.L., Waites, W.M., 1994. Growth of selected cross-contaminating bacterial pathogens on beef and fish at 15 and 35°C. Journal of Food Protection 57, 337-340.
- Feng, P., Weagant, S.D., Grant, M.A., Burkhardt, W., 2002. Bacteriological Analytical Manual : Enumeration of Escherichia coli and the *Coliform* Bacteria, US-FDA. Chapter 4. <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm064948.htm>.
- Geldreich, E.E. and Clarke, N.A., 1966. Bacterial pollution indicators in the intestinal tract of freshwater fish. Applied Microbiology 14 (3), 429-437.
- Leung, C-K., Huang, Y-W., Pancorbo, O.C., 1992. Bacterial pathogens and indicators in catfish and pond environments. Journal of Food Protection 55 (6), 424-427.
- Maturin, L. and Peeler, J.T., 2001. Bacteriological Analytical Manual : Aerobic Plate Count . US.FDA. Chapter 3. <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063346.htm>.

- Musgrove, M.T., Northcutt, J.K., Jones, D.R., Cox, N.A, Harrison, M.A. 2008. *Enterobacteriaceae* and related organisms isolated from shell eggs collected during commercial processing. *Poultry science* 87 (6), 1211-1218.
- Pearson, J., Southam, G.G., Holley, R.A., 1987. Survival and transport of bacteria in egg washwater. *Applied and Environmental Microbiology* (53), 2060-2065.
- Saber, A.E-S., Gijzen, H. J., Nasr, F. A., El-Gohary, F.A., 2004. Microbial quality of tilapia reared in *fecal*-contaminated ponds. *Environmental Research* 95, 231–238
- Salle, A.J., 1964. *Fundamental principles of bacteriology*, 5th York McGraw-Hill-Book Co. ed. New York.
- Schwaiger, K., Schmied, E.-M.V., Bauer, J., 2008. Comparative Analysis of Antibiotic Resistance Characteristics of Gram-negative Bacteria Isolated from Laying Hens and Eggs in Conventional and Organic Keeping Systems in Bavaria, Germany. *Zoonoses and Public Health* 55.331–341.
- Theron, H., Venter, P., Lues, J.F.R., 2003. Bacterial growth on chicken eggs in various storage environments, *Food Research International* 36 (9-10), 969-975.