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The Effect of Different Habitats and Postharvest Treatments of Catfish (*Clarias gariepinus*) on *Escherichia coli* Abundance: A Study in Brebes, Central Java, Indonesia

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Abstract. Catfish (*Clarias gariepinus*) is one of the fishery nutritious commodities that is quite popular in the community because it is cheap and easy to serve. It can be used as an alternative source to substitute protein from land source. Some of fish species, including catfish, contain higher and better protein than animal meat. Catfish are often grown in ponds in a sanitary manner because to the strong market demand. Due to the high bacteriological content of fishpond water, it is essential to identify the appropriate treatment for catfish. The aims of this study were to compare the impact of natural and artificial habitat and effect of different postharvest treatment on *E. coli* abundance. The study examined the bacteriological quality of 36 catfish from three distinct habitats and three different postharvest treatment methods. The result showed that there were no significant different ($p = 0.234$) of the *E. coli* abundance among three different habitats. The number of MPN fecal *coli* varied based on the postharvest treatment of catfish which included washing, cleansing overnight in a water flow, and frying ($p = 0.001$). Of the three post-harvest treatments, frying treatment resulted in significantly different MPN fecal *coli*. However, the frying treatment could not kill the *E. coli* completely. Hence the further study is needed to investigate the appropriate postharvest treatment which can control and reduce pathogenic bacteria spore in catfish.

INTRODUCTION

Catfish processed food is favored by the public because it is cheap, the meat is tasty and delicious, is available in abundance in the market. Not only in Indonesia, but catfish is also favored in Southeast Asian and African countries. Traditionally, in Indonesia catfish are grown in ponds which are usually also used for defecation. The reason for this use is because catfish can clean dirt, and the community has not yet access to latrines. The need for catfish increases every year, with this condition it is interesting to know the content of microbiological contamination of catfish and how to reduce the contamination content through post-harvest handling and processing.

Catfish is one of the most important aquaculture species in the world, including in America, Asia and Africa, [1]. Catfish favored because of the taste of meat a distinctive and delicious. In addition, the nutritional content of each catfish is high, it consisted of protein (17-37% fat (4.8%), minerals (1.2%) consisting of phosphate salts, calcium, iron, copper andiodine, vitamins (1.2%), ie the B complex vitamins are water soluble and vitamin of A, D and E that are fat soluble, [2]. These fish belong to the Order Siluriformes, a different group of ray-finned fish. Catfish represent more than 3,000 species, 478 genera and 36 families. Some species of catfish are farmed, including catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*), Walking Catfish (*Clarias fuscus*), striped catfish (*Pangasianodon hypophthalmus*) Gao, 2012 and catfish (*Clarias gariepinus*).

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The cultivation of catfish in the United States about 3,201,172 tons of production / year with a profit of 4,892,359,000 dollars, [1]. Production of catfish in Indonesia is 4% of the total aquaculture production of essential commodities, in the year of 2014 the production of Indonesia's catfish production are 613,120.0 tones while total aquaculture production 14,521,349 tones [3].

Habitat and ecological life form of catfish are all freshwater aquatic environments, such as reservoirs, dams, wetlands and other freshwater puddles. Catfish can live in ponds with bad quality of water, even without the flow of water at all. This fish can survive and grow quickly, easily adaptable, and effective against the different types and shapes of feed. In addition to these properties, when placed in contaminated water catfish were able to absorb / remove impurities, [4]. The questions to where do these impurities are absorbed? The estimated dirt absorbed into the body catfish (edible). If the dirt is absorbed by the catfish, why catfish not to[4]e ill, what properties does catfish have. Catfish allegedly have an innate immune [4]stem that developed properly. The innate immune system was the only weapon of defenses in invertebrates and plays an instructive role in the adaptive immune system of higher vertebrates. Teleost play a key role as a bridge between the innate and adaptive immune responses in which they are first class of vertebrates that have an element of innate and adaptive immunity. It is known that innate immunity in catfish is a set of comprehensive defense system that has proven to be beneficial increase resistance to pathogens, suc[4]as *Edwardsiella ictaluri* and *Flavobacterium columnar*, [1].

Innate immunity plays an important role in increasing resistance to pathogens. In recent ye[9], the production of catfish has suffered huge financial losses due to spread of pathogens. Outbreaks are caused by enteric septicemia of catfish (ESC), which is caused by Gram-negative intracellular bacterium *Edwardsiella ictaluri,* result in a loss of USD 40-50 million / year on catfish producers [1].

Related to the characteristic of catfish are easily cultivated in any polluted water conditions, In Indonesia, people favored catfish farming, including community in Salem Regency. Brebes Regency has 12,748.16 Ha of pond in 2012. They feed the fish using artificial feed in the form of pellets as well as feces because the latrine is above the pond. This is because not all people have latrine facilities at home and use catfish ponds as latrines. The coverage of latrines in Brebes Regency is around 86.2% or 466,727 units.

Disposal of human excreta into fishponds will result in contamination of water and soil that affect the quality of water resources. Feces containing potentially pathogenic microbes, the main indicator of the presence of pathogenic microbes is the presence of *E. coli* / fecal *coli* form as indicator of enteric or intestinal diseases. *Escherichia coli* were bacterium that is found only originate from feces and warm-blooded animals and humans. Disease caused by the bacterium *Escherichia coli* in humans in the form of gastro enteritis is characterized by symptoms of diarrhea, fever and vomit even sometimes occur death

Several attempts were made to eliminate pathogenic microbes on catfish such as by giving Kaolin, bacteria *Lactobacillus plantarum* C014, and vaccination of fish [5]. Generally, the media for catfish to live in Indonesia is in a fishpond that also serves two functions as to disposal of feces (defecated ponds), intensive fishponds specifically for cultivate of catfish and river waters.

Preliminary test results that have been carried out in the Centre of Laboratory of Health the Regional, Central Java Semarang, water quality conditions in the defecated ponds and river waters have the same amount of bacterial content of fecal *coli* form as high as>240,000 / 100 ml, while the water of intensive ponds have an amount of Fecal *coli* form content as high as 15,000 *coli* form / 100 ml. This value has exceeded the quality standards established for the water bodies, by the Government Regulation no 22 in 2021, as of 1000/100 ml water sample.

High content of fecal *coli*form in water of defecated ponds and river waters affect the quality of catfish that live in those waters. Fecal *coli*form MPN testing on catfish (*Clarias gariepinus*) living in three different habitats, namely river waters, defecated ponds and intensive fishponds showed similar results more than 2400/100 ml sample, and that Standard of MPN fecal *coli*form and positive presence of bacterium *Escherichia coli* exceeded the requirements based on the [6] on Maximum limit of microbial contamination in processed food, fish and fishery products should not be more than 1-10 MPN / gram sample. This shows that all three types of media to live of catfish (*Clarias gariepinus*) did not meet quality standards.

Efforts to reduce the content of *E. coli* and fecal *coli*form on catfish before the fish presented on the dining table can be done through some process namely cleaned or cleansed overnight in water flow, furthermore, before it been meals can be steamed, and fried. This study aims to analyze three different habitats and three postharvest processing type in order to reduce bacterial content of catfish (*Clarias gariepinus*) of the District of Salem, Brebes Regency Three types of habitats namely defecated ponds, catfish eating human feces from the pond to defecate, catfish that eating pellets of an intensive pond and catfish that live wild in the river waters through a three processing types namely cleaned, cleaned overnight in water flow and fried.

MATERIAL AND METHODS

Material

Materials used in this study was a life catfish (*Clarias gariepinus*) measure weight of 100-135 gr /fish, length of 17-22 cm. A The treatments used in this study were 3 x 3 with 4 repetitions. each catfish comes from three different habitats, namely ponds, intensive ponds and river waters, given 3 postharvest processes, namely clean wash, washed overnight in water flow and fry. A total of 36 catfish used. The group will be examined for MPN fecal *coli*form and the presence of *E. coli*.

Method

Cleaning the catfish

Specifically, the viscera and fins catfish Butterfly-shaped or fillet-shaped, then were cleaned and drained. Catfish were cleaned overnight in water flow. A life catfish placed in a basket and flowing water for 24 hours, then follow the cleaning process.

Frying the catfish

Each of the four (4) dead catfish from the three habitats (12 catfish) followed the cleaning process as carried out in the postharvest 1 process (clean and wash) then fried until cooked and drained. Frying process use oil that was always replaces. The process of frying the first conducted for a sample of catfish the which comes from an intensive pond (4 catfish), the oil is replaced to continue the process for frying the second sample of catfish from the river waters of 4 catfish and then the cooking oil is replaced with a new one to fry the catfish sample the which was derived from a defecated pond. The fried catfish that had been stored on the plate was sealed with plastic wrap, aim to avoid the fish are contaminated by bacteria prior to laboratory examination in the following day. The dishes to be used as a place of fried catfish were washed with clean warm water and wrapped using plastic wrap as a cover to keep the catfish remain sterile.

Examination of MPN fecal coliform

MPN Fecal *coli*form examinations conducted in accordance with the order of standard test for MPN *coli*form uses the dilution test, estimation (presumptive test), and a confirmation test (confirmative test) and incubated for 24-48 hours at temperature 44^{o}C. Fresh Catfish uses a variation of 3-3-3 double tube methods, fried Catfish uses a variation of 5-1-1 double tube methods

The presence of Escherichia coli

Examination of the presence of *Escherichia coli* is carried out by using a biochemical test of indole, Voges Proskauer (VP), methyl red (MR), citrate (C), as well as gas production from lactose.

Standard of MPN fecal coliform

Standard of MPN fecal *coli*form for water for aquaculture and fish meat based on government regulations. Water for aquaculture follow [6] concerning the implementation of environmental protection and management of national water quality standards, river water quality standards and the like for second class as of 1000/100 ml water sample. While fish meat and fishery product based on the [7] on Maximum limit of microbial contamination in processed food, fish and fishery products should not be more than 1-10 MPN / gram sample.

Data Analysis

Data were analyzed by the Kruskal-Wallis and Mann Whitney U test because the data not normally distributed, and the Chi-square test on nominal data.

RESULT AND DISCUSSION

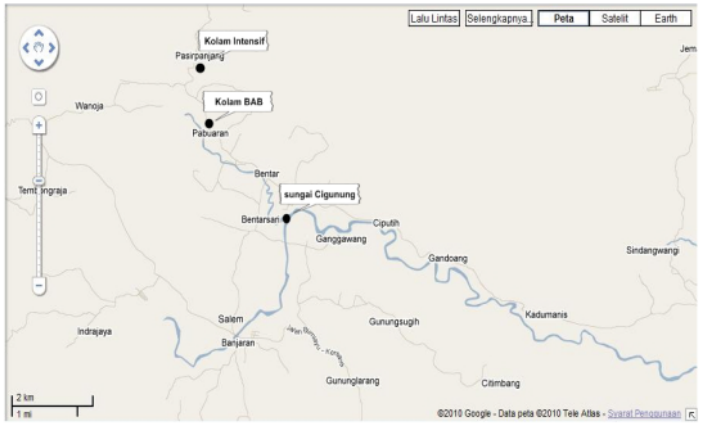

FIGURE 1. Research site's location, Defecated ponds, intensive ponds and river water

Test of Different of MPN Fecal *Coliform* and *E. coli* of Catfish (*Clarias gariepinus*) Among Habitat Type

Figure 1. show the description of location of research. The different test of catfish fecal *coli*form MPN (*Clarias gariepinus*) between habitat treatment, showed the result of different test of catfish fecal *coli*form MPN (*Clarias gariepinus*) has p value = 0.234 (Kruskal-Wallis) no difference the amount of MPN fecal *coli*form among habitats. And the results of the presence of E. *li* from the three habitats using the 2x3 table test showed p = 0.032, when continued with the 2x2 chi square test, the results showed that there was no difference in the presence of *E. coli* between the three with a significance value of p = 1,0000, 0.089 and 1.0000 respectively (Table 2. Presence of *E. coli* between habitat of catfish)

TABLE 1. Different test of catfish fecal coliform MPN (*Clarias gariepinus*) between habitat treatment

Habitat type of catfish	Median	(min-max)	p*
Defecated pond	165	(0 - 2400)	0.234a
Intensive pond	20	(0 - 2400)	
River	1,237.50	(0 - 2400)	

TABLE 2. Presence of *E. coli* between habitat of catfish

Habitat of Catfish	Presence of *E. coli*					
	positive		Negative		Total	
	n	%	n	%	n	%
River water	8	66.7	4	33.3	12	100
Defecated pond	7	58.3	5	41.7	12	100
Intensive pond	2	16.7	10	83.3	12	100

Note: p= 0.032

Test of Different of MPN Fecal *Coliform* and *E. coli* of Catfish (*Clarias gariepinus*) Among Postharvest Processing

Table 3. shows the difference test of fecal *coli*form MPN in catfish (*Clarias gariepinus*) between post-harvest processing, showing the frying process has the lowest number of fecal *coli*form MPNs while the amount in river water is the highest, from the three MPN *coli*form habitats it is significantly different with a significance p = 0.0001 (Kruskal Wallis). When continued with the Mann Whitney U test, each MPN *coli*form number remained significantly different with p 0.028, 0.0001, 0.0001.

The results of the presence of *E. coli* between postharvest treatment processes using a 2x3 chi square test table, showed a significant difference, p = 0.001 and when followed by a 2x2 chi square test, only treatments 1 and 3 (clean-wash and fry) were significantly different from p = 0.001 and RP = 55 (4.3 - 703.4), (Table 5. Prevalence Ratio of the presence of *E. coli* between clean-wash and fry process of catfish). The presence of *E. coli* in treatment 1 (cleaning and washing) was 83.3% greater than the result of the frying process 8.3%. This means that someone who handles catfish in a clean and washed manner has a risk of the prevalence of the catfish being 55 times greater than if it is fried.

TABLE 3. Different Fecal *Coli*form Test of MPN Catfish (*Clarias gariepinus*) between three post-harvest processes.

Postharvest process	Median	(Min-max)	p*	p**
Clean and wash	2,400.00	(20 - 2400)	0.0001	0.028 b
Washed overnight	75	(0 - 2400)		0.0001c
Frying	0	(0 - 38)		0.0001d

TABEL.4 Presence of *E. coli* between postharvest process of catfish

Postharvest process	Presence of *E. coli*					
	Positive		negative		Total	
	n	%	n	%	n	%
Clean and wash	10	83.3	2	16.7	12	100
Washed overnight	6	50	6	50	12	100
Frying	1	8.3	11	91.7	12	100

Note: P= 0.001

TABEL 5. Prevalence Ratio of the presence of *E. coli* between clean-wash and fry process of catfish

Postharvest process	Presence of *E. coli*					
	positive		negative		Total	
	n	%	n	%	n	%
Clean and wash	10	83.3	2	16.7	12	100
Frying	1	8.3	11	91.7	12	100

Note: p= 0.001, RP = 55 (4.3 - 703.4)

From table 2 it is known the presence of *Escherichia coli* in catfish that live in river waters, this is because catfish eat human and livestock waste, because a small number of people in Salem Regency still use rivers for bathing, washing and toilet facilities. While the presence of *Escherichia coli* bacteria in catfish that live in defecation ponds because the owner accidentally defecates in the pond to feed catfish. Meanwhile, in intensive ponds, MPN *coli*forms are obtained, although they have been greatly reduced, they are still far above the allowable threshold.

The results of the clean-wash process showed that 10 (83.3%) catfish contained *E. coli*. If we only handle catfish by cleaning and washing it, the *E. coli* in the catfish is not safe, as is the handling of washing it overnight with running water, although it has been reduced, the presence of *E. coli* from this process is still found. The safest processing if done frying. In frying, we did deep fry until the catfish is crispy and crunchy when consumed.

No difference between the number of MPN fecal *coli* form in catfish (*Clarias gariepinus*) that live in media of defecated ponds with an intensive ponds and river waters, because the content of artificial feed in intensive fishpond in the form of pellets turns impact to reduce water quality drastically because the rest of unconsumed feed (uneaten feed), of residual stool (feces). This is consistent with the preliminary test results stating that the number of bacterial content of fecal *coli* form in the intensive fishpond of 15,000 MPN / 100 ml, which means that the pool water that exceeds the quality standard limits the exposure of water bodies of water established by the Regulation 22, 2021 of

1000 MPN / 100 ml water sample. Decline in water quality due to the decomposition of protein in the feed into ammonium (NH4-N) the level of toxicity depends on the pH of the water. This is maybe the reason why catfish that live in intensive fishpond still contains fecal *coli* form exceeding the prescribed standards. To overcome this would be done running water periodically the physical condition of catfish. Results of this study are not in accordance with [8], which states that the catfish is fried with a temperature of ± 100 ° C, no bacteria can survive. The fact although it has done frying process there are *E. coli* bacteria on catfish that coming from the waters of the river.

Not all bacteria are lost through the frying process. Several alternatives to why *Escherichia coli* is present in fried catfish (*Clarias gariepinus*). First, it may be due to the source of the water used during the washing and overnight washing process, as well as the sanitization of the equipment used during the processing. Poor equipment conditions have the potential to contain germs on the tool. Another alternative can be caused by changes in innate immunity to heat in catfish [1].

Fried catfish from river habitat still contain E.*coli*. This may be due to the character of the catfish which is able to absorb the dirty environment in the water. It is therefore unsafe to consume catfish grown in a 'dirty' environment. the steps needed are not breeding catfish in a dirty environment, feeding catfish sufficiently, the process that needs to be done is to fry or steam properly. The presence of *E. coli* in fried catfish was only discovered in this study, becoming an interesting preliminary study to further deepen the immune system of catfish.

Several alternatives to why *Escherichia coli* is present in fried catfish (*Clarias gariepinus*). First, it may be due to the source of the water used during the washing and overnight washing process, as well as the sanitization of the equipment used during the processing. Poor equipment conditions have the potential to contain germs on the tool. Another alternative can be caused by changes in innate immunity to heat in catfish [1].

Therefore, further research is needed to determine the cause of the presence of *E. coli* in fried catfish by (1) Re-testing the content of *E. coli* in catfish from several habitats and immediately cleaning, washing and putting it into the lab on the same day with safety procedures as described above. It is already done; (2) Doing several ways of frying such as the time it takes to fry, the temperature when frying, the type of frying; (3) identify other bacterial species found from fried catfish meat, especially those that are pathogenic and produce spores; (4) determine the appropriate postharvest treatment that can control and reduce the spores of pathogenic bacteria in catfish.

CONCLUSION

There was no significant difference in fecal *coli* MPN between the three catfish habitats (river water, defecation ponds, intensive ponds but after the post-harvest process, there was a significant difference in fecal *coli* MPN, with frying treatment as the largest decrease in MPN and for that to be the best result). in this research. *E.coli* is still found after the catfish is fried. Frying treatment cannot kill *E. coli* completely. Therefore, further research is needed to determine the appropriate post-harvest treatment that can control and reduce spores of pathogenic bacteria in catfish.

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