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Health status and microbial quality of common carp reared in a pond fed with treated wastewater from a slaughterhouse

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Abstract. Wastewater from slaughterhouses in many countries is still discharged into rivers, without having been adequately treated. Such wastewater contains plenty of organic matter which is an ideal source of nutrients for fish, but also for the development of microorganisms. Thus, usage of wastewater in aquaculture could become a health risk for humans, fish due to the introduction of microorganisms into the aquatic environment. In the available literature, there is insufficient data on health and meat safety regarding common carp reared in purified wastewater. The aim of this study was to assess the health and meat safety of common carp cultivated in a fishpond supplemented with slaughterhouse wastewater that was subjected to tertiary treatment. The number of parasites was not significant and not a single parasitic disease was found in this study, but the number of parasite species detected was as expected and typical for carp production. No spring viraemia of carp or koi herpesvirus disease was found. The carp cultivated were in good health and completely safe for human consumption in terms of the presence of microbial contaminants. The safe use of wastewater for fish rearing should be encouraged, but proper treatment of wastewater must be applied before its use.

1. Introduction

The meat industry is characterized by high water consumption and is undoubtedly a significant source of organic pollution in the environment. The fact that slaughterhouse wastewater is still discharged into natural water bodies without adequate purification is a significant concern from the ecological viewpoint. This practice could be a significant hazard for the environment and consequently for human health. Some authors noted that wastewater could be used as a source of water and nutrients in fish production [1,2,3]. Pelić [4] reported that purified slaughterhouse wastewater could be used in common carp production as a novel approach to aid sustainable aquaculture development. Also, it is useful from the viewpoint of resolving the problem of slaughterhouse wastewater.

The use of fish meat in human diets is highly recommended. Fish is a valuable source of essential amino acids, protein, essential fatty acids and fats [5]. Fish meat is a very valuable source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Since these fatty acids can only be synthesized by aquatic organisms, consumption of fish meat is the only way humans can intake EPA and DHA [6].

However, consumers are increasingly directing their attention towards safety requirements associated with fish consumption due to the presence of different environmental contaminants, microorganisms and parasites, especially if fish are reared in wastewater-fed ponds or integrated production systems [7]. The potential public health risks associated with consumption of fish reared in wastewater include bacterial and parasitic infections, like diarrhoea and skin infections. Untreated wastewater can contain different harmful substances with negative effects on human health and the environment, such as parasites, pathogenic microorganisms, heavy metals, pesticides, antibiotics and hormones [8]. At the same time, the proper use of treated wastewater in fish production has important environmental and economic significance, due to recycling nutrients and reuse of water.

There are both ecological and financial motives for water purification which intertwine and complement each other. The use of available technology to clean the organic load from slaughterhouse wastewater, making it suitable for fish farming, is an ecological solution for water re-use. On the other hand, an integrated system carries some risks, mostly related to the safety of fish meat produced in this way.

The aim of this research was to examine the effect of using appropriately treated slaughterhouse wastewater on the health of carp and safety of carp meat for human consumption.

2. Common carp rearing in pond fed with treated wastewater from slaughterhouse

The research was carried out in several stages. A wastewater purification system was built on the property of a slaughterhouse in Pećinci, Serbia. The efficiency of the wastewater treatment plant was examined by chemical analyses of water at different purification stages. After this, a fishpond was built on the same property. It was mostly fed with water from the slaughterhouse wastewater purification system, but with some added well water. The purified water was first fed to a pre-fishery pond where it was aerated, after which the water moved into a fishpond where part of nutrients from the purifiers was used for carp nutrition. Carp fingerlings in good health were stocked in the fishpond for growth in optimal ambient conditions. After the fishpond, the water was then used to irrigate the soil surrounding the slaughterhouse. Compared with no treatment, the entire system increases the quality of purified wastewater, which reaches the limit concentrations set before it inflows into a natural, recipient water body. The health condition of fish was monitored during the production cycle by diagnostic examination of the causative agents of viral, bacterial and parasitic aetiology. Additionally, cultivated common carp were collected from the fishpond in spring and autumn and assessed for microbial quality.

3. The effect of slaughterhouse wastewater subjected to tertiary treatment on common carp health

The health condition of fish was controlled during the breeding season at least twice a month, having in mind that the results can be beneficially used only if any eventual therapy is administered on time. Monitoring the health of fish primarily involved examination of the body surface (presence of visible changes or injuries on the skin), then examination of the gills (colour change, presence of changes in the gills, appearance of necrosis) and examination of the internal organs. No viral diseases, including spring viraemia of carp and koi herpesvirosis, were detected by clinical examination or laboratory diagnostics. Only sporadic occurrence of erythrodermatitis was detected during the study, but this condition did not cause major health problems or losses, since the usual measures were applied. Zaibel et al. [9] also revealed that wastewater that had undergone tertiary treatment did not affect fish's growth, immune function or disease resistance.

In the current study, external parasites were recorded in small numbers that were characteristic for carp production, and no localised damages on the fish were observed. The following parasites were recorded: *Myxosporidia* spp., *Lernaea cyprinacea*, *Dactylogyrus* spp. and *Ichthyophthirius multifiliis*. The results obtained were in accordance with the results obtained by Novakov et al. [10]. Carp infection with *Lernaea cyprinacea* in spring was significantly higher than in other seasons, as others have stated [11].

Also, infection with *Dactylogyrus* spp. was recorded in all seasons. The current results, although parasite infestation was not significant, support the hypothesis that infestations of parasites including protozoa (*Ichthyophthirius multifiliis*), Monogenea (*Dactylogyrus* spp.) and Copepoda (*Lernaea cyprinacea*) are widespread and cause losses in fish farms in Serbia [10].

4. The potential public health risks associated with using treated slaughterhouse wastewater in fish production

The potential public health risk is the main factor that constrains use of wastewater in fish production. Danso et al. [12] noted that many studies confirmed that there is no evidence of consumer health risks from the consumption of fish cultivated in wastewater-fed fishponds. Primary and secondary treated waste effluents were successfully used to grow Nile tilapia that were safe for human consumption [13]. Furthermore, [14] reported that most of the muscle samples of fish experimentally exposed to *E. coli*, *Aeromonas*, enterococci and faecal coliforms were not contaminated. The same authors did not observe greater contamination under conditions of fish stress, i.e., high organic load, a water temperature of 37°C or low levels of dissolved oxygen. Lan et al. [2] reported there was no significant difference in the number of presumptive thermotolerant coliforms (in the muscle tissue and gut content) between fish farmed in wastewater-fed fishponds and non-wastewater-fed fishponds. Moreover, studies conducted in Egypt and India showed that fish farmed in fishponds supplemented with treated wastewater had lower levels of microorganisms than fish obtained from surface waters [15,16].

5. Microbial quality of common carp reared in a pond fed with treated slaughterhouse wastewater

The counts of all the examined microorganisms in the carp were permissible and did not exceed prescribed hygiene norms. There was no significant difference in the bacteriological quality of fish collected in spring and in autumn. The number of bacteria (total bacterial count, *Enterobacteriaceae*, coliforms and *Escherichia coli*) were highest in digestive tract content, followed by skin and were lowest in fillets. Sulphite-reducing clostridia, *Salmonella, Staphylococcus aureus* and *Listeria* spp. were not detected in the examined fish samples.

Dang and Dalsgaard [17] also reported that the level of faecal contamination was low in fillets of rohu, grass carp and sliver carp obtained in household-based integrated systems where fish farming was integrated with pig farming and horticulture. On the other hand, high levels of *E. coli* were found in the digestive tract of their examined fish. High levels of *E. coli* were also reported in the digestive tracts of fish but low levels in the fillets of fish from fishponds fed with urban wastewater in some Asian countries [18]. Additionally, Lan et al. [2] reported that fish reared in fishponds fertilized with urban wastewater contained very low levels of thermotolerant coliforms in muscle tissue, but high levels of coliforms were present on their skin and digestive tract contents. Proper handling of fish and the prevention of faecal cross-contamination during degutting and preparing fish for consumption at the market or in the home are the main critical points to control the food safety of fish flesh produced from wastewater-fed fishponds.

6. The presence of zoonotic parasites in fish reared in a pond fed with treated slaughterhouse wastewater

In the current study, zoonotic parasites were not detected in the carp. Fishbone zoonotic parasites are food safety hazards associated with usage of wastewater in fish production. According to the available literature data from Serbia, trematodes do not cause a health problem in cultivated carp. In contrast, in Asian countries, the occurrence of zoonotic trematodes in carp is a significant public health risk [19]. In integrated systems, pig manure can be a reservoir for trematodes, and eggs could be introduced into fishponds via infected pig waste [17]. The methods for preventing trematode transmission are the use of commercial feed in pig nutrition and proper heat treatment of the fish before consumption. Phan et al. [20] investigated the risk of fishborne zoonotic trematode infection in fish reared in wastewater-fed ponds in peri-urban areas of northern Viet Nam. The overall prevalence was 5% (6.5% in spring and

2% in autumn). The metacercariae were zoonotic intestinal trematodes of the family *Heterophyidae*. The intensity of infection was relatively low. It should be highlighted that the prevalence was low in comparison to previous findings of zoonotic trematodes in non-wastewater fish in Viet Nam [21]. Also, Hop et al. [22] reported that the prevalence of trematodes in fish reared in wastewater-fed fishponds in Viet Nam was low in comparison with previous findings of trematodes in fish from conventional fishponds. However, the fish reared in wastewater-fed fishponds are certainly at risk of infection with trematode parasites. The main risk for humans is consumption of raw or improperly prepared fish. As the consumption of raw fish meat is not widespread in Serbia, it can be concluded that carp meat produced in the pond that is partially filled with treated wastewater from the slaughterhouse is safe for human consumption from the aspect of the presence of zoonotic parasites.

7. Preventive measures

It is necessary to continuously work on reducing the prevalence of the parasites commonly present in fishponds. This is possible by improving the fish rearing conditions and implementing prophylactic measures such as drying of objects, freezing, mechanical cleaning and disinfection with lime [10]. Visual inspection of fish for parasites before being placed on the market is obligatory. This procedure is the best preventive measure that minimizes the risks associated with consumption of fish farmed in wastewater-fed ponds. Additionally, microbiological analyses of fish are very important. Proper handling of fish in the home and heat treatment are the best preventive measures against microbial and parasitic contaminants in fish. Adequate treatment of wastewater is the main prerequisite for its use in aquaculture.

8. Consumer acceptance of fish cultivated in wastewater

Consumer acceptance of fish reared in wastewater is a very important issue. The main reasons for avoiding consumption of fish from wastewater, except food safety concerns, are consumer behaviour and cultural habits. In Egypt, consumers did not accept fish produced in treated sewage waters despite the fact that the fish were safe for consumption [23]. Research conducted by Suzette et al. [24] in Ghana showed that the factors that influenced consumers' attitudes towards fish in general included food safety (63%), freshness of the fish (51%), taste (44%), packaging (41%) price, size and species of fish. Furthermore, the authors reported that the factors that affected consumers' attitudes to fish produced in treated wastewater were proximity of consumers to the treatment plant, price, consumer's religion and age, and whether or not they consume the fish species. It is interesting that the source of the fish did not significantly affect the preference of the consumer to consume fish cultured in the treated wastewater [24]. Gebrezgabher et al. [25] reported that surveyed consumers generally accept fish reared in treated wastewater if the fish price is suitably low. Danso et al. [26] reported that consumers in Viet Nam want to know if wastewater is used in fish production and if fish is certified by a relevant government agency. The authors highlighted that there is a need for government to provide adequate food safety control and quality control.

Consumers' acceptance of fish from treated wastewater could be increased by their education on the process of wastewater treatment and development of food safety guidelines. Proper treatment of wastewater before its usage in aquaculture production is a prerequisite for consumer acceptance of fish reared in wastewater.

9. Conclusions

The use of purified slaughterhouse wastewater in carp production had no adverse effects on fish health and resulted in production of carp characterized by adequate meat quality that corresponded with that of carp reared in conventional production systems. The results of this research and their comparison with earlier research on carp health strongly suggest the use of properly purified slaughterhouse wastewater results in production of carp without harmful effects. The use of treated slaughterhouse wastewater in fish production is a novel approach to the sustainability of the meat industry and environment protection. The application of this concept at slaughterhouses in Serbia is crucial from the aspect of environment protection, having in mind the requirements and standards aimed at minimizing environment pollution imposed by the EU. In that respect, such requirements have to be fulfilled, and Serbian legislation on environment protection must be harmonized with the EU regulations.

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