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Antibiotics and Sulfonamides in Water, Sediment, and Fish in an Integrated Production System

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Abstract: The aim of the present work was to examine the effect of using slaughterhouse wastewater on the production of carp meat safe for human consumption in terms of antibiotic residue level. The obtained results of the content of tetracycline and sulfonamides in water, sediment, and carp meat samples showed that the measured values were below the limit of detection for each tested parameter. Control of antibiotic residues in foods of animal origin is very important and mandatory to protect the health of end consumers and prevent the spread of antibiotic resistance.

Keywords: antibiotics residue; fish; aquaculture; wastewater; sustainability.

1. Introduction

The slaughterhouse industry, as well as other industrial branches involved in the production and processing of food, is characterized by a high production of wastewater consisting of water from the production process and water needed for washing and cleaning [1]. Water is used in all stages of production in the slaughterhouse, and the amount of water consumed and, consequently, the amount of wastewater generated depends on the technology applied in the slaughterhouse, as well as on its rationality and plant capacity [2]. It should be taken into account that water is a limited resource and therefore it should be used in the slaughterhouse as rationally as possible [3], so according to this principle in the integrated production system purified wastewater is used as a medium for growing fish. Integrated production means connecting several independent production processes (pond, domestic animals, irrigation systems, processing capacities) into a system based on the principle that by-products or waste from one product can be an input component for another production [4]. Quality control of wastewater from the slaughterhouse is primarily considered from the aspect of potential pollution of open water flows that are recipients of wastewater discharged from the slaughterhouse. The level of pollution of wastewater is usually above the permitted limits and purification is necessary [5]. The use of available technologies for growing fish within the slaughterhouse industry represents an ecological solution to the removal of organically loaded water that had been used in the slaughterhouse. On the other hand, such an integrated system also carries certain risks, which are mainly related to the safety of fish meat produced in this way [6]. Antibiotics have been widely used in livestock production and are used in the treatment of diseases, but they are still misused for disease prevention and as growth promoters [7,8].

Moreover, lower doses of antibiotics, especially those from the tetracycline group, are often used as growth promoters in domestic animal production [9]. There is a risk that antibiotic residues will be found in the pond environment, sediment and water and that they will consequently accumulate

in fish tissues [10]. In an integrated system, antibiotics, their residues, and antibiotic-resistant bacteria can enter the pond together with manure or via fish feed and are a potential source of antibiotic-resistant bacteria. The aim of the present research was to examine the impact of using slaughterhouse wastewater on the production of carp meat safe for human consumption in terms of antibiotic residue content.

2. Materials and Methods

A wastewater treatment system as part of the slaughterhouse was built within the Meat Industry in Pećinci. The used purification technology consists of a continuous system, biological type with activated sludge, with preliminary separation of coarse impurities and separation of fat. A fishpond was then built within the slaughterhouse, which is mostly supplied with purified water from the wastewater treatment system, but also with the addition of well water. The water from purifiers goes to pre-fishery where purified water is aerated, after which the water goes into a fishpond where a part of nutrients from purifiers is used for carp nutrition. The water is then used to irrigate the land around the slaughterhouse. In this way, the pond has an impact on increasing the quality of wastewater and reaching the prescribed limit values before being discharged into the natural recipient.

Carp fingerlings in good health were stocked in the fishpond. Carp was produced in optimal ambient conditions by using purified water originating from slaughterhouses and by adding well water. The monitoring of the presence of residues of antibiotics and sulphonamides in sediment, water, and fish was performed together with an assessment of the safety of the carp produced in this way for human consumption. Sampling was carried out in spring (April) and autumn (October).

Water sampling was carried out at six points, namely: water from the purifier, water from the outflow from the purifier, water from the fishery pond, water from pond 1, water from pond 2, and water from the irrigation channel. Sediment sampling was carried out according to the standard procedure, and an Ekman dredger was used to collect surface sediment.

Fish harvesting was carried out by dragging the net on the pond. Seven individuals of common carp were sampled in each sampling. The fish samples were placed in sterile plastic bags and delivered to the laboratory in the shortest possible time. The fish were sacrificed by a quick blow to the head.

Determination of residues of antibacterial substances in water, sediment, and carp meat

The presence of residues of antibacterial substances in carp meat was first determined by a screening method for the detection of antibacterial substances in fresh meat using a modified four-plate method. The disk diffusion microbiological inhibitory test was performed by using three microbiological plates containing *Bacillus subtilis* at different pH values (6, 7.2, or 8) and one plate with *Kocuria rhizophila*.

To confirm the obtained results, the determination of the presence of sulfonamide and tetracycline residues in fish meat was performed by using liquid chromatography (HPLC - FLD). Determination of sulfonamides was performed according to a documented method based on: Determination of sulfonamides Residues in Chicken Muscle by Agilent Bond Elut QuEChERS AOAC Kit HPLC, Application Note, Food Safety. Determination of tetracyclines was performed according to a documented method based on: Determination of Tetracyclines in Chicken by Solid-Phase Extraction and High-Performance Liquid Chromatography, Application Note, Food Safety. Analyzes were performed on an HPLC system, Thermo Ultimate 3000 (Thermo Fisher Scientific), equipped with a photodiode and fluorescence detector (FLD). The HPLC system is controlled by "Chromeleon" software.

3. Results and Discussion

The content of contaminants in fish tissues was observed from two aspects, namely ecological and from the aspect of safety of fish as food. When we consider the content of contaminants in meat and take into account the hygienic safety aspect, in most countries their content is legally defined. Based on the content of contaminants in edible fish tissue, the healthiness of fish meat is assessed, i.e.

satisfaction with the conditions prescribed by regulations. The results of water quality sampled from several points within the slaughterhouse facilities clearly show the efficiency of the purifier and the integrated system. The results of testing the tetracycline and sulfonamide content in water and sediment samples presented in Tables 1 and 2 showed that the measured values were below the method detection limit. The limit of detection for determining tetracycline in water and sediment was 0.01 mg/kg, and the limit of detection for determining sulfonamides was 0.005 mg/kg. The results of testing the content of antibiotics, tetracycline, and sulfonamides in carp meat samples showed that the measured values were below the limit of detection of the applied methods. Complete inhibition of growth on the agar surface around the disk with an inhibition zone of 4 mm on one or more plates indicates a positive result, which was not the case in this study, and on all plates, the inhibition zone was less than 4 mm indicating that there were no presence antibiotic residues in the examined samples of carp meat neither in spring nor in autumn. The negative results were also confirmed by instrumental analysis (HPLC-FLD) and it was determined that the concentration of sulfonamides in the tested samples of fish meat was lower than the detection limit (0.005 mg/kg), as well as that the concentration of tetracycline was lower than the detection limit (0. 01 mg/kg).

Table 1. The residue of tetracycline and sulfonamides in water samples, (mg/kg).

Parameter	Purifier	Outflow from purifier	Prefishery-pond	Pond 1	Pond 2	Irrigation channel
Tetracycline	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Sulfonamides	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

Table 2. The residue of tetracycline and sulfonamides in sediment and fish samples, (mg/kg).

Parameter	Prefishery-pond	Pond 1	Pond 2	Irrigation channel	Common carp samples
Tetracycline	<0.01	<0.01	<0.01	<0.01	<0.01
Sulfonamides	<0.005	<0.005	<0.005	<0.005	<0.005

Fish ponds are most often supplied with water from open-surface water bodies and using well water, while integrated systems use wastewater from farms, treated municipal wastewater, and cooling water from thermal power plants. The previous practice of linking fishing and farm animal production has shown good results both in terms of the full use of resources and the reduction of environmental pollution by recycling farm wastewater before it is discharged into the recipient. The results obtained by Koeypudsa et al. [11] in controlled conditions showed the potential for accumulation of chlortetracycline residues in fish and their environment when manure originating from chickens containing chlortetracycline was used for fertilization of ponds. Tetracyclines are distributed in tissues and can be found in high concentrations in the excretory organs, as well as in the liver and bile, and are eliminated from the body by renal excretion without changing their chemical structure [12]. Environmental conditions of the pond, such as transparency, water quality, and soil quality, i.e. pond bottoms can affect the concentration of antibiotic residues in pond water [13]. The temperature of the water, as well as the pH value, affect the rate of elimination of antibiotics from fish tissues. Rigos et al. [14] proved that antibiotics are eliminated faster from fish tissues if the water temperature is higher. The danger of the spread of antibiotic resistance in integrated production systems must not be ignored either. According to the results obtained by Petersen et al. [15] integrated production systems can contribute to the development of antibiotic-resistant microorganisms in the pond environment. This is contributed by the selective pressure of antibiotics in the pond and/or the introduction of resistant microorganisms from the manure into the pond. The same authors established the occurrence of resistance to chloramphenicol, ciprofloxacin, erythromycin, gentamicin, oxytetracycline, trimethoprim, sulfamethoxazole, and streptomycin in

indicator microorganisms isolated from pond sediment from different integrated production systems. To date, there are no data related to the presence of antibiotic residues in an integrated system in which fish are grown in wastewater originating from slaughterhouses, but there is certainly a risk that antibiotic residues from slaughterhouse waste will be found in pond sediment, water, and in fish grown in such a system.

Antibiotics are among the best-known and most common chemical pollutants that enter the environment and, consequently, the food chain. They are primarily used to suppress the growth of microorganisms, but they have also been used for a very long time as growth promoters in various types of domestic animals. The uncontrolled use of these substances has led to the fact that today they represent a significant danger to the environment and public health. The use of antibiotics and sulfonamides is strictly regulated by law in most countries. Residues of antibiotics can be found in all types of food of animal origin and after their use for therapeutic purposes, as they are deposited in various tissues of treated animals. One of the dangers of using antibiotics is the widespread occurrence of antimicrobial resistance among different bacterial strains that can be found in different animals, including fish [16].

The obtained results are in agreement with the results obtained by Đinović et al. [17]. These authors investigated the presence of veterinary medicaments in the edible tissues of carp caught from Ečka pond. The maximum allowed amount of sulfonamides in food of animal origin is 0.1 μg/g. Also, the maximum allowed residue level (MRL) for antibiotics and sulfonamides is prescribed in our country in articles 11 and 12 of the "Regulation on the quantities of pesticides, metals, metalloids, and other toxic substances, chemotherapeutics, anabolics and other substances that can be found in foodstuffs: (Official Gazette of the SRJ", 5/1992, 11/1992 (correction). It is also a well-known fact that intensive production of fish in ponds can be conducive to the rapid spread of infections, and that antibiotics are often used in aquaculture by adding them to fish feed. As a consequence of the mentioned treatment, there is an increase in the concentration of antibiotic residues in the water and the sediment [18]. Björklund et al. [19] examined the presence of residues and persistence of oxytetracycline in wild fish and sediment from two ponds, after the treatment of fish with this antibiotic. In wild fish, they observed that oxytetracycline residues could be detected 13 days after the end of the therapy.

The half-life of oxytetracycline in pond sediment was 9 and 419 days in the two investigated ponds. This indicates that oxytetracycline can be very persistent in pond sediment. It is important to note that in the pond that was the subject of research in this work, there was no outbreak of disease and antibiotics were not used, so the result obtained according to which the amounts of antibiotic residues in water, sediment, and fish were below the detection limit of the applied methods was completely expected. In addition, the slaughterhouse regularly monitors the presence of antibiotic residues in meat and products, and the antibiotic residues were below the detection limit in the wastewater in the slaughterhouse. There are very few studies related to the presence of antibiotic residues in wastewater from slaughterhouses. Chang et al. [20] tested wastewater samples from hospitals, kindergartens, slaughterhouses, and wastewater treatment plants in China for the presence of antibiotic residues. The authors found that the slaughterhouse, although to a much lesser extent than the hospital, can be a significant polluter when it comes to antibiotics, and also that a large number of tested antibiotic residues from the slaughterhouse were below the detection limit. Cavenati et al. [21] examined wastewater from a slaughterhouse in Portugal for the presence of residues of minocycline, oxytetracycline, tetracycline, enrofloxacin, and ceftiofur. They determined the presence of residues of tetracycline (≤ 15μg/L) and enrofloxacin (<2 μg/L).

With the ever-increasing growth of the world's population and rapid industrialization, there has been an increase in freshwater pollution due to inadequate discharge of wastewater, which is particularly significant for underdeveloped and developing countries and represents an increasingly serious global problem today [2,22]. Compulsory treatment of wastewater from the slaughterhouse is extremely important for its safe and sustainable release into the environment. The discharge of untreated or insufficiently treated wastewater is very common in developing countries and can therefore have extremely harmful effects on the environment. In addition to the extremely high

concern for public health, we should not ignore the economic profit, which is achieved through adequate treatment and proper discharge of wastewater.

4. Conclusions

Determining antibiotic residues in foods of animal origin is very important and mandatory to protect the health of end consumers and prevent the spread of antibiotic resistance. Continuous monitoring of the presence of residues in environmental samples is also very important from the point of view of environmental protection and, consequently, the protection of both animal and human health. The obtained results showed that carp produced in a pond supplied with treated wastewater from a slaughterhouse is safe for human consumption in terms of the presence of tetracycline and sulfonamide residues.

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