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Impact of Freezing Intervals on Oxytetracycline and Ciprofloxacin Residues in Nile Tilapia and Catfish Muscles

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Abstract

ANTIBIOTICS are commonly used in aquaculture to treat or prevent bacterial infections, which may lead to residues in fish tissue when administered improperly. Ciprofloxacin (cip) and oxytetracycline (OTC) residues in experimentally treated Nile Tilapia (*Oreochromis niloticus),* and African catfish (*Clarias gariepinus),* muscles were analyzed to determine the effects of freezing at -18 °C for four months, and analyzed monthly. After freezing for one, two, three, and four months the reduction percent of OTC residue levels in Nile Tilapia were 26.4, 51.9, 76.1, and 76.1%. While in African catfish were 37.2, 64.4, 100 and 100, respectively. Freezing at -18°C Throughout one to four months, Nile Tilapia muscle cip residue concentrations reduced from 30.9 ± 3.3 to 8.24 ± 1.6 ppb, with a reduction percentage of 27.6, 58.8, 80.7, and 80.7. In African catfish tissue, reduction percent were 24.3, 49.4, 72, and 72 after freezing for one, two, three, and four months respectively. Therefore, it is highly recommended to freeze fish before serving it to humans to avoid the possibility of antibiotic residues.

Keywords: Antibiotic residues; Ciprofloxacin; Fish; Freezing; Oxytetracycline, Nile tilapia, Catfish.

Introduction

Fish account for around 17% of animal protein consumption and offer vital amino acids, omega-3 fatty acids, minerals, and vitamins [1, 2]. Nile tilapia is the main species in Egypt's aquaculture industry and according to 2019 statistics, tilapia yield represented 66% of the country's farmed fish production with a total productivity of 1,051,444 tons [3]. While the total amount of African catfish production, which is one of the important freshwater fish farmed in Egypt [4], was 6,836 tons [5].

Intensive farming practices impose pressure on cultured aquatic species, weakening their immunological systems against bacterial and viral diseases, leading to high rates of morbidities and mortalities [6], which necessitate the use of antibacterial medications to manage and treat fish infections. Tetracyclines (TCs) are commonly used antibiotics in Egypt and other African nations due to their widespread availability, low cost, broad spectrum, and effectiveness [7and 8]. Ciprofloxacin belongs to the fluoroquinolone antibiotic category, which is effective against a broad spectrum of Gram-negative and Gram-positive bacteria [9].

Overuse of antibiotics in aquaculture can lead to the build-up of drug residues in fish tissue, resulting in human health risks like; antibiotic resistance, allergic reactions, and various severe pathologies [10, 11and 12]. It is important to adhere to the withdrawal period and maximum residue limit (MRL) for each veterinary medications to prevent adverse consequences [13]. Antibiotic residue detection was conducted on raw animal products without considering any alterations post-processing. Thermal treatments such as heating or freezing alter the chemical composition of medications through degradation [10]. Freezing is one of the most popular and efficient methods of food preservation by inhibiting the growth of microorganisms and enzymatic activity [14, 15]. This study aims to investigate the effect of freezing at -18 °C for four months on oxytetracycline (OTC) and ciprofloxacin (cip) residues in muscles of Nile tilapia and African

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Catfish. Freezing was effective method to degrade ciprofloxacin and oxytetracycline residues.

Material and Methods

The experimental design

A controlled experiment was carried out to investigate the effect of freezing on OTC and cip residues in the musculature of Nile tilapia and African catfish. Nile tilapia weighing 100 ± 5g and catfish weighing 150 ± 10g were obtained from a private fish farm. Individually, they were housed in 500 L fiberglass tanks at the Aquatic Animal Medicine Department, Faculty of Veterinary Medicine, Benha University, Egypt. The fish were kept for approximately one month and underwent regular daily partial water changes to guarantee that they were free from antibiotics. The animal use care was taken in consideration while conduction the experiment following the guideline and periodical monitoring of the committee of Animal ethical use, where they provided the experiment an approval (BUFVTM 07-07-2023).

Fish were then divided into 6 groups as follow:

•Group 1 (Control): Nile tilapia fed on basal diet without any antibiotics.

•Group 2 (Ciprofloxacin): Nile tilapia [n = 10/aquarium) in triplicate receiving ciprofloxacin (Cip) medicated feed.

•Group 3 (Oxytetracycline): Nile tilapia [n = 10/aquarium) in triplicate receiving oxytetracycline (OTC) medicated feed

•Group 4 (Control): catfish (n = 7/aquarium) in triplicate fed on basal diet without any antibiotics.

•Group 5 (Ciprofloxacin): catfish (n = 7/aquarium) in triplicate receiving oxytetracycline medicated feed.

•Group 6 (Oxytetracycline): catfish (n = 7/aquarium) in triplicate receiving cip medicated feed.

Fish received a basal diet with OTC supplementation at a dosage of 100 mg/ kg feed [16]. The fish of all groups were fed 3% from the total biomass regularly 3 times daily for 10 days [17].

•The OTC-feed was prepared according to Julinta et al., (2019) [16]. Briefly, 4 g OTC (oxytetracycline 250 mg, CID, Egypt) was thoroughly mixed with 5 mL vegetable oil. The oil-OTC mixture was then added to one kg of pellet feed and air-dried. The feed without OTC was fed to the control group.

•Cip-feed was prepared following Hal et al., (2021) [18] by mixing 200 mg/kg feed of cip (100% concentration, SANTA CRUZ Company) with vegetable oil, which was then mixed with the diet. At the end of the experiment, OTC and cip residues were determined in each of Nile Tilapia and African catfish samples of all the six groups (Zero time).

Effect of freezing

Fish musculature at zero time were collected, freeze at -18 ºC and examined monthly to determine the effect of freezing on the concentration of antibiotic residues.

Method of quantitation of known antibiotic residues (Oxytetracycline and Ciprofloxacin) according to USDA, FSIS (2011) [19]:

Sample Preparation and Storage:

Tissues were manipulated to minimize the occurrence of freezing and thawing. The muscle tissue samples were removed and placed in bags that were tagged to identify each sample.

Samples and sample extracts must be kept cold at all times with allowances for brief excursions at room temperature during processing and testing. The laboratory should take measures to ensure good housekeeping

Use Tekmar filter bags, Whirl-Pak bags, or equivalent. Label a bag with the sample identification, and buffer pH. Four bags will be required.

Dice tissue into 0.5 cm pieces or homogenize a large enough portion of the sample to complete all anticipated tests. Use a blender (or similar apparatus) to homogenize the samples. The homogenized tissue portion should be frozen. If possible, retain an intact portion of the frozen tissue as a sample reserve.

Weigh sample portions into the four, labeled bags. Weigh 10 0.2 g of tissue (avoid fat) into each bag. Keep the sample to buffer ratio at 1 part sample to 4 parts buffer.

Ten grams of the tissues were cut into 0.5 cm pieces. Then, 40± 1.0 ml of the suitable buffer was poured into each bag and mixed using a blender for 60 seconds. After undergoing stomaching or mixing, the tissue was left undisturbed for a minimum of 45 minutes before being used. Each plate was loaded with a precise volume of 200± 4µl. During the screen test, a different antibiotic reference standard (SRs) was used in one well each day. Plate 1 contained tetracycline, whereas Plate 4* included ciprofloxacin. A volume of 200± 4µl of the SR concentration was transferred into the test well using a pipette. After examining the testing samples, Sensi-Discs can be substituted for reference dilutions on each plate.

Plates 1 and 4 were placed in an incubator at a temperature of 29± 10C for a duration of 16 to 18 hours. Following the incubation period, the presence or absence of zones of inhibition on the plates was documented.

Calculating the concentration of antibiotic residue in tissue:

The diameters of the zones on the test plates were compared to the standard curve in order to determine the amounts of residues in the test sample.

Statistical analysis

Mean, minimum, maximum and standard deviation were calculated for the replicates of each sampling points.

Results

Table (1) showed the mean concentrations of OTC residues in Nile tilapia at Zero time and after four months, which were 23.1 ± 1.67 and 5.53 ± 0.88 ppb respectively, while cip residues at Zero time and after four months were 42.68 ± 3.3 and 8.24 ± 1.6 ppb respectively.

In catfish, the mean concentrations of OTC residues at Zero time was 14.34 ± 0.62 ppb and after four months was not detected. While the mean concentrations of cip residues at Zero time and after four months were 52.8 ± 1.7 and 14.8 ± 1.1 ppb respectively as mentioned in Table (2)

Reduction percent of OTC and cip residues in Nile Tilapia musculature samples after the fourth month of freezing were 76.1 and 80.7 respectively as shown in table (3).

Moreover, table (4) cleared that the Reduction percent of OTC residue in catfish samples after the fourth month of freezing was 100.0 while the Reduction percent of cip residue was 72.0 after the third month of freezing.

Discussion

In the previous study, the survey was applied to elucidate the antibiotic residue in farmed fresh and marine fishes [20], and the findings revealed that 4.67% (seven samples) of the investigated samples had detectable antibiotic residues, whereas 95.33% (143 samples) exhibited no evidence of antibiotic presence. Therefore, it was important to evaluate the impact of freezing on the residue of antibiotics, particularly oxytetracycline and ciprofloxacin.

Freezing is a method used to extend the shelf life of fish and fish products. The process of preservation maintains the taste and nutritional characteristics of food while also reducing the growth of microorganisms and enzymes [21]. Tetracyclines are a broad category of antibiotics that encompass tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline [22]. Oxytetracycline (OTC) is a commonly used broad-spectrum antibiotic in fish farms for the treatment of bacterial infections [23].

In the present study, freezing of fish significantly reduced OTC residues in the muscle of both Nile tilapia and African catfish. Similar results were recorded [24], who found more tetracycline residues in the fresh fish samples that were tested than in the frozen fish samples ($P < 0.05$). In contrast, there was no noticeable effect on TC stability from preserving fish samples frozen at -18 °C for a week in the study conducted by Ahmed et al. (2020) [25]. While, Vivienne et al., (2018) [26] observed that the concentration of OTC residue did not change by freezing at -10 °C after 9 days of storage, this may be due to the differences in the fish species, freezing temperature and the freezing time.

The reduction percentages of OTC residues in Nile tilapia after freezing for four consecutive months were 26.4, 51.9, 76.1, and 76.1 % respectively. Similarly, freezing catfish muscle samples at -18 °C resulted in reduction percentages of OTC of 37.2, 64.4, 100, and 100% for the first, second, third and fourth months respectively. The reduction percentages observed in this study were greater than those reported by Shaltout et al. (2019) [14], who found reductions of 2.05 and 32.38% in OTC levels after preserving chicken slices in a deep freezer at -20 °C for 6 months and 12 months, respectively. In addition, a reduction in OTC residues of frozen beef by 30 and 65% after being frozen for two and four months respectively was reported by Mgonja (2017) [27], that was also less than our results.

Ciprofloxacin is a fluoroquinolone antibiotic derived by the addition of a fluorine atom to the quinolone molecule [28]. Fluoroquinolones (FQs) are effective antibacterial drugs that are particularly useful due to their ability to target a broad spectrum of both Gram-negative and Gram-positive bacteria, as well as mycoplasma. They achieve this by inhibiting the DNA gyrase enzyme, which is necessary for bacterial chromosome replication. Additionally, FQs have the advantage of being easily absorbed orally and distributed throughout the body [29].

In the present study, the impact of freezing at −18 °C on Ciprofloxacin residues, in Nile tilapia and catfish samples, for four successive months were determined. The reduction percentages of cip residues in Nile tilapia after freezing for four consecutive months were 27.6, 58.8, 80.7, and 80.7% respectively. Similarly, freezing catfish muscle samples at -18 °C resulted in reduction percentages of cip by 24.3, 49.4, 72.0, and 72.0% respectively. The findings are consistent with the study conducted by Kryuchkovaet al. (2016) [30], which revealed that the concentration of ciprofloxacin decreased by 20% and exceeded 50% in the muscle tissue of rainbow trout and common carp, respectively, after being stored at a temperature of -18 °C for 15 days and three months, respectively. Freezing chicken slices at -20 °C for six months resulted in a 62.63% reduction in ciprofloxacin residues; and after 12 months of

preservation, the residues were entirely eliminated [14]. While, Mohamed (2019) [31] found that freezing chicken flesh samples at -20 °C had a minimal impact on the breakdown of ciprofloxacin residues, with reduction percentages were 4.7% in breast tissue, 3.9% in thigh tissue, and 5.8% in liver tissue.

Conclusion

The data from our study show differences between Nile tilapia and catfish in the depletion time of OTC and Cip after storage of muscle samples at -18 °C. Storage of tilapia musculature for four months resulted in higher reduction percentages of residues of Cip than that of OTC. On the contrary, in catfish the reduction percentages of residues of OTC were higher than Cip and it was completely disappeared after three months of storage.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; BUFVTM 07-07-2023).

TABLE 1. Concentration (ppb) of Oxytetracycline and Ciprofloxacin residues in Nile tilapia musculature samples at Zero time and Fourth month after freezing at -18 °C.

AB		Oxytetracycline	Ciprofloxacin
	Max	33.4	64.7
Zero time	**Min**	14.1	32.7
	Mean	23.1	42.68
	± SE	± 1.67	± 3.3
After 4th month	**Max**	14.1	8.2
	Min	-	3.2
	Mean	5.53	8.24
	± SE	± 0.88	±1.6

Min: Minimum Max: Maximum
SE: Standard Error AB: Antibiotic

TABLE 2. Concentrations (ppb) of Oxytetracycline and Ciprofloxacin residues in African catfish musculature samples at Zero time and Fourth month after freezing at -18 °C.

AB		Oxytetracycline	Ciprofloxacin
	Max	15.9	57.8
Zero time	**Min**	12.2	45.2
	Mean	14.34	52.8
	± SE	± 0.62	± 1.7
After 4th month	**Max**	ND	20
	Min	ND	11.6
	Mean	ND	14.8
	± SE	ND	1.1

Min: Minimum Max: Maximum
SE: Standard Error AB: Antibiotic

TABLE 3. Reduction % of the Reduction % of the Oxytetracycline and Ciprofloxacin residues in African catfish musculature samples to the effect of freezing at -18 °C.

Type of residue	Zero time	%	After 1st month	Reduction %	After 2nd month	Reduction %	After 3rd month	Reduction %	After 4th month	Reduction %
OTC	23.1	100	17	26.4	11.1	51.9	5.53	76.1	5.53	76.1
Cip	42.68	100	30.9	27.6	17.6	58.8	8.24	80.7	8.24	80.7

TABLE 4. Oxytetracycline and Ciprofloxacin residues in Nile tilapia musculature samples to the effect of freezing at-18 °C

Type of residue	Zero time	%	After 1st month	Reduction %	After 2nd months	Reduction %	After 3rd months	Reduction %	After 4th months	Reduction %
OTC	14.34	100	9	37.2	5.1	64.4	ND	100	ND	100
Cip	52.8	100	40	24.3	26.7	49.4	14.8	72	14.8	72

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تاثير فترات التجميد المختلفة على بقايا أوكسي تيتراسيكلين وسيبروفلوكساسين في عضلات أسماك البلطى النيلى والقرموط الافريقى

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الملخص

عادة ما تستخدم المضادات الحيوية لعلاج أو منع االامراض البكتيرية التى تصيب الاسماك فى الاستزراع السمكى ، التي قد تؤدي إلى متبقيات في أنسجة الأسماك عندما تتستخدم بطريقة غير سليمة. وقد تم تحليل العضلات لتحديد آثار التجميد عند -18 درجة مئوية لمدة أربعة أشهر، وتحليلها شهرياً. ولذلك لتحديد تأثير التجميد على متبقيات كلا من الاوكسى تيتراسيكلين والسيبروفلوكساسين المترسبة بعضلات اسماك البلطي النيلي واسماك القرموط الأفريقي. وبعد التجميد لمدة شهر واحد أو اثنين أو ثلاثة أو أربعة أشهر، انخفضت مستويات رواسب الأوكسي تيتراسيكلين الموجودة في عضلات البلطي النيلي بمقدار 26.4 و51.9 و76.1 و76.1 في المائة. بينما انخفضت متبقيات أوكسى تتراكسلين في أسماك القرموط الأفريقي بعد شهر واحد بمقدار 37.2% وبعد شهرين انخفضت الى 64.4% ولم يكن من الممكن الكشف عنها بعد 3 و4 أشهر من التجميد. وعلى مدى شهر إلى أربعة أشهر عند -18 درجة مئوية، انخفضت تركيزات بقايا السيبروفلوكساسين في عضلات البلطى النيلى من 30.9 ± 3.3إلى 8.24± 1.6 جزء في المليون، مع انخفاض بنسبة مئوية قدرها 27.6 و58.8 و80.7 و80.7 وفي عضلات القرموط الافريقى، انخفضت التركيزات الى النسب المئوية 24.3، و 49.4، و 72، و 72.وذلك بعد تجميدها لمدة أربعة اشهر متتالية . ولذلك، يوصى بشدة بتجميد الأسماك قبل تقديمها إلى المستهلك لتجنب احتمال وجود بقايا المضادات الحيوية.

الكلمات الدالة: المضادات الحيوية ، الأسماك ، الاوكسى تيتراسيكلين ، سيبروفلوكساسين ، متبقيات الادوية، التجميد، البلطى النيلى ، اسماك القرموط.