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Effect of a Peracetic Acid-Based Disinfectant on Growth, Hematology and Histology of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The effects of a peracetic acid-based disinfectant product (Aquastart®) were assessed on some hematological parameters, histological aspects and skin bacterial load of rainbow trout, likewise bacterial load of the rearing tank water. A total of 180 healthy rainbow trout weighing 124.65 ± 10 g were divided into two groups, each in three replicates in flow-through tanks. First group was exposed to Aquastart at 8.9 ppm for 30 min and second group was considered as the control. The fish were then reared for 60 days prior to sampling for hematological and histological studies. The lowest bacterial load level in both water columns and trout skin were observed in the treated trout ($p < 0.05$). Meanwhile, no significant impact on growth performance was recorded between treated and control fish. The immunocompetent cells population size in control fish were significantly lower than treated fish ($p < 0.05$). Histologically, no evidence of abnormality was seen in the gills, kidney, and liver tissues of treated fish. These results showed that application of Aquastart at 8.9 ppm is safe for use in flow-through tanks farming rainbow trout.

Keywords: rainbow trout; hematology; histology; disinfection; peracetic acid

1. Introduction

In the last two decades, unlike landings from capture fisheries, production of farmed fish and shellfish has remarkably increased. Meanwhile, global production of rainbow trout (*Oncorhynchus mykiss*) was reported to be about 762,000 tonnes in 2015 [1] with 144,000 tonnes being produced in Iran. Effective control of infectious diseases using environmentally safe disinfectants is a critical need in aquaculture. Biosecurity in aquaculture is essential to reach a high performance and this can be feasible using some disinfectants to eliminate a wide range of potential pathogens from the equipment, tanks and water column. Since the water resources are also used by other aquatic and domestic animals as well as agricultural activities, the application of disinfectant in water bodies faces some restrictions [2]. Antiseptic agents in aquaculture can be accepted if they meet maximum efficiency plus minimum impact on fish health and the environment [3].

Peracetic acid (PAA) is produced by a reaction between hydrogen peroxide and acetic acid and considered as an ideal antimicrobial agent due to its high oxidizing potential and fat solubility [4–6]. PAA is approved by the Food and Drug Administration (FDA) for sanitizing and disinfection of food processing facilities and direct contact with food, and European approved for use in aquaculture. It becomes popular in aquaculture due to wide-ranging of antimicrobial effects, non-toxic degradation by-products and low environmental side effects [7–9]. In contrast, some hazardous and unsustainable common antimicrobial disinfectants (e.g., potassium permanganate, copper sulphate, chloramine and formaldehyde) can lead to markedly respiratory irritants and carcinogenic by-products in terms of worker safety and also having potential negative environmental impact on the receiving

water body. Wofasteril® and MinnFinn™ solutions are available commercial PAA-based products and have been studied for controlling of white spot disease pathogen [10,11] as well as other freshwater fish pathogens [5,12,13]. In recirculating fish-microalgae system, Liu et al., [14] revealed that commercial PAA-based products with a low H_2O_2/PAA proportion at PAA concentrations up to 2.0 ppm can apply as a safe disinfectant with no adverse effect on microalgae growth behavior. Improving of mirror carp (*Cyprinus carpio*) health status with PAA-based disinfectant was reported [15].

Aquastart product is a commercial aqueous PAA-based product which contains equilibrium mixture of 4% PAA (1 ppm), 10% acetic acid and 23% hydrogen peroxide according to the manufacture material safety data sheet (MSDS) and currently used in aquaculture sectors in Iran. In our previous study, the toxicity of this commercial disinfectant was evaluated in rainbow fry under in vitro condition [16]. Although, many studies reported the efficiency of PAA-based disinfectant products in aquaculture, but it is crucially important to precisely assess the possible side effects of this disinfectant in farmed fish. Therefore, this study was aimed to assess some hematological, histological and growth performances of exposed juvenile rainbow trout to sub-lethal concentration of Aquastart.

2. Results

2.1. Bacterial Load in Fish Skin and Water Column

The results of microbial load in the cultured tank water and skin of fish are given in Table 1. Total bacterial load in the water column significantly ($p < 0.05$) decreased in the PAA-treated tank from $2.0 \times 10^6 \pm 1.1 \times 10^4$ to $1.2 \times 10^4 \pm 4.2 \times 10^2$ colony-forming units (CFU)/mL compared to the control group. Also, total bacterial load on fish skin significantly ($p < 0.05$) decreased in the treated fish from $8.5 \times 10^3 \pm 3.4 \times 10^2$ to $7.5 \times 10 \pm 3.5 \times 10$ CFU/cm^2.

Table 1. Effect of the application of 8.9 ppm of Aquastart for 30 min in the total number of aerobic bacteria present in the water column of fish tanks and skin of juvenile rainbow trout.

Experimental Group	Time	Microbial Population	
		Water Column (CFU/mL)	Fish Skin (CFU/cm^2)
Control	Before exposure	$8.3 \times 10^3 \pm 1.3 \times 10^2$ [a]	$2.4 \times 10^6 \pm 2.2 \times 10^4$ [a]
Treated trout	Before exposure	$6.8 \times 10^3 \pm 6.6 \times 10$ [c]	$2.0 \times 10^6 \pm 1.1 \times 10^4$ [b]
Control	After exposure	$8.5 \times 10^3 \pm 3.4 \times 10^2$ [a]	$2.5 \times 10 \pm 2.2 \times 10^4$ [a]
Treated trout	After exposure	$7.5 \times 10 \pm 3.5 \times 10$ [d]	$1.2 \times 10^4 \pm 4.2 \times 10^2$ [c]

Mean (±standard deviation) values in the same column with different letters are significantly different ($n = 3$, $p < 0.05$). CFU: colony-forming units.

2.2. Growth Performances

Results of growth factors are shown in Table 2. There was no significant difference in the growth factors between treated and control fish ($p > 0.05$). However, the survival rate of treated fish was significantly higher than control group ($p < 0.05$).

Table 2. Growth and survival of rainbow trout treated with 8.9 ppm of Aquastart for 30 min after 60 days.

Experimental Group	*FCR*	*SGR* (%)	*BWG* (%)	*CF* (%)	*SR* (%)
Control	1.43 ± 0.77	0.745 ± 0.01	29.97 ± 1.09	1.22 ± 0.14	53.33 ± 1.92 [b]
Treated trout	1.52 ± 0.13	0.723 ± 0.09	28.47 ± 1.12	1.13 ± 0.13	70.00 ± 1.33 [a]

Mean (±standard deviation) values with different letters are significantly different ($n = 15$, $p < 0.05$). *FCR*: feed conversion ratio; *SGR*: specific growth rate; *BWG*: body weight gain; *CF*: condition factor; *SR*: survival rate.

2.3. Hematological Findings

The results of hematological indices are shown in Table 3. The level of hematocrit, hemoglobin, mean corpuscular hemoglobin and white blood cells were significantly ($p < 0.05$) increased in PAA-treated rainbow trout. Counts of white blood cell in treated fish were significantly ($p < 0.05$) higher than control fish after 30 min exposure as well as end of the trial. Also, the population cells size of lymphocytes in treated fish was significantly ($p < 0.05$) higher than the control group.

Table 3. Hematological indices of rainbow trout treated with 8.9 ppm of Aquastart for 30 min after 0 and 60 days.

	Control	Treatment after 30 min Exposure	After 60 Days	
			Control	Treated Trout
HCT (%)	30.45 ± 0.71 ^{d}	40.40 ± 0.85 ^{a}	32.45 ± 0.63 ^{c}	38.50 ± 0.71 ^{b}
Hb (g/d)	9.82 ± 0.25 ^{d}	13.47 ± 0.28 ^{a}	10.82 ± 0.21 ^{c}	12.83 ± 0.24 ^{b}
RBC (10^6 cells/mL)	4.91 ± 0.13 ^{d}	6.73 ± 0.14 ^{a}	5.41 ± 0.11 ^{c}	6.42 ± 0.12 ^{b}
MCV (fL)	59.75 ± 0.35 ^{a}	59.78 ± 0.32 ^{a}	59.75 ± 0.35 ^{a}	59.75 ± 0.35 ^{a}
MCH (pg)	19.80 ± 0.00 ^{b}	20.00 ± 0.00 ^{a}	20.00 ± 0.00 ^{a}	19.95 ± 0.07 ^{a}
MCHC (g/dL)	32.97 ± 0.52 ^{a}	32.67 ± 0.94 ^{a}	33.17 ± 0.24 ^{a}	33.17 ± 0.24 ^{a}
WBC (10^6 cells/mL)	7.55 ± 0.64 ^{d}	12.50±0.85^{a}	8.60 ± 0.85 ^{c}	10.50 ± 0.71 ^{b}
Neut (%)	9.50 ± 0.71 ^{b}	4.60 ± 0.23 ^{d}	17.35 ± 0.78 ^{a}	8.50 ± 0.71 ^{c}
Lymp (%)	90.00 ± 1.44 ^{c}	94.50 ± 0.90 ^{a}	83.50 ± 0.87 ^{d}	92.50 ± 1.05 ^{b}
Mono (%)	1.50 ± 0.71^{a}	1.50 ± 0.71 ^{a}	N/A	N/A

Dissimilar letters in each row indicate significant difference (Mean ± standard deviation, $n = 9$, $p < 0.05$). HCT: hematocrit; Hb: hemoglobin; RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cells; Lymp: lymphocytes; Mono: monocytes; Neut: neutrophils; N/A: not available.

2.4. Histological Findings

A moderate hyperplasia was seen in gills of PAA-treated fish after 30 min exposure (Figure 1). However, no abnormality was observed in gills samples of both treated and control fish. Also, no tissue damage was observed in the liver (Figure 2). Slight dilation of the Bowman's capsule was observed in the kidney samples of some fish treated with Aquastart (Figure 3).

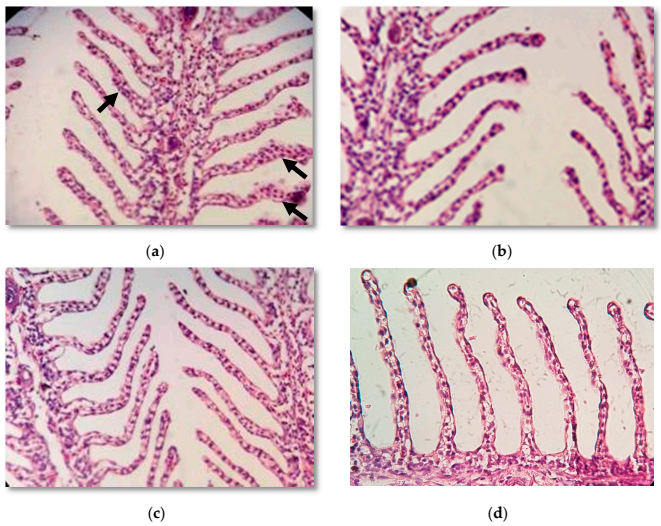

Figure 1. Effect of the application of Aquastart at 8.9 ppm for 30 min in the rainbow trout gills after 0 (**a**) and 60 days (**b**) of rearing, in comparison with the control group after 0 and 60 days of rearing (**c**,**d**, respectively) (hematoxylin and eosin (H&E), ×40). Arrows show the epithelial hyperplasia.

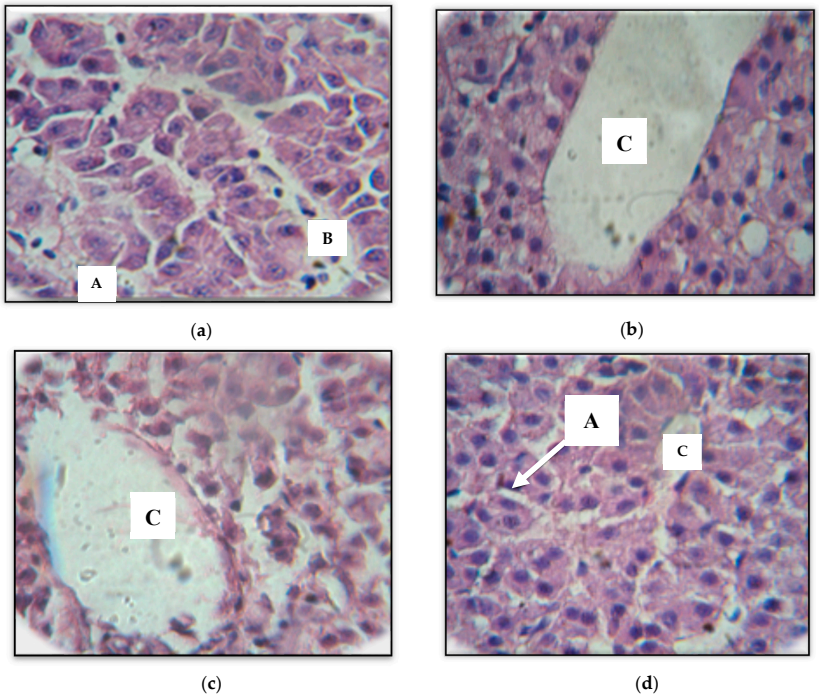

Figure 2. Effect of the application of Aquastart at 8.9 ppm for 30 min in the rainbow trout livers after 0 (**a**) and 60 days (**b**) of rearing, in comparison with the control group after 0 and 60 days of rearing (**c**,**d**, respectively) (H&E, ×100). A: Arteriole, B: Ruptured vessel, and C: Central vein.

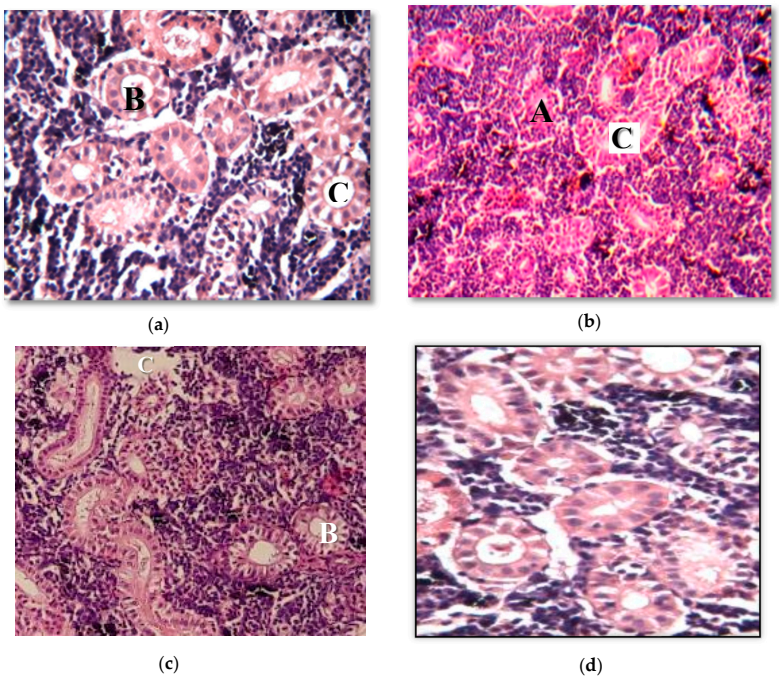

Figure 3. Effect of the application of Aquastart at 8.9 ppm for 30 min in the rainbow trout kidneys after 0 (**a**) and 60 days (**b**) of rearing, in comparison with the control group after 0 and 60 days (**c**,**d**, respectively) (H&E, ×40). A: Glomerulus, B: Complex proximal portion, and C: Complex distal portion.

3. Discussion

The microbial load of both fish skin and water column in treated trout were significantly lower than non-treated rearing water tank and fish skin. In agreement to our findings, Liu et al., [15] showed a

90% reduction of total aerobic bacterial load in PAA-treated culture water tanks by adding 1 mg/L PAA. These reductions in bacterial load are due to the strong oxidative activity of PAA [4], which leads to the destruction of the bacterial cell wall [6] and the inhibition of the initial bacterial colonization [17]. Therefore, such antibacterial effect is due to strong oxidation process by Aquastart.

Application of chemical disinfectants in aquaculture required a precise determination of safe dosage. In this study, no significance difference was seen in the growth factors (i.e., feed conversion ratio (*FCR*), specific growth rate (*SGR*), body weight gain (*BWG*) and condition factor (*CF*)) between treated and control fish, while the survival rate in treated fish was significantly higher than control fish. Liu et al., [18] reported that the stress induced by the PAA-based product Aqua Oxides at high concentrations depends on fish species, being the growth of rainbow trout unaffected.

Hemoglobin, mean corpuscular hemoglobin and mean corpuscular levels were significantly increased in PAA-exposed rainbow trout compared to control fish which is probably due to the oxidative stress of PAA in the exposed fish [15]. However, the level of these parameters was lowered 2 months post-treatment because of the adaptation of fish. PAA-exposed fish with Aquastart showed a higher WBC count compared to untreated fish. This means that Aquastart can stimulate some non-specific immune factors in fish resulting in a better condition for the fish to resist potential pathogens. Liu et al., [15] also described that the application of a PAA-based disinfectant increases the innate immune response of mirror carp kidney leukocytes. Moreover, an increase in the number of circulating lymphocytes was observed in rainbow trout treated with Aquastart. It is probable that optimal water quality resulted in a healthy situation that inhibited the exodus of lymphocytosis from the blood for the wound healing, inflammatory reactions and infection diseases caused by bacterial, viral, and parasite agents [19,20].

Rainbow trout treated with Aquastart for 30 min only showed initially a slight hyperplasia in the gill structure, but no abnormal changes were observed after 60 days of rearing. Also, no tissue damage was observed in the liver of trout exposed to a sub-lethal dose of Aquastart. In some cases, dilation of the Bowman's capsule was observed in the kidney of treated trout. Exposure to PAA-based disinfectant lead to slight hyperplasia in the interlamellar gill region of mirror carp [15]. It seems that such slight changes can be a natural reaction to a chemical substance and thus could be ignored. Therefore, histopathological results obtained in the present study demonstrated that Aquastart as a PAA-based disinfectant can be considered as a safe alternative disinfectant to be applied in fish farms and makes it a suitable choice for aquaculture.

4. Materials and Methods

4.1. Treatment of Fish

A total of 180 apparently healthy rainbow trout (*O. mykiss*) weighing 124.65 $\pm$ 10 g were obtained from a commercial fish farm in Ahmadabad Mostofi (Tehran, Iran), being divided in two experimental groups (control and treated trout) with three replicates. Each replicate contained 30 individuals in a circular tank (1000 L) with continuous flow-through system. Fish were adapted to the new conditions for 8 days prior to the experiment. Water was maintained during the trial at 15 $\pm$ 1 °C, >6 ppm of dissolved oxygen, <0.01 ppm of ammonia, and pH 7. Fish were fed with the commercial trout feed (Chineh, Tehran, Iran) at 3–5% of body weight per day, according to water temperature and fish biometry data. Uneaten feed was rarely observed in the tanks and siphoned out daily approximately 30 min after feeding. Feeding was stopped 24 h before the treatment of trout (Ramyar Shimi Company, Tehran, Iran) at 8.9 ppm [16] for 30 min. The application of Aquastart was based on the water flow in the rearing tanks. A control group without any exposure was also included. Fish were then reared in the same tanks for 60 days.

All the animal experiments complied with ethical standard procedures as determined by the ethical committee of Science and Research University in accordance with the guidelines for "care and use of animals for scientific purposes".

4.2. Growth Performance

The biometry was conducted every 15 days interval after 50% of the fish population in each tank were anaesthetised with extract of clove essential oil at 150 ppm. Growth parameters (*FCR*, *SGR*, *BWG*, and *CF*) were calculated using below equations [21]. Survival rate (*SR*) of the fish was also recorded during the trial.

$$FCR = feed\ consumption(g)/weight\ gain\ (g)$$

$$SGR\ (\%) = \left[\left(\frac{Ln(W_2) - Ln(W_1)}{t}\right)\right] \times 100$$

$$CF = W/L^3 \times 100$$

$$SR\ (\%) = (final\ number\ of\ fish/initial\ number\ of\ fish) \times 100$$

$$BWG\ (\%) = \frac{(W_2 - W_1)}{W_1} \times 100$$

where W_1 and W_2 are the initial and final body weight (g), respectively and L is the length (cm) and t is the number of days.

4.3. Hematological Assays

Blood samples were taken from the peduncle vein [22] of three fish in each tank after being anaesthetised with 150 ppm of clove oil one hour before, after treatment with Aquastart and 60 days post-treatment. Red blood cell (RBC) and white blood cell (WBC) count [23], hematocrit (HCT) and hemoglobin (HB) percentage [24], differential WBC count [22], mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) [25] were measured.

4.4. Quantification of Bacterial Load of Fish Skin and Water Column

One sample trout skin and water column were collected from each tank one hour before and after treatment with Aquastart. The bacterial load was then assessed using the method proposed by Haque et al., [26] with a slight modification. In summary, the water was collected from three different locations of the cultured tank in sterile glass bottles (50 mL) at a depth of 15–20 cm from the surface and were combined to make a homogeneous composite sample. Meanwhile, 1 cm^2 of pectoral trout skin was taken with a sterile scalpel and forceps and placed in a sterile container. Skin samples were then homogenized with 9:1 ratio of sterile saline solution (0.85% w/v of NaCl, Merck, Darmstadt, Germany) to the fish skin. An inoculum of 1 mL of each sample was then spread in duplicates in triptone soya agar (TSA, Merck) plates. After 48 h of incubation at 25 °C, aerobic bacteria were quantified, being the results expressed in terms of CFU/mL and CFU/cm^2 for samples of tank water and trout skin, respectively.

4.5. Histological Studies

Three tissue samples of kidney, liver and gills from each tank were collected after the disinfection treatment and after 60 days of rearing, following the methodology described by Cardiff et al., [27]. Tissue samples were fixed in Bouin's solution (Sigma-Aldrich, Steinheim, Germany) for 24 h prior to dehydration with ascending series of ethanol (70%, 96% and 99%), cleared in xylene and embedded in paraffin. Paraffin blocks were sectioned (5 µm thick) by using a Leica RM2255 (Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. The sections were stained with H&E stain (Merck) prior to examination under a light microscopy (Olympus BH-2, Tokyo, Japan).

4.6. Statistical Analysis

All statistical analyses were performed in the software package IBM SPSS (SPSS 20.0 for Windows; SPSS Inc., Chicago, IL, USA). Significant differences between control and treated trout after 0 and 60 days of rearing were assessed using a two-way analysis of variance (ANOVA). Post-hoc comparisons of means were carried out with Tukey's honestly significant difference (HSD) test. The level of significance used for all tests was $p < 0.05$. All charts and graphs were prepared with Microsoft Excel version 2013.

5. Conclusions

Bacterial load of rainbow trout skin and water columns was significantly reduced by the application of a bath of the peracetic acid-based disinfectant Aquastart at 8.9 ppm for 30 min. This treatment did not show a negative effect on growth and hematological indices of rainbow trout reared for 60 days post-exposure. No abnormal changes in gills, liver and kidney tissues of treated trout were also detected. Therefore, treatment with 8.9 ppm of Aquastart for 30 min can be considered as an effective and safe method for the reduction of bacterial load level in water columns and trout skin. However, further analyses are needed to evaluate the effects of Aquastart at higher and lower concentrations, contact time, different fish size and species.

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