Alternative prophylaxis/disinfection in aquaculture - Adaptable stress induced by peracetic acid at low concentration and its application strategy in RAS

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Abstract

The application of peracetic acid (PAA) at low concentrations has been proven to be a broad-functioning and eco-friendly prophylaxis/disinfection method against various fish pathogens. However, there is lack of knowledge on how to apply PAA in a recirculating aquaculture system (RAS), and whether the application of PAA at low concentration can affect fish welfare. In the present study, PAA was applied in a pilot-scale carp (*Cyprinus carpio*) RAS (comprised of a fish culture tank of 1 m³, a reservoir tank of 600 L and a filter complex of 400 L) every 3 or 4 days for 5 weeks, and the stress response of fish was monitored during every second PAA application by measuring cortisol in water. Results showed that the increase of water cortisol became less pronounced and the decrease of water cortisol occurred earlier after repeated applications of PAA, which indicates an adaptation of the stress response to PAA in the carp. Moreover, the mathematic model showed that the equal distribution of PAA in RAS was a slow progress, which depended on tanks size and flow rate. To avoid potential harm to the biofilter in RAS during PAA application, it's suggested that PAA should be applied only to the fish culture tank at a reduced flow rate.

Keywords

Water cortisol, solid phase extraction, stress, mathematic model

1. Introduction

The development of modern aquaculture trends toward increasing productivity in a sustainable manner. The concept of a recirculating aquaculture system (RAS) includes water reuse and reduced environmental impact. However, the potential risk of disease outbreaks is inherent to RAS use. Peracetic acid (PAA) has been proven to be effective against various fish pathogens at low concentrations (Pedersen et al., 2013). Pedersen et al. (2009) suggest bypassing the biofilter when applying PAA in RAS, because PAA as low as 2 mg L⁻¹ can harm the nitrifying microorganisms in the biofilter. This research describes an easy alternative strategy for applying PAA into a RAS to ensure the safety of the biofilter.

The use of PAA is considered eco-friendly, because it degrades spontaneously and releases no harmful residues in the environment (Wagner et al., 2002). However, fish welfare should also be considered when PAA is applied. Previous studies related to fish welfare have mainly focused on acute toxicity of PAA or mortality/gill damage caused by *in vitro* treatments (Straus et al., 2012; Marchand et al., 2013). Fish welfare, however, is a much broader concept than simply avoiding mortality. Ashley (2007) suggested that stress is a possible indicator of poor welfare. Therefore, the aim of the present study is to detect whether regular applications of PAA at recommended concentrations (≤ 2 mg L⁻¹) can induce stress to fish.

2. Material and methods

2.1. Fish and rearing conditions

Twenty carp (649 $\pm$ 183 g) were reared in a pilot-scale RAS. The system consisted of a fish culture tank (1000 L), a reservoir tank (600 L) and a biofilter complex (400 L). Water was circulated among these tanks at a flow rate of 10 L min⁻¹. The day/night photoperiod was 12/12 hours (day: 6 am to 6 pm) using artificial lights (OSRAM L 18W/865 daylight, OSRAM GmbH, Munich, Germany). The water temperature was 20 $\pm$ 1°C; the pH was 7.7 $\pm$ 0.1 and the

oxygen concentration was 8 - 9 mg L⁻¹. The carp were acclimated in the RAS for 3 months prior to the experiment with a daily feeding rate of 1% body mass.

2.2. PAA application and water sampling

The PAA-product Wofasteril® E400 (40 % PAA, 12 % H₂O₂; Kesla Pharma Wolfen GmbH, Greppin, Germany) was applied in the culture tank every 3 - 4 d for a total of 5 weeks. The planned concentration was 1 mg PAA L⁻¹ in the entire RAS, which was realized by diluting 5 ml product into 50 ml distilled water before adding into the inlet of the culture tank within 15 seconds. Water was sampled using 1 L glass bottles (Schott Duran®, DURAN Glastechnik GmbH, Wertheim/Main, Germany) at the outlet of the culture tank every second PAA application. For each sampling period, the control (0 h) water samples were taken immediately prior to PAA application (n=3) and in 0.5, 1, 2, 4, 6 and 24 h (n=3) afterwards. Two days before each water sampling period, the reservoir tank and the filter complex were rinsed and refilled with preheated tap water. Meanwhile, feed was withheld till the end of sampling period.

The degradation of PAA/H₂O₂ in the culture tank was measured using the method described by Liu et al. (2015). To understand how PAA was distributed in this RAS, a mathematic model was developed as follows based on assumptions that substances in each individual tank were distributed equally and the PAA decay was ignored.

$$d/dt (C_F) = Q/V_F * (C_B - C_F)$$

$$d/dt (C_R) = Q/V_R * (C_F - C_R)$$

$$d/dt (C_B) = Q/V_B * (C_R - C_B)$$

In these equations, C_F, C_R, C_B and V_F, V_R, V_B were PAA concentrations and water volumes of the fish culture tank, the reservoir tank and the biofilter complex, respectively; Q was the flow rate.

2.3. Measurement of water cortisol

The determination of cortisol in the water has been proven as a reliable alternative tool instead of plasma cortisol to monitor the stress response of various fish species (Ruane and Komen, 2003; Ellis et al., 2004; Ellis et al., 2007; Scott and Ellis, 2007; Sebire et al., 2007; Fanouraki et al., 2008; Wong et al., 2008). The cortisol extraction and following measurement were performed based on the procedure described by Brüning et al. (2015).

2.4. Statistics

Differences among the sampling periods were compared via one-way ANOVA with Tukey test. The 0 h (control) and 24 h cortisol concentrations of the same sampling period were compared by independent T-test. Both ANOVA and T-test were performed on IBM® SPSS® Statistics (Version 21.0.0.1; Chicago, Illinois, USA). The PAA distribution model was analyzed on Berkeley Madonna modelling software (Version 8.3). The curve fitting of the PAA degradation and distribution model was analyzed on SigmaPlot (Version 12.5; Systat Software GmbH, Erkrath, Germany).

3. Results

The measured PAA concentration directly after application was about 2 mg L⁻¹. Following a second order reaction ($r^2 > 0.999$), PAA in the fish culture tank degraded from 2 mg L⁻¹ to almost 0 mg L⁻¹ by 6 h after application. H₂O₂ degraded slower, and about 40% remained in the water by 6 h after application (Fig. 3). According to the distribution model, PAA was slowly distributed from the fish culture unit to the reservoir tank and biofilter complex. An equal distribution of PAA was achieved within about 150 min after application, which was affected by tank size and flow rate (Fig. 4).

In general, water cortisol concentrations showed a transient increase after PAA application. However, cortisol peak concentrations decreased with repeated PAA applications (Fig. 1). The increase of cortisol became quicker but milder during subsequent sampling periods. In contrast, the decrease of

water cortisol occurred earlier during following sampling periods. During the fifth sampling period, cortisol began to decrease within 30 min after PAA application.

The basal (0 h) cortisol concentration remained higher after the second sampling period (ANOVA, $F_{4,25}=60.579$, $P<0.001$) and stabilized at an intermediate level after the third sampling period (Tukey test, $P=0.244$). The 24 h cortisol concentration was always higher than the respective 0 h cortisol concentration (independent T-test, $df=10$, $P<0.001$) except at the fifth sampling period, when the 0 h and 24 h cortisol concentrations were equal (independent T-test, $df=10$, $P=0.738$) (Fig.2).

4. Discussion

4.1. PAA distribution and application strategy in RAS

The dose of the PAA –product E400 was calculated using the total water volume of the RAS and the resulting concentration was expected to be 1 mg L⁻¹. In contrast, onsite measurements showed that the initial PAA concentration in the fish culture tank was 2 mg L⁻¹. It indicates that PAA was equally distributed only in the culture tank immediately after application.

The degradation of PAA in aqueous solutions normally shows a first order reaction to the time (Pedersen et al., 2009; Liu et al., 2014). However, results indicated that PAA degradation in the fish culture tank fit a second order reaction to the time. This indicated a dilution effect of the water from the other tanks in relationship to time. The distribution model indicated that the PAA concentration in the culture tank and the reservoir tank fit a first-order exponential decay and increase, respectively ($r^2>0.99$). In contrast, the PAA concentration in the biofilter complex fit a first-order sigmoidal increase ($r^2>0.99$). We excluded PAA decay in our distribution model, because the PAA decay is affected by various parameters (Liu et al., 2014) and there is no universal formula to quantify it. However, taking PAA decay into qualitative consideration, we expected a lesser PAA increase and a lower PAA

concentration in the reservoir tank and biofilter complex than shown in the distribution model.

In commercial RAS, potential harm to the biofilter due to PAA disinfection should be addressed (Pedersen et al., 2009). Based on the present model, we suggest applying PAA at the inlet of each fish culture tank with occasional mixing to improve distribution. The PAA concentration should be calculated based on the culture tank volume instead of whole system volume. A reduced flow rate (hence increased retention time) during the PAA application can reduce the concentration of PAA reaching the biofilter and reduce potential harmful effects.

4.2. Adaptation of the carp to repeated PAA applications

Decreasing water cortisol concentration peaks were observed with subsequent PAA applications. This indicates that the plasma cortisol peak was decreasing, which could be the result of inhibited cortisol production, or enhanced cortisol catabolism, or both. Compared to the first sampling period, we observed an earlier increase and faster decrease of water cortisol concentration in ensuing sampling periods. This indicates both production and metabolism of plasma cortisol were advanced by repeated PAA applications. A less pronounced increase of water cortisol concentration was observed with subsequent PAA applications. During the fifth sampling period (ninth PAA application), the water cortisol increased weakly and returned to the basal level within 24 h. These results suggest that the carp adapted to the PAA treatments and showed a faster, but less severe, stress response, after repeated PAA applications. Additionally, no mortality was observed during the study. It can be speculated that, if the present experiment had been extended, the carp would fully adapt to PAA applications and would show no or only negligible stress responses.

It was also noticed that the basal water cortisol increased moderately after PAA applications. This might be induced by the PAA applications; further

investigations are needed on this subject. Goos and Consten (2002) found a similar adaptation of male common carp to repeated temperature shocks as in the present study. Noteworthy, their control group showed a variance on plasma cortisol level along with time. This variance is probably due to the circadian rhythms of plasma cortisol that can be found in many fish species (Montoya et al., 2010; López-Olmeda et al., 2012; Oliveira et al., 2013). Even with fixed day/night photoperiod in the present experiments, inherent biological variability of fish may have caused the minor fluctuation of cortisol.

5. Conclusions

We conclude that the first application of PAA at 2 mg L^{-1} can induce a stress-response in naive carp. However, the stress of the carp, as measured by water cortisol concentration, kept decreasing and became insignificant after repeated applications of PAA. Therefore, long-term, regular applications of PAA up to 2 mg L^{-1} are adaptable stressor to the carp. To minimize risk of biofilter impairment, applying PAA in the fish culture tank and concurrently reducing the flow to the biofilter is recommended.

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Figure 1: Water cortisol measurements at 0.5, 1, 2, 4, 6 and 24 h after a 1 mg L⁻¹ PAA applications for each sampling period (n=3, error bar indicates standard error). The 24 h sample was not taken during the first sampling period.

Figure 2: Comparison of control (0 h) cortisol and 24 h cortisol during sampling periods. (***: $P < 0.001$, $n = 3$, error bar indicates standard error; the letter a, b and c indicate different homogenous subsets after Tukey HSD test). The X-axis represents the sampling period order over five weeks with 1 mg L^{-1} PAA applications biweekly and sampling weekly.

Figure 3: Degradation of PAA and H₂O₂ in the fish culture tank within 6 h after application with 1 mg L⁻¹ PAA. The treatment was calculated for the whole system (2 m³) and was applied to the fish culture tank (1 m³).

Figure 4: Theoretical PAA distribution model ignoring PAA decay in the pilot-scale RAS of the present study.

References

- Ashley, P.J., 2007. Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science* 104, 199-235.
- Brüning, A., Hölker, F., Franke, S., Preuer, T., Kloas, W., 2015. Spotlight on fish: Light pollution affects circadian rhythms of European perch but does not cause stress. *Science of The Total Environment* 511, 516-522.
- Ellis, T., James, J.D., Stewart, C., Scott, A.P., 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. *Journal of Fish Biology* 65, 1233-1252.
- Ellis, T., James, J.D., Sundh, H., Fridell, F., Sundell, K., Scott, A.P., 2007. Non-invasive measurement of cortisol and melatonin in tanks stocked with seawater Atlantic salmon. *Aquaculture* 272, 698-706.
- Fanouraki, E., Papandroulakis, N., Ellis, T., Mylonas, C.C., Scott, A.P., Pavlidis, M., 2008. Water cortisol is a reliable indicator of stress in European sea bass, *Dicentrarchus labrax*. *Behaviour* 145, 1267-1281.
- Goos, H.J.T., Consten, D., 2002. Stress adaptation, cortisol and pubertal development in the male common carp, *Cyprinus carpio*. *Molecular and Cellular Endocrinology* 197, 105-116.
- López-Olmeda, J.F., López-García, I., Sánchez-Muros, M.J., Blanco-Vives, B., Aparicio, R., Sánchez-Vázquez, F.J., 2012. Daily rhythms of digestive physiology, metabolism and behaviour in the European eel (*Anguilla anguilla*). *Aquaculture International* 20, 1085-1096.
- Liu, D., Straus, D.L., Pedersen, L.-F., Meinelt, T., 2015. Comparison of the Toxicity of Wofasteril Peracetic Acid Formulations E400, E250, and Lspez to *Daphnia magna*, with Emphasis on the Effect of Hydrogen Peroxide. *North American Journal of Aquaculture* 77, 128-135.
- Liu, D., Steinberg, C.E.W., Straus, D.L., Pedersen, L.-F., Meinelt, T., 2014. Salinity, dissolved organic carbon and water hardness affect peracetic acid (PAA) degradation in aqueous solutions. *Aquacultural Engineering* 60, 35-40.
- Marchand, P.A., Straus, D.L., Wienke, A., Pedersen, L.F., Meinelt, T., 2013. Effect of

-
- water hardness on peracetic acid toxicity to zebrafish, *Danio rerio*, embryos. *Aquaculture International* 21, 679-686.
- Montoya, A., López-Olmeda, J.F., Garayzar, A.B.S., Sánchez-Vázquez, F.J., 2010. Synchronization of daily rhythms of locomotor activity and plasma glucose, cortisol and thyroid hormones to feeding in Gilthead seabream (*Sparus aurata*) under a light–dark cycle. *Physiology & Behavior* 101, 101-107.
- Oliveira, C.V., Aparício, R., Blanco-Vives, B., Chereguini, O., Martín, I., Javier Sánchez-Vazquez, F., 2013. Endocrine (plasma cortisol and glucose) and behavioral (locomotor and self-feeding activity) circadian rhythms in Senegalese sole (*Solea senegalensis* Kaup 1858) exposed to light/dark cycles or constant light. *Fish Physiol Biochem* 39, 479-487.
- Pedersen, L.F., Meinelt, T., Straus, D.L., 2013. Peracetic acid degradation in freshwater aquaculture systems and possible practical implications. *Aquacultural Engineering* 53, 65-71.
- Pedersen, L.F., Pedersen, P.B., Nielsen, J.L., Nielsen, P.H., 2009. Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems. *Aquaculture* 296, 246-254.
- Ruane, N.M., Komen, H., 2003. Measuring cortisol in the water as an indicator of stress caused by increased loading density in common carp (*Cyprinus carpio*). *Aquaculture* 218, 685-693.
- Scott, A.P., Ellis, T., 2007. Measurement of fish steroids in water—a review. *General and Comparative Endocrinology* 153, 392-400.
- Sebire, M., Katsiadaki, I., Scott, A.P., 2007. Non-invasive measurement of 11-ketotestosterone, cortisol and androstenedione in male three-spined stickleback (*Gasterosteus aculeatus*). *General and Comparative Endocrinology* 152, 30-38.
- Straus, D.L., Meinelt, T., Farmer, B.D., Beck, B.H., 2012. Acute toxicity and histopathology of channel catfish fry exposed to peracetic acid. *Aquaculture* 342, 134-138.
- Wagner, M., Brumelis, D., Gehr, R., 2002. Disinfection of wastewater by hydrogen

peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent. Water environment research : a research publication of the Water Environment Federation 74, 33-50.

Wong, S., Earley, R., Campbell, J., Dykstra, M., 2008. Measuring water-borne cortisol in convict cichlids (*Amatitlania nigrofasciata*): is the procedure a stressor? Behaviour 145, 1283-1305.

Statement of Relevance:

Fish welfare becomes more and more important in aquaculture. However, there is a lack of knowledge if routine prophylaxis/disinfectant might affect fish welfare. In the present study, we tested how fish responded to repeated applications of peracetic acid (PAA) in a pilot-scale recirculating aquaculture system (RAS) via measurement of cortisol in water. The results showed that fishes reduced their stress during subsequent PAA application, indicating adaptation of fish to repeated prophylaxis/disinfection with PAA. Moreover, we established a model and measured the PAA concentration onsite to investigate the distribution of PAA in RAS. We found out that by simply adding PAA only to the fish culture tank with a reduced flow rate can avoid the potential harm on the biofilter. Findings from the current study provide insights that may prove of benefit to future treatment based studies