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The efficacy of *Aeromonas hydrophila* GPl-04 feed-based vaccine on African catfish (*Clarias gariepinus*)

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Abstract. *Mulia DS, Utomo T, Isnansetyo A. 2022. The efficacy of* Aeromonas hydrophila *GPl-04 feed-based vaccine on African catfish (*Clarias gariepinus*). Biodiversitas 23: 1505-1510. Aeromonas hydrophila* is a pathogenic bacterium to African catfish (*Clarias gariepinus*). Vaccination is one of the strategic efforts to control this type of bacterial attack. The objective of this study was to determine the effectiveness of *A. hydrophila* GPl-04 feed-based vaccine for African catfish. This study used *A. hydrophila* GPl-04 strain isolate as vaccine material. Sample used was African catfish measuring 10-13 cm length, weighing 16-25 g, taken from aquaculture ponds in the Purbalingga area, Central Java, Indonesia. This study applied experiments under the completely randomized design (CRD) method with 5 treatments and 3 replications. The treatments consisted of P0: feed without vaccine (control); P1: feed with 10 mL/100 g dose of vaccine given for 10 days; P2: feed with 10 mL/100 g dose of vaccine given for 15 days; P3: feed with 15 mL/ 100 g dose of vaccine given for 10 days; and P4: feed with 15 mL/ 100 g dose of vaccine given for 15 days. The challenge test was carried out on week 3, by injecting 0.1 mL of *A. hydrophila* suspension at 105CFU/mL dose per fish. The main parameters included the fish antibody titer, survival rate (SR), relative percent survival (RPS), mean time to death (MTD), and growth rate. Supporting parameters included water quality such as water temperature, water pH, and dissolved O_2 levels. The main parameter data were analyzed using analysis of variance (ANOVA) and Duncan multiple range test (DMRT) at 5% test level, and the supporting parameter was descriptively quantitative analyzed. The results showed *A. hydrophila* GPl-04 feed-based vaccine could increase antibody titer, SR, RPS, and MTD of African catfish ($P<0.05$). In addition, vaccination did not adversely affect the growth. The *A. hydrophila* GPl-04 feed-based vaccine was effective in protecting African catfish from *A. hydrophila* attack where 10 mL/100 g dose, given for 10 days (P1) was indicated as the most effective compared to other vaccination treatments.

Keywords: Catfish, feed-based vaccine, fish immune system, relative percent survival, survival rate

INTRODUCTION

African catfish (*Clarias gariepinus*) is a widely cultivated and consumed freshwater fish. Its advantages include easy cultivation and relatively fast growth (Olatoye and Basiru 2013). It plays an important role as a profit source for freshwater fish cultivators (Anyanwu et al. 2015). However, problems in cultivating this type of fish arise when it is left unchecked and causes crop failure as well as economic losses due to disease. One of the frequent diseases attacking African catfish is a bacterial disease caused by Motile Aeromonas Septicaemia (MAS) or Aeromoniasis caused by *Aeromonas* sp. bacterium (Emeish et al. 2018).

Aeromonas sp. is a Gram-negative, rod-shaped, facultatively anaerobic, oxidase positive bacterium with the capability of fermenting glucose and normally lives in the aquatic environment (Martínez-Murcia et al. 2008; Erdem et al. 2011; Parker and Shaw 2011; Percival and Williams 2014). This type of bacteria attacks various types of fish, including catfish (*Clarias* sp.), gourami (*Osphronemus gouramy*), tilapia (*Oreochromis niloticus*), goldfish (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), eel (*Anguilla japonica*), koi (*Anabas testudineus*), pirarucu (*Arapaima gigas*), and milkfish (*Chanos chanos*) (Sari et al. 2013; Borty et al. 2016; Dias et al. 2016; Guo et al. 2016; Simon et al. 2016; Soltani et al. 2016; Rozi et al. 2018; Sukenda et al. 2018; Mulia et al. 2020). There are numerous species of *Aeromonas* sp. and most of them are pathogenic (Fernandez-Bravo and Figueras 2020). This is because these bacteria have virulent genes (Mulia et al. 2020). In addition, *Aeromonas* sp. is also known to be resistant to several antibiotics, which is thought to be caused by having resistance genes (Mulia et al. 2021). One species that is also known as pathogenic and causes MAS in *C. gariepinus* is *A. hydrophila* (Anyanwu et al. 2015; Wamala et al. 2018; Wulandari et al. 2019).

Aeromonas hydrophila is ubiquitous in water and also an opportunistic pathogen with a high level of virulence (Rasmussen-Ivey et al. 2016; Stratev and Odeyemi 2016). It causes MAS disease in fish and other aquatic biotas in a broad spectrum (Tipmongkolsilp et al. 2012; Yano et al. 2015; Wimalasena et al. 2017). Symptoms shown by African catfish infected with *A. hydrophila* include skin discoloration and hyperaemic spots, haemorrhagic skin ulcers, hyperaemic patches on the fins, caudal fin congestion, hemorrhagic and congested liver, kidney and spleen, and abdominal cavity (Anyanwu et al. 2015; Emeish et al. 2018).

Fish disease control can be done by considering aquaculture management, feeding management, and good water quality management. In general, safe efforts of fish disease control can be carried out by providing immunostimulants and probiotics (Newaj-Fyzul and Austin 2014; Isnansetyo et al. 2016), in addition to vaccination (Luzardo-Alvarez et al. 2010; Coscelli et al. 2015; Rauta et al. 2017). Vaccination is one of the methods to intentionally provide stimuli or antigens that will enable the body to improve immune system function by producing antibodies against germs or pathogens (Mulia et al. 2016). It is an important disease management strategy used to maintain human and animal health worldwide. Its use in fish farming can reduce mortality rates due to pathogenic infections, thereby reducing the use of antibiotics and minimizing the emergence of pathogen resistance to antibiotics (Taukhid et al. 2014).

The vaccine is made from killed or attenuated bacteria by using subunit vaccine (purified antigenic determinant), bacterial toxin (toxoid), and plasmid (pDNA) (Besnard et al. 2016). *Aeromonas hydrophila* vaccine can improve the immune system by increasing antibody titers in African catfish (*C. gariepinus*), catfish (*Pangasius hypophthalmus*), nile tilapia (*Oreochromis niloticus*), and rainbow trout (*Oncorhynchus mykiss*) (Dehghani et al. 2012; Mulia 2012; Aly et al. 2015; Olga et al. 2020). In this study, the vaccine was given through injection and immersion. Fish vaccination can be done by intramuscular (im) and intraperitoneal (ip) injections, immersion (dip or bath), and oral (Brudeseth et al. 2013; Embregts and Forlenza 2016). A breakthrough in providing easier vaccines is essential on-field application, such as by designing a feed vaccine (feed-based vaccine) where the vaccine is given together with feed to avoid fish stress (Dalmo et al. 2016). The addition of polyvalent of *A. hydrophila* vaccine to fish feed exhibited an increase of antibody titer in African catfish (Mulia et al. 2015). The objective of this study was to determine the effectiveness of A. *hydrophila* GPl-04 feed-based vaccine in African catfish (*C. gariepinus*).

MATERIALS AND METHODS

Samples

This study used *A. hydrophila* strain GPl-04 isolate as the vaccine ingredient and African catfish samples measuring 10-13 cm, weighing 16-25 g, taken from aquaculture ponds in the Purbalingga area, Central Java, Indonesia.

Research design

This study applied an experimental method with a completely randomized design (CRD), 5 treatments, and 3 replications. The treatments consisted of P0: feed without vaccine (control); P1: feed with 10 mL/100 g dose of vaccine, given for 10 days; P2: feed with 10 mL/100 g dose of vaccine, given for 15 days; P3: feed with 15 mL/100 g dose of vaccine, given for 10 days; and P4: feed with 15 mL/100 g dose of vaccine, given for 15 days.

Preparing the *Aeromonas hydrophila* vaccine

Aeromonas hydrophila vaccine was made in the form of whole cells by inactivating the bacteria using 2% formalin. Its GPl-04 strain was cultured on glutamate starch phenyl (GSP) medium (Merck) at 30ºC for 24 h. Then, one colony was grown in 10 mL of tryptic soy broth (TSB) medium (Merck) and incubated at the same temperature and time. The vortexed bacterial suspension was then poured on tryptic soy agar (TSA) medium (Merck) in a large petri dish and incubated at 30ºC in 24 h. Furthermore, the bacteria were harvested by dredging them slowly using a drigalski and added with phosphate buffered saline (PBS) so that all bacteria could be taken, then added 2% formalin and shaken for 24 h. Then it was centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded, and the pellet was added with 3 mL of PBS before re-centrifuged. Washing was done using PBS in 3 times to obtain a formalin-free vaccine. The viability of *A. hydrophila* was tested by regrowing the bacteria on GSP medium. Then, *A. hydrophila* bacteria were declared ready to be used as vaccines when they did not grow within GSP medium.

Preparing the feed-base *Aeromonas hydrophila* vaccine

A total of 100 g of feed pellets were smeared with 10 mL of egg white until evenly distributed. Then, the feed was sprayed with a vaccine that had been previously suspended using sterile PBS solution at 108 CFU/mL density based on the selected treatment dose before being aerated to dry.

Challenge test

The challenge test was carried out at week 3, by injecting 0.1 mL of *A. hydrophila* suspension per fish at a dose of 105 for all treatments. The dose was obtained from the Lethal Dose50 (LD50) test results. Observations were made by observing the clinical symptoms and survival of African catfish within one week.

Observed parameters

Observed parameters consisted of main parameters such as antibody titer, survival rate, relative percent survival (RPS), mean time to death (MTD), and growth rate (weight and length gain of fish). Supporting parameters included water quality parameters such as temperature, pH, and dissolved oxygen levels (DO).

Data analysis

The main parameter data were analyzed using variance (ANOVA) if the data are normally distributed and Duncan multiple range test (DMRT) at 5% test level. If the data are not normally distributed, it would use an alternative test such as Friedman's ANOVA. The supporting parameter data were analyzed in a descriptive-quantitative manner.

RESULTS AND DISCUSSION

Antibody titer

This study performed oral vaccination to African catfish through *A. hydrophila* feed-based vaccine. The feed-based

vaccine was given for 10 and 15 days, depending on the selected treatment. The results showed increased production of African catfish antibody titer (Table 1). On week 0, it showed a low antibody titer with an insignificant difference (P=0.93; P>0.05). On week 1 (one week after being given with feed-based vaccine), antibody titer increased due to feed-based vaccine (P1-P4) treatments and indicated a significant difference (P=0.007; P<0.05) to the control (P0). On week 2, antibody titers continued to increase in comparison to the control (P=0.001; P<0.05), and week 3 antibody titers too continued to increase in comparison to the control (P=0.000; P<0.05). On week 4, feed-based vaccine treatment showed decreased antibody titer, although it was still significantly different from the control (P=0.000; P<0.05).

The results showed a significant increase in antibody titers compared to the controls (Table 1). Previous studies have also succeeded in increasing antibodies of *Clarias batrachus*, *Labeo rohita*, *Carassius auratus gibelio*, *Channa striata* by using *A. hydrophila* oral vaccination (Nayak et al. 2004; Swain et al. 2007; Tu et al. 2010; Siriyappagouder et al. 2014; Kaur et al. 2021). Oral vaccines have been shown to increase or extend protection (Ballesteros et al. 2014), although antibody response is transient and usually lasts about 3 months (Firdaus-Nawi et al. 2013).

Oral vaccination by incorporating vaccines into feed is an ideal method of vaccination with several advantages and conveniences, such easy to provide, stress-free to fish, applicable for various sizes of fish and suitable for fish in large numbers (Sommerset et al. 2005; Plant and LaPatra 2011; Mutoloki et al. 2015; Mohamad et al. 2021). However, there are also various disadvantages of oral vaccination such as destructing antigens in the intestine, weak efficacy and short protection period in comparison to injection (Hart et al. 1988) due to the breakdown of antigens in the harsh gastric environment and the highly tolerogenic intestinal environment (Rombout and Krion 2014). However, oral vaccination so far has been the solution to protect fish against diseases, including MAS in African catfish. Oral feed-based vaccines for *A. hydrophila* can induce mucosal and systemic immune responses, thus protecting fish from pathogens and controlling systemic infection outbreaks (Han et al. 2018; Kahieshesfandiari et al. 2019; Kaur et al. 2021). Together, a feed-based bivalent vaccine of *Streptococcus* sp. and *Aeromonas* sp. can also protect tilapia (*Oreochromis* spp.) from streptococcosis and MAS (Monir et al. 2021).

Survival rate, relative percent survival (RPS) and mean time to death (MTD) of catfish

The catfish survival rate reached the highest survival value in P4 by 100%, and P3, P1 and P2 reached 96.67%, and 93.33%, respectively, while the control only reached 56.67%. (Table 2). The results showed that feed-based vaccine had a significant effect on the survival rate of catfish given with vaccination treatment (P=0.001; P<0.05) compared to the control (Table 2). However, there was no significant difference between vaccination treatments. Previous studies have also succeeded in increasing the survival rate of *Clarias batrachus*, *Oncorhynchus mykiss*, *Channa striata*, and *Oreochromis niloticus* after being given with *A. hydrophila* vaccination (Nayak et al. 2004; Bastardo et al. 2012; Siriyappagouder et al. 2014; Aly et al. 2015).

The RPS of African catfish in treatment P4 reached the highest relative percent survival of 100.00%, while P3, P1 and P2 reached 92.30%, and 84.60%, respectively. However, each of the four vaccination treatments was not significantly different (P=0.752; P>0.05). RPS is an important index to assess vaccine efficacy (Monir et al. 2021). Results of this study showed that feed-based vaccine could protect African catfish in a wide range, which was 93.33-100.00% that proved high efficacy of *A. hydrophila* vaccine. In this study, the *A. hydrophila* feed-based vaccine at a dose of 10-15 mL/100g for 10-15 days gave a relatively similar protective effect. Such high RPS value was due to the fact that vaccination could increase antibody production that brought positive impact on the protection against pathogens when a challenge test with active *A. hydrophila* was carried out.

Previous studies reported the role of *A. hydrophila* feed-based vaccine in increasing the RPS values with varying results. It produced RPS values of 77.78±3.85% in red hybrid tilapia (*O. niloticus* × *O. mossambicus*). Also, feed-based bivalent vaccines of *Streptococcus iniae* and *A. hydrophila* also exhibited the same RPS values in these types of fish (Monir et al. 2021). Oral yeast-based DNA vaccine protected crucian carp (*Carassius auratus*) with RPS value of 46.7% (Han et al. 2018). However, *A. hydrophila* biofilm oral vaccine protected *Clarias batrachus* with higher RPS values by 90.8-100% (Nayak et al. 2004). Similar to other studies, *A. hydrophila* biofilm oral vaccine protected *Channa striata* and *Labeo rohita* with RPS values of 88% and 83.4%, respectively (Vinay et al. 2013; Siriyappagouder et al. 2014).

Table 1. Measurement results of antibody titers

Treatment	Week				
	0	1	2	3	4
P0	5.31^{a}	6.68^{a}	6.68^{a}	5.31^{a}	2.33^{a}
P1	5.31^{a}	21.41^{b}	96.34^{b}	53.45^{b}	53.45^{b}
P2	5.31^{a}	32.00^{b}	74.54^{b}	85.63^{b}	64.00^{b}
P3	6.68^{a}	53.45^{b}	85.63^{b}	96.34^{b}	42.81^{b}
P4	5.31^{a}	21.41^{b}	106.89^{b}	149.09^{b}	53.45^{b}

Note: the average number with the same superscript letters shows an effect that is not significantly different at the 5% test level.

Table 2. Survival rate, RPS and MTD

Treatment	Survival rate (%)	RPS (%)	MTD (days)
P0	56.67±11.55^{a}		3.33±0.58^{a}
P1	93.33±11.55^{b}	84.60±26.67^{a}	0.67±1.16^{b}
P2	93.33±11.55^{b}	84.60±26.67^{a}	0.67±1.16^{b}
P3	96.67±5.77^{b}	92.30±13.33^{a}	0.33±0.56^{b}
P4	100.00±0.00^{b}	100.00±0.00^{a}	0.00±0.00^{b}

Table 3. Growth rate of African catfish

Treatment	Growth rate	
	Weight gain (g)	Weight gain (cm)
P0	11.80±5.26[a]	2.43±0.06[a]
P1	12.83±3.63[a]	2.20±0.06[a]
P2	15.90±0.78[a]	1.80±0.56[a]
P3	13.50±1.95[a]	2.10±0.44[a]
P4	14.53±3.41[a]	1.93±0.35[a]

Table 4. Parameter of water quality

Treatment	Parameter of water quality		
	Temperature (^{0}C)	Dissolved Oxygen (ppm)	Acidity (pH)
P0	26.1 - 28.8	6.0 - 8.9	7.0 - 7.9
P1	26.5 - 28.6	6.3 - 8.6	6.7 - 8.0
P2	26.0 - 28.8	6.6 - 8.5	6.9 - 8.0
P3	26.0 - 28.4	6.9 - 8.6	7.0 - 8.0
P4	26.0 - 28.5	6.2 - 8.8	7.0 - 7.9

The MTD value of African catfish in P4 treatment was 0.00 days, which was an indication of zero death. In P3, as well as P1 and P2, the values were 0.33 days and 0.67 days, respectively, while P0 was 3.33 days. There was no significant difference in MTD between vaccination treatments, but it had a significant difference with the control (P=0.004; P<0.05). Results of this study showed that the application of feed-based vaccines was quite effective in controlling *A. hydrophila*, thus, it could minimize the number of deaths and had an impact on MTD value. Previous studies also reported that the application of *A. hydrophila* vaccine had a significant effect on MTD of African catfish (*Clarias* sp.) and snakehead fish (*Ophiocephalus striatus*) (Mulia et al. 2006; Olga and Fatmawaty 2013).

Catfish growth rate

Fish growth is indicated by increased weight and length throughout the pisciculture. The weight gain of African catfish in P2 treatment reached 15.90 g, followed by P4, P3, P1, and P0 with 14.53, 13.50, 12.83, and 11.80 g, respectively (Table 3). However, the weight gain between treatments was not significantly different (P=0.636; P>0.05). The increasing length under P0 treatment was 2.43 cm, followed by P1, P3, P4, and P2 by 2.20, 2.10, 1.93, and 1.80 cm, respectively, with each that was not significantly different (P=0.327; P>0.05). This showed that vaccination could increase the immune response but not affect the overall growth. Similarly, findings had also been reported in previous studies (Sughra et al. 2021). The results of this study showed that the growth of vaccinated fish was not disturbed by the vaccine. Skinner et al. (2008) also reported that vaccination had no adverse effect on the growth of Atlantic salmon. Based on these results, it can be concluded that vaccination could improve the fish's immune system but did not have a negative impact on fish growth.

Parameter of water quality

The effectiveness of vaccination is influenced by water quality factors, namely temperature. Water temperature, size, and species of fish directly influence the immune response of fish and should always be considered at the time of vaccination (Olga and Fatmawaty 2013). The results of the measurement of water quality parameters, namely water temperature ranging from 26.0-28.8ºC, Dissolved Oxygen 6.0-8.9 ppm, and pH 6.7-8.0 (Table 4). This shows that there is a slight variation between treatments, but it is still within the normal range. The optimal water temperature for the maintenance of African catfish ranges from 25-30ºC and the optimum dissolved oxygen content ranges from 3-20 ppm (Nugrahajati et al. 2013). African catfish can live well in water with a pH ranging from 6.5-8 and (Saparinto and Susiana 2013).

In the conclusion, feed-based vaccine of *A. hydrophila* GPl-04 was successful in increasing the antibody titer of African catfish. Giving vaccinated feed at 10-15 mL/100 g dose for 10 to 15 days obtained a relatively good immune response. It also succeeded in protecting by the highest survival value of 100.00% in P4 treatment, while P1-P3 ranged between 93.33-96.00%. High survival rate had a positive impact on RPS values that ranged between 84.60-100.00%. In addition, this study also resulted in significant MTD values between vaccinated and non-vaccinated (control) treatments but had no effect on the growth rate. This indicated that vaccination did not adversely affect fish growth. This study concluded that *A. hydrophila* GPl-04 feed-based vaccine effectively protected African catfish from *A. hydrophila* attack. Feed-based vaccine treatment in 10 mL/100 g dose given for 10 days (P1) was the most effective in comparison to other vaccination treatments. This study succeeded in revealing that the feed-based vaccine *A. hydrophila* strain GPl-04 has the potential to increase the immune system and survival of African catfish.

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