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The Efficacy of *Aeromonas veronii* bv *veronii* BmCL-03 Vaccine to Control Motile *Aeromonas* Septicemia (MAS) Disease on African Catfish (*Clarias gariepinus*)

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ABSTRACT

This study aimed to determine the efficacy of the *Aeromonas veronii* bv *veronii* BmCL-03 vaccine to control the MAS disease of African catfish (*Clarias gariepinus*). The research used an experimental method with a completely randomized design (CRD), five treatments, and three replications. The treatments consisted of T1: intramuscular (i.m) injection; T2: intraperitoneal injection (i.p); T3: oral; T4: immersion; T5: without vaccination (control). Booster vaccination was carried out one week after using the same method, except for oral vaccination, which was given during the first ten days. In the third week, each fish was given 0.1mL of *A. veronii* bv *veronii* suspension at a 10^7 CFU/mL dose for all treatments as part of the challenge test. Antibody titer, survival rate (SR), relative percent survival (RPS), mean time to death (MTD), and growth rate are among the research factors. The data were analyzed using analysis of variance (ANOVA) and Duncan multiple range test (DMRT) at a test level of 5%. The results showed that the *A. veronii* bv *veronii* BmCL-03 vaccine was significantly different ($P<0.05$) and could increase antibody titer, SR, RPS, and weight gain of African catfish but was not significantly different ($P>0.05$) to fish length and MTD. Vaccination does not hurt the growth of African catfish. The vaccine of *A. veronii* bv *veronii* effectively protects African catfish, and the i.m injection treatment is the most effective. The *A. veronii* bv *veronii* vaccine has good prospects as a vaccine product that can improve the immune system and protect African catfish.

Key words: *Aeromonas veronii* bv *veronii*, African catfish, Vaccine

INTRODUCTION

The African catfish (*Clarias gariepinus*) is a freshwater fish with potential for cultivation. Its features include easy cultivation and rapid growth (Spirina et al. 2021; Mulia et al. 2023). African catfish have a high level of productivity and a low feed conversion ratio (FCR) (Olatoye and Basiru 2013; Abraham et al. 2018). The nutritional content of African catfish includes 17.7% protein, 4.8% fat, 0.3% carbohydrates, and 1.2% minerals (Apriansyah et al. 2021). In the Banyumas area, African catfish production continues to increase in line with market demand. In 2023, the total production of African catfish will reach 3,860,008kg; and in 2024, it will increase to 3,994,346kg (Banyumas Regency Fisheries and Livestock Service 2024).

However, one of the challenges in cultivating African catfish is bacterial pathogens, especially the *Aeromonas* genus (Mulia et al. 2020; 2023). Bacteria of the genus *Aeromonas* are pathogenic and very dangerous in intensive fish farming (Austin and Austin 2016; Pessoa et al. 2019). Cultivation with high stocking densities triggers opportunistic *Aeromonas* activity (Stratev and Odeyemi 2016). This bacteria causes Motile *Aeromonas* Septicemia (MAS) disease, which triggers mass deaths and significant losses (Emeish et al. 2018). MAS disease can cause up to 100% fish death within one week (Shameena et al. 2020).

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In most cases, clinical signs of fish infected with *Aeromonas* spp. including melanosis, ulcers, fin and tail-rot, fin congestion, hyperemia, hemorrhagic, exophthalmia, abdominal cavity, abdominal dropsy, abdominal ascites, congested liver and kidney, and necrotic (Emeish et al. 2018; Gallani et al. 2020; Assane et al. 2021; Mazumder et al. 2021; Mulia et al. 2023; 2024). Safely controlling fish diseases can be done by administering probiotics and immunostimulants (Isnansetyo et al. 2016; Amenyogbe 2023). Fish vaccination can also be carried out (Rauta et al. 2017; Du et al. 2022). Vaccination is one effort to control attacks by *Aeromonas* spp. (Coscelli et al. 2015; Mulia et al. 2022). Vaccination is an environmentally friendly technology because it is derived from live organisms, does not contaminate the environment, and is precisely targeted. Vaccination intentionally provides stimuli or antigens to boost the body's immune system by creating antibodies (Mulia et al. 2016; Du et al. 2022). Vaccination is effective against pathogens, so it positively impacts increasing fish production (Nayak 2020). Vaccination is effective in controlling *Aeromonas* spp. in African catfish (*C. gariepinus*), crucian carp (*Carassius auratus*), and red hybrid tilapia (*Oreochromis* sp.) (Mulia et al. 2021; Song et al. 2022; Ali et al. 2023).

The results of research in the field show that MAS disease is not only caused by *A. hydrophila* but several other *Aeromonas* species, one of which is *A. veronii* bv *veronii* (Jagoda et al. 2014; Mulia et al. 2023). The virulence level of *A. veronii* bv *veronii* reaches 66.67 to 100% in catfish (Mulia et al. 2023). *A. veronii* is a significant bacterial pathogen in aquatic animals. It can cause major global morbidity and mortality in loaches (Seo et al. 2020). In recent years, *A. veronii* infection has occurred, causing substantial economic losses to the aquaculture industry (Xu et al. 2019). The *A. veronii* vaccine has been tried by Zhang et al. (2020) and has proven effective against *A. veronii* infection in loach fish (*Misgurnus anguillicaudatus*). The survival rate of vaccinated loaches reached 65.66% after the challenge test, while the control group was 0%. Research on the efficacy of recombinant bacteria *Lactobacillus casei*, which expresses OmpAI from *A. veronii* C5-I as a molecular adjuvant in enhancing immunity in crucian carp (*C. carassius*), showed that *A. veronii* vaccination could provide strong protection against MAS disease with survival reaching 73.3%, compared to the control of 0% (Zhao et al. 2021). Therefore, in this study, *A. veronii* bv *veronii* vaccination will be carried out using several vaccination methods, namely intramuscular injection (i.m), intraperitoneal injection (i.p), oral administration with the feed-based vaccine, and immersing to determine its efficacy in controlling MAS disease in African catfish.

MATERIALS AND METHODS

Samples

The vaccine material used in this study was an *A. veronii* bv *veronii* isolate from strain BmCL-03. African catfish (*C. gariepinus*) measuring 15-17cm long and weighing 44-58g were collected from agriculture ponds in Banyumas, Central Java.

Design research

The study employed an experimental approach with a completely randomized design (CRD), five treatments, and three replications. Treatment consists of T1: intramuscular (i.m) injection; T2: intraperitoneal injection (i.p); T3: oral; T4: immersion; T5: without vaccination (control). Each sample unit contained ten African catfish.

Preparing the *Aeromonas veronii* bv *veronii* vaccine

The *Aeromonas veronii* bv *veronii* vaccine was made based on a modification by Mulia et al. (2022). The vaccine was made in whole cell form by inactivating bacteria using 3% formalin. *A. veronii* bv *veronii* strain BmCL-03 was grown in GSP medium (Merck) at 30ºC for 24 hours. Then, one colony was cultured in 10mL of TSB medium (Merck) and incubated at the same temperature and duration. The bacterial suspension was vortexed, put onto tryptic soy agar (TSA) medium (Merck) in a giant petri dish, and incubated at 30ºC for 24 hours. The bacteria were then harvested by gently dredging with a drigalsky and adding PBS to ensure that all of the bacteria were collected. The collected bacteria were mixed with 3% formalin and agitated at 150rpm for 24 hours. After centrifuging at 3000rpm for 20min, supernatant was removed and 3mL of PBS were added.

Preparing the feed-based *Aeromonas veronii* bv *veronii* vaccine

100g of feed pellets (FF999, PT Central Proteina Prima, Surabaya) were smeared with 10mL egg white until equally dispersed. The vaccine was then sprayed into the feed using a sprayer, suspended in a sterile PBS solution at a density of 10^8 CFU/mL and up to 100mL in volume. The vaccine feed was then aired until dry (Mulia et al. 2022).

Vaccination of *Aeromonas veronii* bv *veronii* to African catfish

Vaccination was carried out at week 0 using several vaccine methods. Intramuscular injection was carried out by injecting the vaccine into the fish's body intramuscularly at a dose of 0.1mL at a density of 10^8 CFU/mL; intraperitoneal injection was carried out by injecting the vaccine into the fish's body at a dose of 0.1mL at a density of 10^8 CFU/mL, oral administration was carried out by giving vaccinated feed as much as 5% of the fish's body weight per day for ten days; immersing was done by immersing the fish in the vaccine suspension for 30 minutes. The vaccine formulation was 10mL at a 10^8 CFU/mL density mixed with 990mL of PBS solution. The booster was carried out in the 1st week, using the same method and dosage as for the injection and bath treatment.

Challenge tests

The challenge tests were carried out on all treatments in the 3rd week by injecting 0.1mL of active *A. veronii* bv *veronii* bacteria per fish at a dose of 10^7 CFU/mL. Observations were collected by examining African catfish's clinical symptoms and survival for one week.

Research parameters

The main parameters used in research are antibody titer, survival rate (SR), relative percent survival (RPS), mean time to death (MTD), and growth rate (fish weight

and length increase). Water quality measures, such as temperature, pH, and dissolved oxygen levels, support the research.

Data analysis

The main parameter data were examined using analysis of variance (ANOVA) and the Duncan multiple range test (DMRT) at a 5% level. The supporting parameter data were evaluated descriptively and quantitatively.

RESULTS AND DISCUSSION

Titer antibody

This research has successfully vaccinated African catfish with *A. veronii* bv *veronii* by im and ip injection, oral, and immersion (Brudeseth et al. 2013; Embregts and Forlenza 2016). The antibody titers produced were significantly different between the vaccination treatment and the control, which were observed every week, and there was a trend of increasing antibody titers in vaccinated fish until the end of the study (Table 1). On week 0, the antibody titer was still low, ranging from 2^0 - 2^1, and was not significantly different (P>0.05). On week 1 (one week after vaccination), there was an increase in the antibody titer of vaccinated fish (P<0.05). The intramuscular injection vaccination treatment was significantly different from the control. However, the other vaccination protocols were not significantly different (P>0.05). On week 2 (one week after the booster immunization), the vaccination treatment had a higher antibody titer than the control group (P<0.05). At the same time, the im and ip injections were not significantly different (P>0.05). On week 3 (two weeks after booster vaccination), antibody titers increased significantly compared to controls (P<0.05).

Table 1: Measurement results of antibody titers

Treatments	Replicates	Weeks				
		0	1	2	3	4
T1	1	2^1	2^3	2^6	2^8	2^{10}
	2	2^1	2^2	2^6	2^9	2^9
	3	2^0	2^3	2^7	2^8	2^{10}
	ñ	$\mathbf{2^{0.74a}}$	$\mathbf{2^{2.74a}}$	$\mathbf{2^{6.42a}}$	$\mathbf{2^{8.42a}}$	$\mathbf{2^{9.74a}}$
T2	1	2^1	2^3	2^6	2^9	2^{10}
	2	2^0	2^2	2^6	2^8	2^9
	3	2^0	2^2	2^7	2^8	2^8
	ñ	$\mathbf{2^{0.41a}}$	$\mathbf{2^{2.42ab}}$	$\mathbf{2^{6.42a}}$	$\mathbf{2^{8.42a}}$	$\mathbf{2^{9.22ab}}$
T3	1	2^0	2^3	2^4	2^4	2^5
	2	2^1	2^2	2^3	2^4	2^4
	3	2^1	2^2	2^3	2^3	2^4
	ñ	$\mathbf{2^{0.74a}}$	$\mathbf{2^{2.42ab}}$	$\mathbf{2^{3,42c}}$	$\mathbf{2^{3.74b}}$	$\mathbf{2^{4.42c}}$
T4	1	2^1	2^2	2^5	2^7	2^8
	2	2^1	2^2	2^4	2^7	2^8
	3	2^1	2^3	2^5	2^8	2^9
	ñ	$\mathbf{2^{1a}}$	$\mathbf{2^{2.42ab}}$	$\mathbf{2^{4.74b}}$	$\mathbf{2^{7.42a}}$	$\mathbf{2^{8.42b}}$
T5	1	2^1	2^1	2^1	2^0	nd
	2	2^0	2^1	2^1	2^0	nd
	3	2^1	2^2	2^2	2^1	nd
	ñ	$\mathbf{2^{0.74a}}$	$\mathbf{2^{1.42b}}$	$\mathbf{2^{1.42d}}$	$\mathbf{2^{0.41c}}$	nd

Note: nd = no data. The average number with the same superscript letters shows an effect that is not significantly different at the 5% test level.

The antibody titer produced by injection and immersion treatments was higher than oral, while the antibody titer produced by the control decreased from the previous week. On week 4 (one week after the challenge), antibody titers continued to increase for all vaccination treatments (P<0.05). The intramuscular and intraperitoneal injection treatments showed no meaningful difference (P>0.05). They produced the highest antibody titers, ranging from $2^{9.22}$ to $2^{9.74}$, while the immersion treatment was relatively the same (P>0.05) as intraperitoneal injection and significantly different from oral (P<0.05). However, antibody titer data for the control group was not available because, after the challenge test, all the control fish died.

On week 0, antibody titers tended to be low because all those treated had not been vaccinated. In nature, various natural antigens in the form of bacteria and organic compounds can stimulate fish to form antibodies so that naturally (without vaccination), they will have antibodies even though they are deficient. The antibody titer formed in week 0 is a natural response of the fish's body (Zhang et al. 2020; Mulia et al. 2022; Wu et al. 2024).

The research results revealed that vaccination could increase the immune response of fish, either by injection (im, ip), oral, or immersion (Table 1). Previous research expanded the antibody titer of *Carassius auratus* and *Misgurnus anguillicaudatus* after being vaccinated with *A. veronii* using a different vaccine administration route (Zhang et al. 2020; Wu et al. 2024). Giving a vaccine (antigen) into the host's body will stimulate the immune system to produce antibodies. The body stimulates diverse immune responses to protect itself from the detrimental effects of invading pathogens or infectious organisms. The host immune system recognizes foreign molecules, and the following immune response is related to macrophages, B-cells, or T-cells (Parija 2023).

Booster vaccination with several vaccination methods can increase antibody titers, and more antibodies are formed than before the booster (Pereira et al. 2015). Under optimum conditions, which are two or three weeks following stimulation, specific antibodies will provide immunity (Wu et al. 2024). Previous research also reported that booster vaccination could increase antibody titers for *C. gariepinus* and *Pangasius hypothalamus* (Mailani et al. 2020; Mulia et al. 2022).

Vaccination by injection is more effective than the oral and immersion methods, as seen from the higher antibody titers at the end of the study. This is thought to be because the diffusion of the vaccine by injection into the body is constant to stimulate antibodies and protect the body of African catfish against bacteria (Mulia et al. 2016). This is based on previous research, where the highest vaccine efficacy was produced by injection, followed by immersion, and then oral (Sugiani et al. 2015). However, in contrast to Wu et al. (2024), *A. veronii* vaccination can increase *C. auratus* antibody titers with the highest value resulting from i.p injection, followed by i.m and oral injection, and the lowest is immersion. An i.m. injection is an injection into a muscle, typically the muscle at the base of a fish's dorsal fin or tail fin, whereas an i.p. injection is commonly performed in the peritoneum near the base of the pelvic fins. The injection procedure produces substantial immune protection and long-lasting immunity; nevertheless, the injection process is time-consuming, labor-intensive, and highly stressful for the fish (Zhang et al. 2021).

Oral vaccination entails placing the vaccine into the feed and giving it while the fish are fed. This vaccination method is easy to administer, can save a lot of energy, can avoid fish stress, can be done on various sizes of fish, and can be given to many fish (Zhang et al. 2021; Gonçalves et al. 2022). Oral *A. veronii* bv *veronii* vaccination can increase *C. auratus* antibody titers until the 4th week. Vaccinated feed-based fish can produce mucosal and systemic immune responses, which protect fish from pathogens and limit systemic infection outbreaks (Kaur et al. 2021). However, the oral technique needs fishing operations, utilizes vast amounts of vaccine, and the vaccine components are easily destroyed by gastrointestinal proteases, losing immunogenicity and resulting in a relatively modest immune protection effect; the protection time is short compared to injection (Hart et al. 1988; Zhang et al. 2021). This is related to the degradation of antigens in the harsh stomach environment and the highly tolerogenic intestinal environment (Rombout and Krion 2014).

Immersion vaccination involves immersing the fish in water carrying the vaccine for a set period, which requires less effort, allows for immunization during transportation, causes less injury to the fish, and even avoids catching the fish during vaccination. However, immersion immunization requires enormous volumes of vaccines and frequently provides low protection and a short duration of immunity (Zhao et al. 2019). Several vaccine methods positively impact the safety of fish from disease attacks, but each has its weaknesses. Therefore, it is necessary to consider the vaccination method chosen, adjusted to the size of the fish, number of fish, level of difficulty, and human skills (Pessoa et al. 2019).

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

The survival rate of catfish at T1 reached the highest value, namely 90.00%, T4 reached 80.00%, T2 reached 53.33%, and T3 reached 36.67%, while the control group (T5) was 0% (nothing survived) (Table 2). The study found that providing the *A. veronii* bv *veronii* vaccine significantly improved catfish survival rates ($P<0.05$) compared to the control group. Previous research also reported that loach fish vaccinated with *A. veronii* antigen produced the highest survival rate of up to 65.66%, compared to the control group of 0% (Zhang et al. 2020). Crucian carp (*C. carassius*) vaccinated with *A. veronii* resulted in 73.3% survival, compared to 0% in the control group, as all fish died after the challenge test (Zhao et al. 2021).

Table 2: Survival rate, RPS, and MTD of catfish

Treatment	Survival rate (%)	RPS (%)	MTD (day)
T1	90.00±10.00^{a}	90.00±10.00^{a}	1.17±1.04^{a}
T2	53.33±5.77^{b}	53.33±5.77^{b}	1.37±0.15^{a}
T3	36.67±.5.77^{c}	36.67±5.77^{c}	1.88±0.24^{a}
T4	80.00±10.00^{a}	80.00±10.00^{a}	1.94±0.42^{a}
T5	0.00±0.00^{d}		1.30±0.10^{a}

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

The results of the investigation revealed that i.m injection (T1) and immersion (T4) treatments produced the highest survival (80-90%) and were significantly different from i.p injection (T2) with lower survival reaching 53.33% and oral (T3) reaching 36.67% ($P< 0.05$). This is because the difference in effectiveness of oral vaccines is lower compared to injection and immersion methods. Oral vaccination in rainbow trout (*Oncorhynchus mykiss*) is less effective than immersion and injection vaccination, resulting in a more minor and statistically insignificant immune response (Jaafar et al. 2019). Vaccination using the immersion method is more effective, with higher survival values, namely 46.7 and 53.3%, compared to oral, which only reaches 20%. On the other hand, administering vaccines by injection is a more effective method because it has a higher survival rate, reaching 58-76% (Kole et al. 2019).

Another obstacle in oral vaccination with vaccinated feed is an insufficient and inconsistent response due to antigen damage in the intestine (Hølvold et al. 2014). Oral vaccination is less optimal than injection and immersion methods due to the large surface area of the intestine, the possibility of antigen breakdown in the gastrointestinal tract, and a tolerogenic environment because the antigen will be digested by gastrointestinal enzymes (Embregts et al. 2018). Several other problems associated with administering oral vaccines are competition between more significant and more substantial fish that will prey on more food and that each fish has a different appetite (Sugiani et al. 2015). African catfish with a good appetite and eating more food will have better immunity, while other fish have lower immunity.

The i.m injection vaccination resulted in higher survival than i.p ($P<0.05$). Vaccination with intraperitoneal injection has the potential to bypass the initial barrier of defense (skin and mucous) and enter directly into the blood arteries and interior organs, thereby increasing survival outcomes and RPS (Theeraporn et al. 2020). However, in this study, the results of peritoneal injection were lower than those of intramuscular injection. This is thought to be due to injury to the internal organs of the stomach during the injection. In line with research by Wal et al. (2021), intraperitoneal injection of vaccines can cause injury to the fish's peritoneal cavity, so when infected with bacteria, it can cause bleeding in the liver and cause death of the fish. The research results of Noia et al. (2014), injecting fish with a needle aimed at the anterior peritoneal cavity causes damage and internal adhesions in turbot fish (*Scophthalmus maximus*).

RPS is crucial for evaluating vaccine efficacy (Monir et al. 2021). Treatment T1 achieved the highest level of protection, with an RPS value of 90.00%, T4 reached 80.00%, T2 reached 53.33%, and T3 reached 36.67%. The research results show that vaccination protects from attacks by *A. veronii* bv *veronii* with different vaccination methods. The i.m injection therapy had the highest RPS and was substantially different ($P<0.05$) from the i.p and oral injections but not from immersion ($P>0.05$). Each treatment has a different level of protection against MAS disease attacks. In line with research by Zhang et al. (2020), the RPS against *A. veronii* infection in the injection group with a dose of 0.1mL 10^{7} CFU/mL was 65.66%, while the immersion group with a dose of 2×10^{7} CFU/mL in 2L aerated water was 50.78%, with a level survival in the control group was 0%. Research by Kole et al. (2019) shows that the relative protection level of vaccination with the immersion method is more effective, with an RPS of

46.7% (without booster) and 53.3% (with booster), compared to oral vaccination with an RPS of 20%. *A vaccine* is considered good if it produces RPS ≥50% (Sughra et al. 2021). The RPS value produced by African catfish shows that vaccination can increase the immune response by forming antibodies to protect the body so that the fish are more resistant to bacterial attacks during the challenge test (Mulia et al. 2022).

After the challenge test, the MTD of African catfish varied from 1.17 to 1.94 days, with no significant difference between treatments (P<0.05). The research results demonstrate that the vaccination successfully controls *A. veronii* bv *veronii*, reducing the number of deaths and influencing the MTD value. Previous studies also found that immunization did not significantly affect the MTD value of African catfish (*C. gariepinus*) and *Pangasius hypophthalmus* (Mulia and Purbomartono 2007; Mailani et al. 2020). Vaccination only protects fish from bacterial attacks, and if vaccinated fish are attacked, then the vaccination treatment has no natural effect on the development of the disease. As a result, the MTD of vaccinated fish does not differ from that of unvaccinated fish (Mulia and Purbomartono 2007). Therefore, although vaccination is a valuable tool in disease prevention, it may not guarantee complete immunity in all methods. However, it can still significantly impact disease in vaccinated fish.

The growth rate of African catfish

Fish growth is characterized by an increase in the weight and length of the fish during maintenance. The weight gain of African catfish in the T3 treatment was 32.40g, followed by T4, T2, T1, and T5 at 25.10, 24.90, 23.90, and 21.70g (Table 3). The T2, T3, and T4 treatments differed considerably (P<0.05) from the control (T5). The immunization significantly increased the weight gain of African catfish (P<0.05). Vaccination directly affects the immune system and stimulates metabolism so that fish growth is optimal (Pane et al. 2021). Vaccination in crucian carp (*C. auratus*) resulted in considerable weight gain (P<0.05) compared to the control group (Kong et al. 2020). Vaccination, however, did not affect the increase in fish length, as previously reported (Sughra et al. 2021; Mulia et al. 2022). The findings of this study suggest that administering the vaccination does not interfere with the growth of vaccinated fish. Skinner et al. (2008) also found that immunization had no harmful influence on Atlantic salmon development. Based on these findings, it may be inferred that vaccination can boost the fish's immune system without negatively impacting fish growth.

Table 3: Growth rate of African catfish

Treatment	Growth Rate	
	Weight gain (g)	length gain (cm)
T1	23.90±0.66^{ab}	4.40±0.89^{a}
T2	24.90±2.32^{b}	5.80±0.36^{a}
T3	32.40±1.20^{c}	4.70±1.57^{a}
T4	25.10±1.00^{b}	3.80±0.49^{a}
T5	21.70±1.14^{a}	3.50±0.12^{a}

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

Parameter of water quality

Several factors influence the effectiveness of vaccination in African catfish cultivation. Water temperature, size, and fish species directly affect the fish's immune response and should always be considered when vaccinating (Olsen et al. 2024). Table 4 displays the results of testing water quality indicators, including temperature (25.7-29.9ºC), dissolved oxygen (6.2-8.4ppm), and pH (6.6-8.3). The results demonstrated a tiny fluctuation between treatments but are still within normal limits. The oxygen content value that meets the quality standards for African catfish, according to the National Standardization Agency (NSA), is >3ppm, and the pH ranges from 6.5 to 8.5 (Jailani et al. 2020). The range of dissolved oxygen levels that is good for the growth of African catfish is 4.2-7.7mg/L (Jailani et al. 2020).

Table 4: Parameter of water quality

Treatment	Parameter of water quality		
	Temperature (°C)	Dissolved oxygen (ppm)	Acidity (pH)
T1	25.7 – 29.4	6.2 – 8.2	6.6 – 7.9
T2	25.9 - 29.8	6.2 – 8.2	6.8 – 8.0
T3	25.8 – 29.5	6.7 – 8.2	6.9 – 8.0
T4	26.4 – 29.3	6.6 – 8.4	6.7 – 8.2
T5	26.0 – 29.9	6.5 – 8.3	6.8 – 8.3

Conclusions

This study successfully documented the vaccine effectiveness of *A. veronii* bv *veronii* BmCL-03 in increasing African catfish antibody titers. The *A. veronii* bv *veronii* vaccine also protected fish with the best survival rate, 90.00% by i.m injection (T1) and 80% by immersion (T4), which differed considerably from i.p injection (T2) and oral (T3). The high survival rate positively impacted the RPS value, ranging from 80.00-90.00% for i.m. injection and immersion, 53.33% for i.p. injection, and 36.67% for oral administration. This study found that MTD values did not change significantly between immunization regimens. Vaccination has no harmful impact on the growth of African catfish. In comparison to other immunization methods, intramuscular injection is the most effective. The *A. veronii* bv *veronii* vaccine is a potential vaccine product that can improve African catfish's immune system, SR, and RPS.

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Authors contribution

DSM: conceived and designed, and performed the experiments, analyzed the data, prepared figures and tables, authored and revised the manuscript, and approved the final draft. AZL: performed the experiments, analyzed the data, and approved the final draft. S: supervised the experiment, reviewed the manuscript, and approved the final draft. CP: reviewed the drafts and approved the final manuscript. AS: reviewed drafts of the paper and approved the final manuscript. O: reviewed the drafts and approved the final manuscript.

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