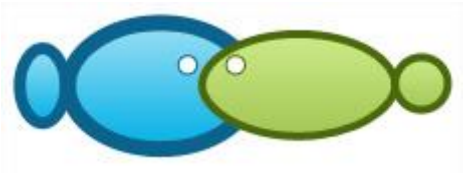

The efficacy of *Aeromonas jandaei* BmSL-02 vaccine to control Aeromoniasis disease on North African catfish (*Clarias gariepinus* (Burchell 1822))

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Abstract. *Aeromonas jandaei* is a Gram-negative bacterium of the genus *Aeromonas* that causes aeromoniasis or motile *Aeromonas* septicemia (MAS) in North African catfish (*Clarias gariepinus* (Burchell 1822)). Vaccination can be chosen to prevent adverse effects due to attacks by pathogenic bacteria. The study aims to determine the *Aeromonas jandaei* BmSL-02 vaccine's efficacy in controlling MAS disease in North African catfish. The type of vaccine is a whole-cell vaccine and is an attenuated vaccine. The samples used were North African catfish measuring 17.35-18.95 cm in length, and weighing 29.80-44.60 g, obtained from aquaculture ponds in the Banyumas area, Central Java. The study used an experimental method with a completely randomized design (CRD), five treatments, and three replications. The treatments consisted of J1: intramuscular injection (i.m); J2: intraperitoneal injection (i.p); J3: oral; J4: immersion; 5: without vaccination (control). Vaccination injection was done by injecting the vaccine into the fish's body with a dose of 0.1 mL at a density of 10^8 CFU mL^{-1}. A feed-based vaccine was prepared by spraying a vaccine suspension with a density of 10^8 CFU mL^{-1} in 100 mL of sterile PBS into 100 g of feed coated with 10 mL of egg white. The vaccine formulation for immersion was 10 mL at a density of 10^8 CFU mL^{-1} mixed with 990 mL of PBS solution. Booster vaccination was carried out one week after using the same method, except oral administration was given for the first 10 days. Challenge tests were carried out in the third week by injecting 0.1 mL of *A. jandaei* suspension per fish with a 10^7 CFU mL^{-1} dose in all treatments. The main parameters of the study included 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, namely water temperature, pH, and dissolved O_2 levels. The main parameter data were analyzed by analysis of variance (ANOVA) and Duncan multiple range test (DMRT) at a test level of 5%. Supporting parameter data were descriptively and quantitatively analyzed. The results showed that the *A. jandaei* BmSL-02 vaccine was significantly different ($P<0.05$) and could increase antibody titer, survival value, RPS value, MTD, and length gain of North African catfish. However, fish weight gain was not significantly different ($P>0.05$). Vaccination has no negative impact on the growth of North African catfish. *A. jandaei* vaccine can improve North African catfish's immune system, survival, and RPS. *A. jandaei* vaccine is one of the potential vaccine products that can control aeromoniasis in North African catfish.
Key Words: *Aeromonas jandaei*, fish disease, Gram-negative bacteria, fish species, physiological status.

Introduction. One of the bacterial diseases that often attacks freshwater fish is *Aeromonas* spp. and the disease is called aeromoniasis or motile *Aeromonas* septicemia (MAS). Several species of *Aeromonas* spp. are known to be pathogenic and infect farmed fish (Pessoa et al 2019; Eid et al 2022; Hu et al 2023; Mulia et al 2023; Abdella et al 2024). One species that has been successfully isolated from freshwater fish is *Aeromonas jandaei* (Azzam-Sayuti et al 2021a; Mulia et al 2024). *A. jandaei* infection causes clinical signs of depigmentation on the

body, erosion, lesions on the back, ulcers all over the body, brittle fins, hyperemia, hemorrhage, and abscesses with a mortality rate of 100% (Dwi et al 2023). *A. jandaei* is not as popular as *A. hydrophila*, but recent findings have shown that *A. jandaei's* activity causes fish to become sick and eventually die (Azzam-Sayuti et al 2021a; Dwi et al 2023).

The ability of *Aeromonas* spp. to infect the host is thought to be influenced by its pathogenicity (Ahangarzadeh et al 2022). The pathogenicity mechanism of *Aeromonas* is quite complex, one of which is related to virulence factors (Mulia et al 2020; Azzam-Sayuti et al 2021b). The results of molecular identification of bacteria that cause catfish disease found five strains of *A. jandaei* with varying virulent genes, namely *aer/haem*, *flaa*, and *lafa* (Mulia et al 2024), while four strains of *A. jandaei* isolated from Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852), were known to have virulent genes *aeraA*, *hlyA*, *eprCAI*, and *nuc*, but the *ast*, *act*, *ahp*, and *lip* genes were not detected. Meanwhile, the *lafB* and *alt* genes were only detected in one strain, and the *ascV* gene was detected in two strains. In another study, *A. jandaei* isolated from Arapaima, *Arapaima gigas* (Schinz, 1822), detected the virulent genes *aerA*, *gcat*, *lip*, *Dnase*, and *hlyA*, but no *alt*, *act*, and *ser* genes were found (Proietti-Junior et al 2020).

Aeromonas infection is detrimental and causes mass mortality in farmed fish reaching 80%-100% (Shameena et al 2020). Clinical signs of fish infected with *A. jandaei* include changes in skin color accompanied by loss of scales, exophthalmia, erosion of the fins, lesions on the back, hyperemia, hemorrhagic, ulcers, abscesses, ulcers, fin and tail rot, necrotic liver, hemorrhagic kidneys and liver, yellow exudate, decreased appetite, and weight loss, abnormal behavior such as irregular swimming, including excessive mucus secretion on the fish's body (Assane et al 2021; Mazumder et al 2021; Dwi et al 2023). To overcome the attack of *Aeromonas* spp. can be done by improving growth technology management, and providing immunostimulants and probiotics (Isnansetyo et al 2016; Krishnan & Raja 2021). In addition, fish vaccination can also be carried out (Zhang et al 2020; Yuan et al 2022).

Vaccination is one way to provide stimulation or antigens intentionally so that the body can increase the immune system by producing antibodies against a disease germ or pathogen (Du et al 2022; Mulia et al 2022). For decades, research on the *A. hydrophila* vaccine has proven to be able to control aeromoniasis in fish (Austin & Austin 2016; Mulia et al 2022). In recent years, *A. veronii* infection has caused huge losses to the aquaculture industry (Xu et al 2019), therefore research on *A. veronii* vaccines has also been widely carried out and the results are significant (Zhang et al 2020; Zhao et al 2021; Wu et al 2024). A bivalent vaccine of *A. hydrophila* and *A. veronii* has been tested to control aeromoniasis in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). The results showed that this bivalent vaccine candidate was effective against *A. veronii* and *A. hydrophila* infections in tilapia. However, to obtain promising vaccine efficacy, it is necessary to optimize the vaccine formulation and validate the vaccine efficacy in field trials (Anantasuk et al 2024). Research related to the *A. jandaei* vaccine has not been conducted. However, the virulence potential of this bacteria is very large, so that this research is important to do. Therefore, a study of *A. jandaei* vaccination has been conducted with several vaccination methods, namely intramuscular injection (i.m), intraperitoneal injection (i.p), oral with feed-based vaccine, and immersion. The study aims to determine the efficacy of the *A. jandaei* BmSL-02 vaccine in controlling aeromoniasis in North African catfish (*C. gariepinus*).

Materials and Method

Samples. The vaccine material used is an isolate of *A. jandaei* strain BmSL-02. The sample used is North African catfish (*C. gariepinus*), measuring 17.35-18.95 cm long and weighing 29.80-44.60 g, obtained from aquaculture ponds in Banyumas, Central Java.

Design research. Design research using experimental method with completely randomized design (CRD), five treatments, and three replications. Treatments consist of J1: intramuscular injection (i.m); J2: intraperitoneal injection (i.p); J3: oral; J4: immersion; J5: without vaccination (control). Each sample unit is kept with ten North African catfish.

Preparing Aeromonas jandaei BmSL-02 vaccine. The *A. jandaei* vaccine was made based on the modification of Mulia et al (2022). Whole-cell vaccine is made by inactivating bacteria using 3% formalin. Isolate *A. jandaei* BmSL-02 was streak cultured on glutamate starch phenyl (GSP) medium (Merck) at 30ºC for 24 h. One colony was grown on 10 mL of tryptic soy broth (TSB) medium (Merck) and incubated at the same temperature and time. The bacterial suspension was vortexed and then poured onto tryptic soy agar (TSA) medium (Merck) in a giant petri dish and incubated at 30ºC for 24 h. Furthermore, the bacteria were harvested by gently scraping using a Drigalsky spatula with the addition of phosphate buffer saline (PBS) so that all bacteria could be taken. The harvested bacteria were added with 3% formalin and shaken at 150 rpm for 24 h. The culture results were centrifuged for 20 minutes at 3,000 rpm. The supernatant was discarded, while 3 mL of PBS was added to the pellet (debris) and centrifuged again. Washing with PBS was carried out 3 times to obtain a vaccine free from formalin. The viability of *A. jandaei* was tested by regrowing the bacteria in a GSP medium and incubating for 48 h. *A. jandaei* bacteria were declared ready as a vaccine if they did not grow in the GSP medium.

Preparing the feed-based Aeromonas jandaei BmSL-02 vaccine. The feed-based vaccine was made by adding the vaccine to the fish feed (FF999, PT Central Proteina Prima, Lampung). A total of 100 g of feed was mixed with 10 mL of egg white until evenly distributed, then sprayed with the vaccine suspended in a sterile PBS solution of as much as 100 mL at a density of 10^8 CFU mL^{-1}. The vaccine feed was then aired until dry (Mulia et al 2022).

Vaccination of Aeromonas jandaei BmSL-02 on North African catfish. The vaccination method was carried out in several ways, namely intramuscular injection by injecting the vaccine into the fish's body intramuscularly with a dose of 0.1 mL at a density of 10^8 CFU mL^{-1}; intraperitoneal injection by injecting the vaccine into the fish's body with a dose of 0.1 mL at a density of 10^8 CFU mL^{-1}, oral is done by giving feed-based vaccine as much as 5% of the fish's body weight per day for 10 days; and immersion is done by immersing the fish in the vaccine suspension for 30 minutes. The vaccine suspension is 10 mL at a 10^8 CFU mL^{-1} density mixed with 990 mL of PBS solution. After the fish are vaccinated, a week later, a booster vaccination (repeat vaccination) is carried out in the same manner and dose as the injection and immersion treatments.

Challenge test on North African catfish after vaccination. The challenge test was carried out on all treatments in the third week by injecting 0.1 mL of active *A. jandaei* BmSL-02 bacteria per fish with a dose of 10^7 CFU mL^{-1}. Observations were made by observing clinical signs and survival of North African catfish for one week.

Research parameters. The main parameters used in the research were antibody titer, survival rate, relative percent survival (RPS), mean time to death (MTD), and growth rate (weight and length gain of fish). The supporting parameters for the research were water quality parameters, such as water temperature, water pH, and dissolved O_2 levels.

Data analysis. The main parameter data were analyzed using analysis of variance (ANOVA) and Duncan multiple range test (DMRT) at a test level of 5% (Zhu et al 2021). The supporting parameter data were descriptively and quantitatively analyzed.

Results

Titer antibody. The results showed that in week 0, antibody titers were still low, ranging from 2^0 to $2^{1.72,}$ and were not significantly different ($P>0.05$) (Table 1). In week 1 (one week after vaccination), there was an increase in antibody titers of vaccinated fish ($P<0.05$) compared to the control. The i.m injection vaccination treatment was not significantly different from the i.p injection but was higher and significantly different from the immersion and oral ($P>0.05$). In week 2 (one week after booster vaccination), there was an increase in antibody titers of all vaccination treatments compared to the control ($P<0.05$). However,

there was no significant difference between vaccination treatments (P>0.05). In week 3 (two weeks after booster vaccination), antibody titers continued to increase compared to the control (P<0.05); the i.m. and i.p.injection treatments were not significantly different (P>0.05) and higher (P<0.05) compared to immersion and oral. In the fourth week (one week after the challenge test), antibody titers continued to increase for all vaccination treatments (P<0.05), while all control fish died. The i.p. injection treatment produced higher antibody titers (29.74) but was not significantly different from the i.m injection (29.42) (P>0.05). However, both injection methods produced higher antibody titers than oral (28.12) and immersion (27.74) (P<0.05). However, there was no data on antibody titers for the control group because all control fish died after the challenge test.

Table 1

Measurement results of antibody titers

Treatment	*Replicates*	*Weeks*				
		0	*1*	*2*	*3*	*4*
J1	1	2^{0}	2^{5}	2^{6}	2^{8}	2^{9}
	2	2^{0}	2^{5}	2^{7}	2^{9}	2^{10}
	3	2^{1}	2^{4}	2^{6}	2^{7}	2^{9}
	ñ	$2^{0,42a}$	$2^{4.74d}$	$2^{6.42b}$	$2^{8.22c}$	$2^{9.42ab}$
J2	1	2^{0}	2^{5}	2^{7}	2^{8}	2^{10}
	2	2^{1}	2^{4}	2^{6}	2^{9}	2^{10}
	3	2^{0}	2^{4}	2^{6}	2^{8}	2^{9}
	ñ	$2^{0.42a}$	$2^{4.42cd}$	$2^{6.42b}$	$2^{8.42c}$	$2^{9.74b}$
J3	1	2^{2}	2^{3}	2^{7}	2^{7}	2^{9}
	2	2^{2}	2^{4}	2^{7}	2^{7}	2^{8}
	3	2^{1}	2^{3}	2^{5}	2^{5}	2^{6}
	ñ	$2^{1.72a}$	$2^{3.42bc}$	$2^{6,59b}$	$2^{6,59b}$	$2^{8.12a}$
J4	1	2^{0}	2^{3}	2^{7}	2^{7}	2^{8}
	2	2^{0}	2^{2}	2^{6}	2^{6}	2^{7}
	3	2^{0}	2^{2}	2^{6}	2^{6}	2^{8}
	ñ	2^{0a}	$2^{2.42b}$	$2^{6.42b}$	$2^{6.42b}$	$2^{7.74a}$
J5	1	2^{0}	2^{0}	2^{0}	2^{0}	nd
	2	2^{1}	2^{1}	2^{1}	2^{1}	nd
	3	2^{1}	2^{1}	2^{1}	2^{0}	nd
	ñ	$2^{0.72a}$	2^{a}	2^{a}	2^{a}	nd

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

Survival rate, RPS, and MTD of North African catfish. The results showed that the i.m. injection (J1) and i.p injection (J2) treatments produced the highest survival (66.67%-70.00%) and were significantly different (P<0.05) from immersion (J3) and oral (J4) with survival reaching 36.67%. Meanwhile, all control fish died (survival 0%) after the fish were challenged (Table 2). The i.m injection (J1) and i.p injection (J2) treatments achieved the highest level of protection, with RPS values of 66.67% and 70.00%, while the immersion (J3) and oral (J4) treatments had lower RPS, which was 36.67%. MTD of North African catfish after the challenge test ranged from 1.93-2.68 days, and the injection treatment was not significantly different from the control (P>0.05). However, it significantly differed from the oral and immersion treatments (P<0.05).

Table 2

Survival rate, RPS, and MTD of North African catfish

Treatment	*Survival Rate (%)*	*RPS (%)*	*MTD (day)*
J1	66.67±5.77^{c}	66.67±5.77^{b}	1.81±0.17^{a}
J2	70.00±10.00^{c}	70.00±10.00^{b}	1.83±0.29^{a}
J3	36.67±5.77^{b}	36.67±5.77^{a}	2.68±0.18^{b}
J4	36.67±5.77^{b}	36.67±5.77^{a}	2.57±0.35^{b}
J5	0.00±0.00^{a}		1.93±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.

The growth rate of North African catfish. The results showed that the weight gain of North African catfish treatment J5 reached 31.70 g, followed by J4, J3, J2, and J1, each 30.70, 30.17, 27.47, and 25.87 g, but between treatments were not significantly different (P>0.05) (Table 3). However, the increase in the length of North African catfish experienced a significant difference between treatments J1, J3, and J5 which were significantly different from J2 and J4 (P<0.05). The growth rate of treatment J5 (control) was obtained from the difference between the data from week 0 and week 3.

Table 3

The growth rate of North African catfish

Treatment	*Growth Rate*	
	Weight Gain (g)	*Length Gain (cm)*
J1	25.87±0.95^{a}	3.72±0.12^{a}
J2	27.47±0.90^{a}	4.67±0.55^{b}
J3	30.17±8.81^{a}	3.48±0.67^{a}
J4	30.70±7.50^{a}	5.48±0.76^{c}
J5	31.70±4.40^{a}	3.28±0.41^{a}

Parameter of water quality. Water quality parameters are strictly checked to maintain optimal maintenance environmental conditions. The water quality parameter measurements results, namely water temperature ranges from 25.4-29.8ºC, dissolved oxygen 6.4-8.2 ppm, and pH 6.5-8.2 (Table 4).

Table 4

Parameter of water quality

Parameter of Water Quality	*Treatment*					*NSA (2000)*	*FADC (2016)*
	J1	*J2*	*J3*	*J4*	*J5*		
Temperature (^{0}C)	25.5-29.2	25.8-29.8	25.9-29.6	25.4-29.2	26.8-29.6	25-30	20-30
Dissolved Oxygen (mg L^{-1})	6.7-8.1	6.6-8.0	6.7-8.0	6.7-8.3	6.4-8.2	>4	3-6
Acidity (pH)	6.7-7.9	6.5-8.0	6.7-8.1	6.5-8.0	6.9-8.2	6.5-8.5	6.5-8.5

Discussion. *A. jandaei* vaccination on North African catfish successfully increased the antibody titer of North African catfish for each week and was significantly different between vaccination treatments and controls (Table 1). Vaccination was carried out using four vaccine methods, such as intramuscular (i.m) and intraperitoneal (i.p) injections, oral, and immersion (Embregts & Forlenza 2016; Mulia et al 2022; Wu et al 2024). The vaccination results also showed an increasing trend in the antibody titer of vaccinated fish until the end of the study.

In week 0, the fish had not been vaccinated, so the antibody titer was low, namely 20-21.72 (Table 1). Naturally, all organisms have a body defense system that can protect their bodies from pathogen attacks, so they can produce antibodies even though they are deficient. The antibody titer formed in week 0 is the fish's natural body response. Likewise, in yellow-head catfish (*Pelteobagrus fulvidraco*) and *C. gariepinus*, the antibody titer formed tends to be low because it has not been vaccinated with *Aeromonas* spp. (Zhang et al 2020; Zeng et al 2021; Mulia et al 2022; Wu et al 2024).

The increase in antibody titer began to appear in the first week. The study's results showed that vaccination could increase the immune response of fish, either by injection (i.m, i.p), oral, or immersion. The vaccine (antigen) is inserted into the fish's body to stimulate the body's immune system to produce antibodies. Antibodies will bind to antigens to eliminate foreign substances harmful to the body, including pathogenic bacteria. The immune response is related to macrophages, T, and B cells (Parija 2023).

Vaccination can be done using several methods. Previous studies reported that different vaccination methods could increase antibody titers of *Misgurnus anguillicaudatus* (Cantor, 1842) and *Carassius auratus* (Linnaeus, 1758) after being vaccinated with *A. veronii* (Zhang et al 2020; Yuan et al 2022; Wu et al 2024). The results of the study showed that vaccination by injection produced higher antibody titers than the oral and immersion methods until the end of the study (week 4), and there was no significant difference between i.m and i.p injections ($P>0.05$). Although in the first week, the antibody titer produced by the oral treatment was higher than the immersion, until the end of the study, the two treatments were not significantly different ($P>0.05$). Injection vaccination is carried out by inserting the vaccine into the body through the bloodstream and constantly running to stimulate better antibodies and protect the fish's body against bacteria (Mulia et al 2016; Monir et al 2020). Previous studies reported that injection vaccination had the highest efficacy, followed by immersion, then oral (Mulia et al 2006; Sugiani et al 2015). However, other studies showed different things, *A. veronii* vaccination with the i.p injection method can increase the antibody titer of *C. auratus* with the highest value, then i.m injection and oral, and the lowest is immersion (Wu et al 2024). Vaccination of *A. caviae* with i.p injection can increase the antibody titer of crucian carp (*C. carassius*) (Yuan et al 2022).

Intramuscular injections are done on the muscles at the base of the dorsal or fishtail fin. Intraperitoneal injections are usually done in the peritoneum, which is located at the base of the abdominal fin. Although the injection requires a lot of work, a long time, and significant stress for fish, this method provides strong and durable immune protection (Monir et al 2020; Nayak 2020; Zhang et al 2021). Oral vaccination is given to fish by inserting a vaccine into the feed and given when the fish is fed. This method is easy to do, saves much energy, avoids stress for fish, and can be given to many fish (Mohamad et al 2021; Zhang et al 2021). Vaccination *A. hydrophila* orally can increase antibody titers *C. gariepinus* (Mulia et al 2022). Antibody titer production increases significantly in *C. auratus* until the fourth week after being vaccinated with *A. veronii* bv *veronii* orally (Wu et al 2024). Feed-based vaccines can protect fish from pathogens and control systemic infection outbreaks by triggering mucosa and systemic immune responses (Kahieshesfandiari et al 2019; Kaur et al 2021). However, gastrointestinal protease can easily damage the vaccine inserted into feed, and immunogenicity is reduced, thus providing less immune protection and shorter protection time than the injection vaccine (Zhang et al 2021). This is caused by the breakdown of antigens in a robust stomach environment and a very tolerant intestinal environment (Rombout & Krion 2014).

Unlike injection vaccination, technical immersion vaccination applications are more accessible and do not require particular expertise. Vaccination with immersion requires many vaccines and often provides low protection and a short duration of immunity. However, this method does not require much labor, allowing vaccination during transportation and minimizing stress, fish, and injury (Zhao et al 2019). Several vaccine methods positively impact the protection of fish from disease attacks, but each has its weaknesses. Therefore, it is necessary to consider the selected vaccination method, adjusted to the size of the fish, the number of fish, the level of difficulty, and human skill (Monir et al 2020).

In the treatment with the i.m and i.p injection vaccination, as well as immersion, a booster vaccination was carried out one week after the first vaccination. Each treatment's booster vaccination method is carried out according to the method used during the initial vaccination. Booster vaccination with several vaccination methods can increase antibody titers and antibodies that are formed more than before the booster (Mulia et al 2006; Pereira et al 2015). Specific antibodies provide immunity to fish in ideal conditions two or three weeks after stimulation (Wu et al 2024). Previous research also reported that booster vaccination could increase antibody titers in *C. gariepinus* and *Pangasianodon hypophthalmus* (Sauvage, 1878) (Mulia et al 2016; Mailani et al 2020). Vaccination efforts to *A. jandaei* showed significant protection against antigens with injection methods that are more effective than other methods. However, vaccinations with various vaccine modes can increase the vaccinated fish's immune system.

The results of the study showed that administering the *A. jandaei* vaccine had a significant effect on the survival rate of North African catfish ($P<0.05$) compared to the control. Previous studies also reported that crucian carp (*C. carassius*) vaccinated with *A. veronii* survived 73.3%, compared to 0% in the control group (Zhao et al 2021). The results showed that the i.m injection (J1) and i.p injection (J2) treatments produced the highest survival (66.67%-70%) and were significantly different ($P<0.05$) from oral (J3) and immersion (J4) (36.67%). This difference was due to the lower effectiveness of the oral and immersion vaccines than the injection method (i.m and i.p). However, in contrast to previous studies, the intraperitoneal vaccine produced a survival of *C. auratus* of 64%, the intramuscular vaccine of 56%, the oral vaccine of 52%, and immersion of 48%. In this study, i.p injection was better than i.m injection, followed by oral and immersion immunization (Wu et al 2024).

This study showed that i.m and i.p injection vaccinations produced higher survival than other vaccination methods ($P<0.05$). The intraperitoneal injection has the potential to bypass the first line of defense (skin and mucous membranes) and enter directly into the blood vessels and internal organs, thereby improving survival outcomes and RPS (Pulpipat et al 2020). The.m injection has the potential to rapidly introduce vaccines through the body system to trigger specific immune responses. At the same time, antigens administered by immersion must first pass through the mucosal barrier before entering the system (Monir et al 2020). Oral vaccination (combining antigen with fish feed) is theoretically the ideal route of vaccine administration (Monir et al 2020). However, the disadvantages of this vaccination are poor and inconsistent responses due to antigen breakdown in the intestine, possible antigen breakdown in the digestive tract and/or a highly tolerogenic environment because gastrointestinal enzymes will digest the antigen, does not induce humoral immunity as well as injection vaccination (Holvold et al 2014; Embregts et al 2018; Monir et al 2020). Another problem associated with oral vaccination is fish competition. Farming technologies involve repeated sorting of fish and grouping them into homogeneous sizes. However, during their growth, some fish have a greater appetite and grow faster, so they will eat more feed-based vaccines (Sugiani et al 2015). North African catfish that have a good appetite and eat more food will have better immunity, while other fish have lower immunity. Vaccination is effective in controlling the specific *A. jandaei* bacterial challenge, while the control group died after the challenge test with active *A. jandaei* bacteria.

The efficacy of the *Aeromonas* spp. vaccines can be indexed from the RPS value (Monir et al 2021; Mulia et al 2022). The study results showed that vaccination protects against *A. jandaei* attacks through different vaccination methods. The i.m injection (J1) and i.p injection (J2) treatments achieved the highest level of protection, with RPS values of 66.67% and 70.00%, while the oral (J3) and immersion (J4) treatments were not significantly different ($P>0.05$) and had a lower RPS, which was 36.67%. This shows that each treatment has a different level of protection against MAS disease attacks. In line with the research of Zhang et al (2020), the RPS against *A. veronii* infection in the injection group was 65.66%, while the immersion group was 50.78%, with a survival rate in the control group of 0%. *A.hydrophila* GPI-04 vaccination resulted in an RPS of 93.33% -100% in vaccinated North African catfish (*C. gariepinus*), while the control was 0% (Mulia et al 2022). Another study using the *A. caviae* vaccine can significantly increase relative

protection by 45%-65% in crucian carp compared to the control (Yuan et al 2022). A vaccine is considered good if it produces an RPS of at least 50%. The RPS value of North African catfish shows that vaccine administration can increase the immune response by producing antibodies to protect the body. This will make fish more resistant to bacterial attacks during the challenge test (Mulia et al 2022).

MTD of North African catfish ranged from 1.81-2.68 days after challenge with active *A. jandaei*. The average time to death of the i.m and i.p vaccine groups was faster than that of the oral and immersion groups. The injection method (i.m and i.p) can easily injure fish compared to oral and immersion methods (Monir et al 2020). However, the vaccine treatment was quite effective in controlling *A. jandaei* so that it could minimize the number of fish deaths. Previous studies have also reported that vaccination significantly affects the MTD value of North African catfish compared to controls (Mulia et al 2022). Vaccination is an essential tool to prevent disease. Although specific methods may not guarantee complete immunity, vaccination can affect serious diseases in vaccinated fish.

Fish growth is an essential process in the life cycle. Fish growth is characterized by an increase in weight and length of fish during maintenance. The results showed that North African catfish experienced weight gain at the end of the study, but there was no significant difference between treatments ($P>0.05$) (Table 3). However, vaccination significantly affected the increase in fish length ($P<0.05$). This indicates that vaccination does not hurt the weight gain of North African catfish. Vaccination directly affects the immune system and stimulates metabolism so that fish growth is optimal (Pane et al 2021). The effect of injection vaccination does not hurt the growth rate of fish. This is different from previous studies, vaccination in crucian carp (*C. auratus*) had a significant impact on weight gain ($P <0.05$) compared to controls (Kong et al 2020). The results of this study indicate that vaccination does not stop the growth of vaccinated fish. Vaccination does not affect the growth of Atlantic salmon (Skinner et al 2008). From these findings, it can be concluded that vaccination can improve the fish's immune system but does not affect fish growth.

Several factors in the North African catfish cultivation process can affect the success rate of vaccination. In the vaccination process, water temperature, size, and fish species directly impact the immune response of fish (Olsen et al 2024). The results of water quality parameter measurements showed that, although there was slight variation between treatments, it was still within the normal range. Previous research results measuring the suitability of groundwater quality for North African catfish cultivation showed a water temperature of 27-28°C, dissolved oxygen levels ranging from 5.8-6.7 mg L^{-1}, and water pH between 7.1-8.1 (Suwarsito et al 2020). According to the National Standardization Agency (NSA) (2000), the quality standard for dissolved oxygen content in North African catfish cultivation is more than four mg L^{-1}, a water temperature between 25-30°C, and a water pH between 6.5 and 8.5, while according to Freshwater Aquaculture Development Center (FADC) (2016), dissolved oxygen levels are 3-6 mg L^{-1}, water temperature between 20-30°C, and water pH between 6.5 and 8.5.

Conclusions. This study successfully documented the effectiveness of the *A. jandaei* BmSL-02 vaccine in increasing the antibody titer of North African catfish. In addition, the *A. jandaei* BmSL-02 vaccine also successfully protected fish with the highest survival rate, which was 66.67% -70% with i.m injection (J1) and ip (J2), and was significantly different from oral (J3) and immersion (J4), which was 36.67%, while the control was 0%. The RPS value produced by the i.m and i.p injection groups was also the highest, 66.67% -70%, while the oral and immersion were 36.67%. This study produced significantly different MTD values between vaccination treatments. Vaccination can significantly differ on the increase in fish length but does not hurt the weight gain of North African catfish. The results of this study concluded that the *A. jandaei* BmSL-02 vaccine is effective in protecting North African catfish from *A. jandaei* attacks. The i.m and i.p injection treatments were the most effective compared to other vaccination treatments. *A. jandaei* vaccine is one of the potential vaccine products that can improve the immune system, survival, and RPS of North African catfish. Vaccination can be used to develop fish health management strategies to protect fish from pathogens and increase cultivation productivity.

Acknowledgements. This research was supported by the PGB program, UMP (No. A.11-III/6405-S.Pj./LPPM/II/2024).

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Received: 4 November 2024; Accepted:27 December 2024; Published online: 27 December 2024.
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How to cite this article:
Mulia D. S., Cahyani G. D., Suwarsito, Purbomartono C., Isnansetyo A., Hanafie A., 2024 The efficacy of *Aeromonas jandaei* BmSL-02 vaccine to control Aeromoniasis disease on North African catfish (*Clarias gariepinus* (Burchell 1822)). AACL Bioflux 17(6):2982-2993.