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Efficacy of *Aeromonas veronii* bv *veronii* BmSG-03 Feed-Based Vaccine on the Immunity of North African Catfish (*Clarias gariepinus* (Burchell 1822))

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ABSTRACT

Motile *Aeromonas* septicemia, caused by *Aeromonas veronii* bv. *veronii* poses a serious threat to freshwater aquaculture. This study evaluated the efficacy of *A. veronii* bv *veronii* BmSG-03 feed-based vaccine on the immunity of North African catfish (*Clarias gariepinus* Burchell 1822). An experimental approach was conducted using a completely randomized design (CRD) with five treatments and three replications. The treatments consisted of: P0 (control, without vaccination); P1 (feed-based vaccine for three consecutive days [days 1–3], with a booster on days 14–16); P2 (vaccine for four consecutive days [days 1–4], with a booster on days 14–17); P3 (vaccine for five consecutive days [days 1–5], with a booster on days 14–18); and P4 (vaccine for seven consecutive days [days 1–7], with a booster on days 14–20). Each experimental unit comprised ten catfish. The main parameters measured were antibody titer and survival rate, while supporting parameters included water temperature, pH, and dissolved oxygen. Primary parameter data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 5% significance level, whereas supporting parameters were analyzed descriptively. The results demonstrated that the *A. veronii* bv. *veronii* BmSG-03 feed-based vaccine was effective in increasing the immunity of North African catfish, enhancing both antibody titers and survival rates ($P< 0.05$) compared with the control. Among the treatments, administration of the vaccine for seven days followed by a seven-day booster (P5) was the most effective. These findings suggest that feed-based vaccines of *A. veronii* bv. *veronii* represent a promising immunoprophylactic strategy for freshwater aquaculture. Moreover, feed-based vaccination offers a practical, economical, and environmentally friendly approach to improving fish health, productivity, and sustainability, particularly in preventing Motile Aeromonas Septicemia in North African catfish farming.

INTRODUCTION

The North African catfish "*Clarias gariepinus* (Burchell, 1822)" is one of the most important freshwater fish species widely cultured in Africa and Southeast Asia, including Indonesia **(FAO, 2025)**. In Indonesia, catfish production has continued to increase, reaching 1,101,625 tons in 2022 and rising by 3.17% to 1,136,619 tons in 2023 **(Central Bureau of Statistics, 2025)**. Among the cultured species, the North African catfish is considered highly promising due to its ease of culture and relatively fast growth performance **(Essien *et al.*, 2024)**. However, the sustainability of its aquaculture is often threatened by infectious diseases, particularly Motile Aeromonas Septicemia (MAS), which is caused by *Aeromonas* spp. MAS is a severe systemic disease affecting freshwater, brackish, and marine fish, with high mortality rates **(Fernández-Bravo & Figueras, 2020)**. Infections with *Aeromonas* spp. can be fatal, causing mortality rates ranging from 80 to 100% in cultured fish **(Shameena *et al.*, 2020; Matter *et al.*, 2024)**. Moreover, this bacterium is zoonotic in humans, associated with gastrointestinal disorders, bacteremia, diarrhea, and hepatobiliary infections **(Wu *et al.*, 2019; Yuwono *et al.*, 2021)**.

Several *Aeromonas* spp. associated with MAS have been isolated from diseased fish, including *A. hydrophila, A. salmonicida, A. caviae, A. sobria, A. veronii, A. jandaei*, and *A. dhakensis* **(Mulia *et al.*, 2020, 2023, 2024; Azzam-Sayuti *et al.*, 2021; Jiang *et al.*, 2023; Rahman *et al.*, 2023)**. Among these, *A. veronii* has emerged as a significant pathogen infecting freshwater fish, with recent reports of large-scale outbreaks **(Li *et al.*, 2020)**. This pathogen causes severe morbidity and mortality in loaches worldwide, leading to significant economic losses in aquaculture **(Xu *et al.*, 2019; Seo *et al.*, 2020)**. In China, *A. veronii* has been reported to infect *Carassius auratus*, *Cyprinus carpio*, *Ctenopharyngodon idella*, and *Silurus asotus* **(Li *et al.*, 2020)**, while studies from Malaysia showed infections in the walking catfish (*Clarias batrachus*), the Nile tilapia (*Oreochromis niloticus*), and the striped catfish (*Pangasianodon hypophthalmus*) **(Azzam-Sayuti *et al.*, 2021)**.

Aeromonas veronii consists of two biovars, namely *A. veronii* bv. *sobria* and *A. veronii* bv. *veronii* **(Shameena *et al.*, 2020)**. Based on *16S rRNA* gene analysis of diseased catfish from several culture centers in West Java, Central Java, and Yogyakarta, as well as gourami from Central Java, *A. veronii* bv. *veronii* was identified among the pathogenic species **(Mulia *et al.*, 2023, 2024)**. This pathogen has been implicated in substantial economic losses in catfish farming due to high morbidity and mortality rates. Conventional control strategies in aquaculture have relied heavily on antibiotics. However, excessive and improper use of antibiotics has resulted in the emergence of antimicrobial-resistant (AMR) bacterial strains, reducing the efficacy of standard treatments and posing a serious risk to animal and public health **(Santos & Ramos, 2018)**. Recent reviews confirm that antibiotics, resistant bacteria, and antimicrobial resistance genes (ARGs) are widely distributed in aquatic environments (water,

sediments, and organisms), thereby increasing the risk of both horizontal and vertical resistance transfer **(Yuan *et al*., 2024)**.

As a sustainable and environmentally friendly approach, vaccination is increasingly recognized as an effective preventive strategy to enhance fish immune responses and to reduce the risk of disease outbreaks. The principle of vaccination involves the controlled administration of antigens to stimulate the immune system, enabling the production of pathogen-specific antibodies **(Mondal & Thomas, 2022; Monir *et al*., 2022; Mulia *et al*., 2022)**. Vaccination in aquaculture has proven effective in reducing pathogen-induced mortality, minimizing antibiotic use, and lowering the risk of antimicrobial resistance development **(Barnes *et al*., 2021)**.

Previous studies reported the efficacy of inactivated *A. veronii* vaccines delivered intraperitoneally in improving the immune response of largemouth bass (*Micropterus salmoides*) **(Zhang *et al*., 2025)**. Likewise, a formalin-inactivated *A. veronii* bv. *veronii* BmCL-03 vaccine was shown to enhance the immune response of North African catfish (*C. gariepinus*) when administered through intraperitoneal and intramuscular injection, immersion, and oral (feed-based) routes **(Mulia *et al*., 2025)**. Among these methods, feed-based vaccination offers several advantages over injection or immersion. It is more practical, as it can be incorporated into routine feed without intensive handling, thereby minimizing fish stress **(Mubeen *et al*., 2025)**. Additionally, oral vaccination allows for mass immunization at lower costs while stimulating mucosal immunity in the gastrointestinal tract, the first line of defense against pathogens, unlike injections that mainly target systemic immunity **(Radhakrishnan *et al*., 2023; Tammas *et al*., 2024)**. Other advantages include reduced risk of mechanical injuries, secondary infections, and improved farmer compliance **(Wu *et al*., 2024)**. Feed-based monovalent *A. hydrophila* vaccines have effectively enhanced immune responses and protected *Ctenopharyngodon idella* against *A. hydrophila* **(Mubeen *et al*., 2025)**. Similarly, the *A. hydrophila* GP1-04 feed-based vaccine improved immune responses and provided disease protection in North African catfish (*C. gariepinus*) **(Mulia *et al*., 2022)**. Based on this background, the present study aimed to evaluate the efficacy of the *A. veronii* bv. *veronii* BmSG-03 feed-based vaccine on the immunity of North African catfish (*C. gariepinus*). The findings are expected to contribute to developing efficient vaccination strategies in catfish aquaculture and promote sustainable fish health management practices.

MATERIALS AND METHODS

Ethical approval

The management, conditions, and procedures of the experiment in this study were approved by the Ethical Clearance Commission of University of Gadjah Mada (approval # certificate: 00137/04/LPPT/I/201).

Samples

The vaccine material used in this study was an *A. veronii* bv *veronii* isolate from strain BmSG-03. The North African catfish (*C. gariepinus*) measuring 10- 13cm long and weighing 11- 12g were collected from agriculture ponds in Sidabowa, 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 P0: without vaccination (control); P1: feed-based vaccine was administered for three consecutive days (days 1–3), followed by a booster on days 14–16 (three days); P2: feed-based vaccine was administered for four consecutive days (days 1–4), followed by a booster on days 14–17 (four days); P3: feed-based vaccine was administered for five consecutive days (days 1–5), followed by a booster on days 14–18 (five days); P4: feed-based vaccine was administered for seven consecutive days (days 1–7), followed by a booster on days 14–20 (seven days). Each sample unit contains ten North African catfish.

Preparation of 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 is made in whole cell form by inactivating bacteria using 3% formalin. *A. veronii* bv *veronii* strain BmSG-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 30°C for 24 hours. The bacteria were then harvested by gently dredging with a drigalski and adding PBS to ensure that all bacteria were collected. The collected bacteria were mixed with 3% formalin and agitated at 2.52 $\times g$ for 24 hours. After centrifuging at 3000 rpm for 20 minutes, the supernatant was removed and 3mL of PBS was added.

Preparation of the feed-based *Aeromonas veronii* bv *veronii* vaccine

Exactly, 100g of feed pellets (Richmade, CJ Feed & Care Indonesia) were smeared with 10mL of 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 100 mL in volume. The vaccine feed was then aired until dry **(Mulia *et al*., 2022)**.

Feed-based vaccine administration to the North African catfish

Feed-based vaccines and boosters (repeat/booster vaccines) were administered according to the treatment. In addition to the vaccine day, all vaccine-treated animals were fed non-vaccinated feed on other days. The control group was fed non-vaccinated feed. The feed-based vaccine and non-vaccinated feed were administered at a rate of 5% of the fish's weight per day.

Research parameters

The main parameters used in the research were antibody titer and survival rate (SR). Water quality measures, such as water temperature, pH, and dissolved oxygen levels, supported the research. Antibody titer measurements were based on the study of **Anderson (1974)**. Determining fish survival rates was carried out by counting the number of living fish at the end of this study, based on the instructions of **Effendi (2002)**:

$$SR = (N_t / N_0) \times 100$$

SR = survival rate (%)
Nt = number of animals at the end of the study
N_0 = number of animals at the beginning of the study

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

Titer antibody

The results indicated that at week 0 (prior to the administration of feed-based vaccination), the mean antibody titer was 2^0, with no significant differences observed among treatments ($P > 0.05$) (Table 1). By week 1, antibody titers in the vaccinated groups increased to $2^{3,73} – 2^{5,22}$, compared to 2^0 in the control group ($P < 0.05$). Treatment P1 did not differ significantly from P2 and P3, but differed significantly from P0 and P4. Similarly, treatments P2 and P3 were not significantly different from P1 or P4, yet both were significantly higher than P0. These findings demonstrate that a feed-based vaccine administered in week 1 successfully induced antibody formation and enhanced antibody titer production in North African catfish.

Table 1. Measurement results of antibody titers on North African catfish

Treatment	Replicate	Titer Antibody Titers on Week			
		0	1	2	3
P0	1	2^0	2^0	2^0	2^0
	2	2^0	2^0	2^0	2^0
	3	2^0	2^0	2^0	2^0
	Ñ	2^{0a}	2^{0a}	2^{0a}	2^{0a}
P1	1	2^0	2^3	2^5	2^7
	2	2^0	2^4	2^5	2^8
	3	2^0	2^4	2^6	2^7
	Ñ	2^{0a}	$2^{3,73b}$	$2^{5,41b}$	$2^{7,41b}$

P2	1	2^{0}	2^{4}	2^{5}	2^{7}
	2	2^{0}	2^{4}	2^{6}	2^{8}
	3	2^{0}	2^{5}	2^{6}	2^{8}
	Ñ	2^{0a}	$2^{4,41bc}$	$2^{5,41b}$	$2^{7,41b}$
P3	1	2^{0}	2^{4}	2^{7}	2^{8}
	2	2^{0}	2^{4}	2^{7}	2^{8}
	3	2^{0}	2^{5}	2^{6}	2^{7}
	Ñ	2^{0a}	$2^{4,41bc}$	$2^{6,73c}$	$2^{7,73b}$
P4	1	2^{0}	2^{4}	2^{7}	2^{8}
	2	2^{0}	2^{5}	2^{7}	2^{8}
	3	2^{0}	2^{6}	2^{8}	2^{9}
	Ñ	2^{0a}	$2^{5,22c}$	$2^{7,41c}$	$2^{8,41b}$

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

In week 2 (two weeks after the administration of feed-based vaccination), antibody titer production in the vaccinated groups increased to $2^{5,41} – 2^{7,41}$ compared with 2^{0} in the control group (P<0.05). No significant difference was observed between treatments P1 and P2; however, both significantly differed from P3 and P4, while P3 and P4 did not differ significantly. These results indicate that feed-based vaccination effectively stimulated antibody formation, with higher vaccine doses in the feed resulting in correspondingly higher antibody titers in North African catfish.

In week 3 (three weeks after the administration of feed-based vaccination), antibody titer production further increased to $2^{7,41} – 2^{8,41}$ compared with 2^{0} in the control group (P<0.05). However, no significant differences (P>0.05) were observed among the vaccinated treatments. This finding indicates that feed-based vaccination continued to influence antibody formation and enhancement in North African catfish.

Survival rate of the North African catfish

The survival rate of the North African catfish varied across treatments. The lowest survival was observed in P0, at 80.00 ± 10.00%. Treatments P1, P2, and P3 resulted in survival rates of 83.30 ± 5.77%, 90.00 ± 0.00%, and 93.30 ± 5.77%, respectively, while the highest survival was recorded in P4, reaching 96.70 ± 5.77% (Fig. 1). Statistical analysis revealed that P0 did not differ significantly from P1 and P2 (P>0.05), but was significantly different from P3 and P4 (P<0.05). No significant difference was observed between P3 and P4 (P>0.05). Treatment P1 differed significantly from P4 (P<0.05), whereas P2, P3, and P4 were relatively similar (P>0.05). These results indicate that feed-based vaccination positively influenced the survival rate of North African catfish, particularly in P3 and P4.

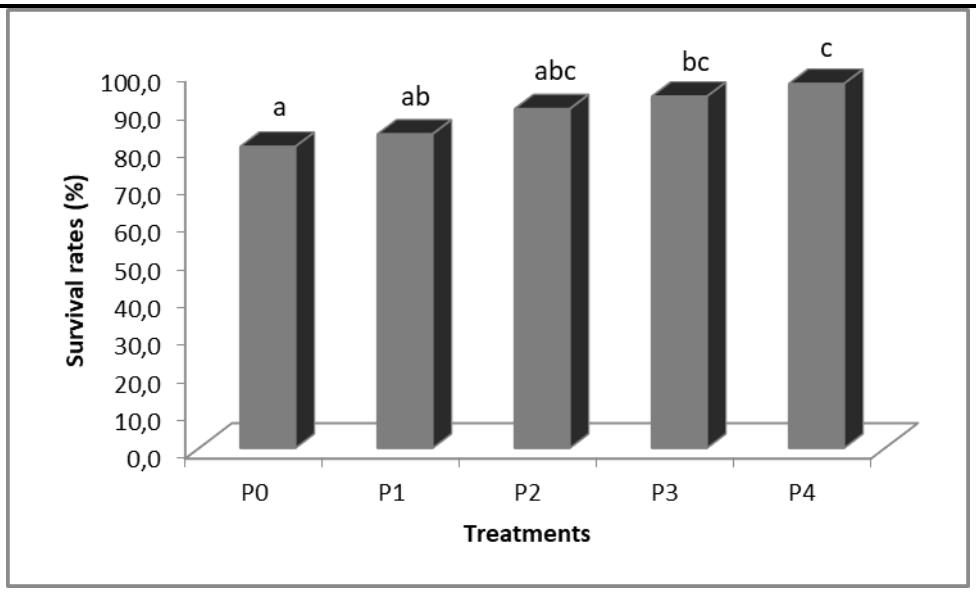

Fig. 1. Survival rate of the North African catfish under different treatments
Note: columns with the same superscript letters shows an effect that is not significantly different at the 5% test level.

Water quality parameters

The water quality parameters monitored during the experiment included water temperature, pH, and dissolved oxygen. The results showed that all parameters remained within the optimal range for catfish culture, with water temperature ranging from 26.8 to 28.9°C, pH ranging from 6.5 to 7.9, and dissolved oxygen levels between 6.2 and 8.6mg L^{-1} (Table 2).

Table 2. The parameters of water quality

The Parameters of Water Quality	Treatment					NSA (2024)
	P0	P1	P2	P3	P4	
Temperature (˚C)	26.9–28.9	27.5–28.9	26.8–28.6	26.8–28.8	27.5–28.7	25–30
Acidity (pH)	6.5–7.6	6.6–7.9	6.8–7.8	7.0–7.9	6.8–7.9	6.5–8
Dissolved Oxygen (mg L^{-1})	6.6–8.4	6.2–8.7	6.8–8.2	6.7–8.4	6.3–8.6	>2

DISCUSSION

Vaccination is a strategic approach to strengthen the immune system, thereby protecting fish from pathogenic infections. In aquaculture, vaccination can be administered through intramuscular (IM) and intraperitoneal (IP) injections, immersion (dip or bath), or orally via feed **(Du *et al*., 2022; Gao *et al*., 2025; Mulia *et al*., 2025)**. In this study, vaccination against *A. veronii* bv *veronii* in North African catfish (*C. gariepinus*) was successfully performed using an oral feed-based vaccine. The results demonstrated that administration of the feed-based *A. veronii* bv *veronii* BmSL-03 vaccine significantly enhanced the immune response in North African catfish.

The vaccination protocol involved different treatment durations for vaccination and booster administration, namely 3, 4, 5, and 7 days. Vaccinated fish exhibited higher antibody titers than the control group across all weekly observations, showing a consistent upward trend until the end of the experiment (Table 1). At the final sampling point, antibody titers in the vaccinated groups reached $2^{8.41}$, while the control group remained at 2^{0}.

At week 0, antibody titers were low and showed no significant differences among treatments ($P>0.05$). By week 1, however, a significant increase in antibody titers was observed in all vaccinated groups (P1–P4) compared to the control (P0) ($P<0.05$). At week 2, the highest antibody titers were recorded in P3 and P4, significantly different from the other treatments, although P1 and P2 still exhibited higher values than the control. At week 3, antibody titers continued to rise compared to the control ($P<0.05$), but no significant differences were detected among the vaccinated groups ($P>0.05$).

These findings indicate that feed-based vaccination effectively stimulated the immune response of African catfish through both mucosal and systemic pathways. Antigens delivered via feed reached the intestine and were recognized by mucosal immune cells in the gut-associated lymphoid tissue (GALT). This process activated B cells to differentiate into plasma cells that produce antibodies, particularly IgM and IgT/IgZ **(Du *et al*., 2022; Radhakrishnan *et al*., 2023)**.

The results of this study are consistent with previous reports showing that oral vaccination enhanced the immune response of the Nile tilapia (*O. niloticus*) and grass carp (*C. idella*) **(Argayosa *et al*., 2024; Mubeen *et al*., 2025)**. Similarly, feed-based vaccines against *A. veronii* have improved immune responses in cultured goldfish (*C. auratus*) and North African catfish (C. *gariepinus*) **(Wu *et al*., 2024; Mulia *et al*., 2025)**. In North African catfish, simultaneous systemic and mucosal immunity stimulation is significant, since *A. veronii* commonly infects through both the skin and gastrointestinal tract. The observed increase in antibody titers further supports the effectiveness of oral vaccination in providing protective immunity against *A. veronii* infections.

Recent studies have demonstrated that oral vaccination induces local mucosal immunity and systemic immune responses, with intestinal mucosal immunity being the dominant component **(Oliveira *et al*., 2022)**. Compared to injectable vaccines, feed-based vaccines provide a non-invasive approach that minimizes handling stress while reducing the risks of mechanical injuries and secondary infections associated with physical manipulation of fish **(Wu *et al*., 2024)**. Furthermore, oral vaccination enables mass immunization with lower operational costs, making it more feasible for intensive aquaculture systems by reducing labor expenses and supporting animal welfare standards **(Embregts & Forlenza, 2016; Mohamad *et al*., 2021; Ali *et al*., 2024; Tammas *et al*., 2024)**. Another important advantage is the stimulation of mucosal immunity in the gastrointestinal tract, which serves as the first line of defense against pathogen invasion **(Radhakrishnan *et al*., 2023)**.

Despite these benefits, oral vaccination also has certain limitations, including antigen degradation by digestive enzymes, lower efficacy, and shorter duration of protection when compared to injectable vaccines **(Du *et al*., 2022)**. The possible immunological mechanism can be explained as follows: macromolecular antigens are internalized by hindgut epithelial cells. In contrast, minor soluble antigens infiltrate the bloodstream through intercellular gaps in the intestinal mucosa. In contrast, larger particulate antigens are phagocytized by macrophages, undergo intracellular processing, and are subsequently transported to the appropriate lymphatic tissues or immune cells. From there, they enter the circulatory system and are eventually delivered to the lymph nodes *via* blood or lymphatic fluid, where they stimulate the appropriate immune responses **(Schep *et al*., 1999**).

The results of this study demonstrated that the feed-based vaccine *A. veronii* bv *veronii* BmSG-03 effectively protected fish, as evidenced by higher survival rates (SR) in vaccinated groups compared to the control, particularly in treatments P5 (Fig. 1). Previous studies also reported similar findings, where loaches vaccinated with *A. veronii* antigen achieved a survival rate of up to 65.66%, while the control group showed 0% survival **(Zhang *et al*., 2020)**. Likewise, the crucian carp (*Carassius carassius*) vaccinated with *A. veronii* reached 73.3% survival compared to 0% in the control group, where all fish died after challenge exposure **(Zhao *et al*., 2021)**. In North African catfish, feed-based vaccination with *A. vero*nii bv *veronii* BmCl-03 resulted in survival rates ranging from 53.33% to 90%, while unvaccinated controls experienced 0% survival **(Mulia *et al*., 2025)**.

When incorporated into feed, the vaccine stimulates mucosal immunity in the gastrointestinal tract, the primary entry point for aquatic pathogens. The activation of gut-associated lymphoid tissue (GALT) promotes local immune responses through enhanced mucosal IgM secretion, followed by systemic antibody production. This dual mechanism strengthens fish resistance against pathogen invasion and explains the significantly higher SR observed in vaccinated groups compared to the control **(Ali *et al*., 2024; Wu *et al*., 2024)**. Recent studies further suggest that feed-based vaccines, including genome-free and microencapsulated formulations, enhance non-specific enzyme activity (e.g., lysozyme) and serum IgM levels, thereby reinforcing immunity and improving SR in vaccinated fish **(Ali *et al*., 2024; Lakshmi *et al*., 2025)**. Another advantage of oral administration is the reduction of handling-related stress. Unlike injection-based methods requiring individual fish manipulation, oral vaccination can be delivered during routine feeding. Oral administration reduces physiological stress, which is known to suppress immune responses and increase disease susceptibility, thereby lowering overall mortality **(Wu *et al*., 2024; Miryala & Swain, 2025)**.

From a practical standpoint, fish farmers more easily adopt feed-based vaccines. Unlike injection or immersion methods requiring skilled labor and specialized facilities, oral vaccination can be integrated into routine feeding practices. Oral vaccination

improves farmer compliance and reduces operational costs. Nevertheless, oral vaccination has limitations, particularly antigen degradation within the digestive tract, which can compromise vaccine efficacy. Advances in encapsulation technologies and biofilm-based delivery systems offer promising strategies to enhance fish antigen stability and immune responses **(Radhakrishnan *et al.*, 2023)**.

Several extrinsic factors, including water quality, fish size, and species, also play critical roles in influencing vaccination outcomes and must be considered during application **(Olsen *et al.*, 2024)**. This study maintained water quality parameters within the optimal range, with water temperature ranging from 26.8– 28.9°C, pH between 6.5–7.9, and dissolved oxygen (DO) levels from 6.2 to 8.6mg L^{-1} (Table 2). These values remained slightly variable among treatments but were within the acceptable limits. According to the National Standardization Agency **(NSA, 2024)**, the optimal water temperature for North African catfish is 25– 30°C. Similarly, water pH requirements are between 6.5 and 8 **(NSA, 2024)**. The DO concentrations recorded in this study were also suitable, as the **NSA (2024)** standard is >2 mg L^{-1}. Therefore, the water quality conditions during the experiment were well maintained and supportive of vaccination effectiveness in North African catfish.

CONCLUSION

The feed-based vaccine *A. veronii* bv *veronii* BmSG-03 has been shown to be effective in enhancing both mucosal and systemic immune responses in fish, resulting in significantly higher survival rates compared to the control group. This approach shows strong potential to reduce mortality and decrease reliance on antibiotics in aquaculture. Feed-based vaccine *A. veronii* bv *veronii* is a promising immunoprophylaxis strategy for freshwater aquaculture.

Future studies are required to optimize feed formulations, such as through encapsulation techniques and incorporating suitable adjuvants, while extending evaluations across multiple fish species and diverse culture conditions. Furthermore, developing multivalent vaccines to address the risk of co-infections, along with comprehensive economic analyses, will be essential to ensure the feasibility and scalability of this strategy for industrial aquaculture applications.

Conflict of interest

The author team has no conflict of interest to declare in this research and worked with full dedication and responsibility.

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