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The role of *Aeromonas hydrophila* GPL-04 feed-base vaccine in gourami fish (*Osphronemus gourami*) vaccination and immunity

C. Purbomartono[1], A.W. Iriana[2], A. Husein[2], D.S. Mulia[2]

[1]Department of Aquaculture, Faculty of Agriculture and Fisheries, [2]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Purwokerto, Purwokerto, Indonesia

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Correspondence:
C. Purbomartono
cpmartono@gmail.com

Abstract

This study aims to determine the effect of vaccinated feed using *Aeromonas hydrophila* GPL-04 on immunity in gourami fish. The study employed an experimental laboratory method, utilizing a completely randomized design with four treatments and three repetition, each with 15 fish. The treatments given were vaccinated feed with doses of T1 (10 mL 100 g^{-1} feed for 10 days), T2 (10 mL 100 g^{-1} feed for 15 days), T3 (15 mL 100 g^{-1} feed for 10 days), and T4 (15 mL 100 g^{-1} feed for 15 days) and control (T0). The immunity parameters observed were antibody titer, survival rate (SR), relative percent survival (RPS), and mean time to death (MTD). Observations of immunity parameters were conducted on days 0, 7, 14, 21, and 28 by taking blood through the vena cava caudalis. The results showed that administering feed vaccinated to gourami fish for 28 days of the study could significantly increase antibody titer and SR, but not significantly for the RPS and MTD. The optimum dose of vaccinated feed for gourami fish in this study was 15 mL/100 g^{-1} feed for 15 days of administration (T4).

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Introduction

Gurami fish (*Osphronemus gourami*) is a type of freshwater fish that is widely consumed by the Indonesian people because of its delicious meat taste and high nutritional content (1), especially its protein content reaching 64.73% (2). In addition, economically, gourami fish has several advantages such as a fairly high and stable price, and a clear target market (3). Gourami cultivation is also supported by land that is quite suitable in Indonesia, especially the availability of sufficient fresh water such as rivers, lakes, ponds, and ponds. Most gourami cultivation in Indonesia is still carried out with simple or traditional technology, but a few still carry out semi-intensive, intensive, and super-intensive cultivation (4).

The increasing consumption of gourami fish with its delicious taste and high nutritional content, supported by stable prices, has caused consumer demand for gourami fish to increase. This has caused gourami fish farming to develop, but it also has the potential to pose a threat of infectious diseases caused by *Aeromonas hydrophila*. To prevent the emergence of this infectious disease, vaccination can be done. Vaccination can increase the immunity of fish to certain pathogens so that the mortality rate can be reduced as small as possible (5). Vaccination provides an effective and inexpensive solution to combat the disease risk in fish farming. The right application of vaccination to prevent bacterial diseases provides the right solution due to the use of harmful antibiotics (6). Vaccines are a powerful method, proven to provide a solution to prevent disease and increase fish survival. Vaccines, in addition to reducing dependence on antibiotics and the level of losses caused by disease, are known to improve fish health and performance, suppress disease outbreaks, and provide long-term protection against disease, while not leaving harmful residues in products or the environment (7). Vaccination can be called an environmentally friendly technology because it does not pollute the environment, is right on target, and does not cause

negative impacts on fish, the environment, or consumers. Vaccination can also be done on various sizes of fish from seeds to broodstock. Disease control through vaccination has good prospects for disease prevention in the future (8).

The results of the study on African catfish proved that antibody titers can be increased by administering *A. hydrophila* GPl-04 feed-based vaccines. The application of vaccinated feed at a dose of 10 mL and 15 mL of 100 g^{-1} feed for 10 days and 15 days was able to induce a good non-specific immune response in African catfish, protecting with the highest survival reaching 100.00% at a dose of 15 mL 100 g^{-1} feed for 15 days of administration. The high survival rate positively impacts the relative percent survival RPS value's achievement, ranging from 84.60-100.00% (9). Furthermore, (10) reported that the implementation of the vaccine with a dose of 10^6 cells mL^{-1} can increase the immune response, such as the total number of leukocytes, although not significantly different, but the survival is quite high, reaching 54.17%. The formation of the body's immune response is stimulated by the entry of antigens into the body, which are then fought by macrophage cells. Macrophage cells attach to antigens and perform recognition and characterization, which are then phagocytosed.

According to (11), administering feed-based vaccines using different *Aeromonas* species, namely *A. veronii bv veronii* BmCL-03, can also increase antibody titers, protecting with survival rates of up to 90.00% by intramuscular (i.m) injection and 80% by immersion and significantly different from intraperitoneal (i.p) and oral injections. Achieving high survival rates positively impacts the relative percent survival 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. Compared to other immunization methods, i.m injection is the most effective.

The results of another study (12) stated that administering a killed *A. hydrophila* vaccine to gourami fish can result in improvements in antibody titers and survival rates of up to 95% with an optimal vaccine dose of 10^4 CFU mL^{-1}. This study aims to determine the specific immune response of gourami fish given a feed-based vaccine (vaccinated feed), namely the *A. hydrophila* vaccine. The immune response parameters measured include antibody titer, survival rate, relative protection level, and average time to death.

Materials and methods

Ethical approval

The ethics committee for the care and use of research animals, the Muhammadiyah University of Purwokerto, Ministry of Higher Education and Science Technology, approved the experimental protocol for this study, with reference number KEPK/UMP/34/II/2020.

Experimental design

This research was conducted at the Microbiology Laboratory, Department of Biology, Universitas Muhammadiyah Purwokerto. This study used 4-month-old gourami fish measuring 9-13 cm purchased from the Technical Implementation Unit (UPT) of the Sidaboa Fish Seed Center, Patikraja District, Banyumas Regency. The materials used in the study were Phosphate Buffer Saline (PBS) solution, *A. hydrophila* strain GPL-04 vaccine, egg white, and commercial pellet feed. In this research, the vaccine was made based on a method developed by (9). The vaccine was made using the *A. hydrophila* strain GPL-04 (Aerovac vaccine). *A. hydrophila* bacteria were cultured on Glutamate Starch Phenile (GSP), Tryptone Soya Broth (TSB), Tryptone Soya Agar (TSA) and Phosphate Buffer Saline. The Phosphate Buffer Saline solution used in making the vaccine consisted of NaCl, Na_2HPO_4, NaH_2PO_4, 2% formalin, and distilled water.

Materials

The study used a completely randomized design (CRD) consisting of 4 treatments, each with 3 replications, conducted for 28 days with the following design: T0: unvaccinated feed; T1: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 10 days; T2: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 15 days; T3: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 10 days; T4: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 15 days.

To measure antibody titers, blood samples were taken on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} days after being given vaccinated feed. Meanwhile, to calculation survival rate (SR), relative protection level (RPS) and mean time to death (MTD) were carried out at the end of the study (day 28).

Purification of *A. hydrophila* culture

A. hydrophila strain GPL-04 bacterial culture was obtained at the Microbiology Laboratory of the Biology Department, Universitas Muhammadiyah Purwokerto. The bacterial culture was grown on GSP media in streak and incubated for 18-24 hours. The morphology of the bacterial colonies that grew was observed to ensure that the isolate was *A. hydrophila* by looking at the shape, color, and edge of the colony. The separated single bacterial colonies were then cultured and inoculated using the zig-zag streak method in TSA slant media. The culture was incubated at 37 °C for 24 hours and stored in the refrigerator for use as stock culture and working culture. The stock culture was subcultured every 6 months, while the working culture was cultured once a month.

The purified bacterial culture of *A. hydrophila* strain GPL-04 from the working culture was taken using a needle then transferred to 10 mL of liquid TSB medium and incubated at 37 °C for 18-24 hours. The *A. hydrophila* culture was used to make vaccines.

Aeromonas hydrophila vaccine preparation

A. hydrophila bacterial culture from 10 mL medium that has been incubated for 24 hours is then vortexed, then poured into a large petri dish containing TSA media, and incubated again for twenty-four hours at 37°C. The bacterial culture is harvested by gently scraping using a Drugalsky glass rod. The harvested bacteria are washed using PBS solution 3 times. The harvested bacteria are put into an Erlenmeyer flask plus 2% formalin and shaken for 24 hours, then put into a conical tube and centrifuged for 20 minutes. The liquid at the top of the conical tube (supernatant) is discarded, while the sediment is washed with PBS and centrifuged again 3 times. The viability of the *A. hydrophila* bacteria is tested by replanting it in a GSP medium in a streak manner. *A. hydrophila* bacteria are called vaccines if not grown in the GPS medium (killed vaccine).

Preparation of *A. hydrophila* vaccinated feed

Commercial fish feed or pellets as much as 100 g are given 10 mL of albumin from chicken egg until evenly distributed and dry, then sprayed with a vaccine suspended with sterile PBS solution with a dose of 10^5 CFU mL^{-1} as much as 10 mL using a sprayer. When spraying the pellets, the vaccine is spread evenly using a brush and aired until dry.

Challenge test

The challenge test was conducted on the 21st day after vaccination. This action aims to determine the resistance of gourami fish to *A. hydrophila* by infecting gourami fish through injection of *A. hydrophila* at a dose of 0.1 mL of each fish. Vaccination was carried out on both control and vaccinated feed treatments. The dose of *A. hydrophila* challenge test used was obtained from the Lethal Dose 50 (LD50) results. LD50 is the concentration value of bacteria that can cause 50% fish death. After being infected with the bacteria, a challenge test was carried out by observing the appearance of visible symptoms and the total number of gourami fish that survived for 1 week or until the 28th day.

Observed parameters

The main parameter observed was the magnitude of antibody titer, while other parameters were SR, RPS, and MTD.

Data analysis

Data were analyzed using analysis of variance (ANOVA) to determine the effect of treatment. If there is a significant difference, then it is continued with the Duncan Multiple Range Test (DMRT) at a test level of 5%.

Results

The purpose of giving vaccinated feed to gourami fish is to increase immunity. The immunity obtained is used to defend against the threat of pathogenic agents such as *A. hydrophila* and other pathogenic bacteria. Immunity parameters can be determined by measuring the antibody titer in the blood serum of vaccinated fish. In this study, vaccinated feed from *A. hydrophila (Aerovac*) was used to increase antibody titer in the blood serum.

Antibody titer

In the antibody titer produced, the increase in antibodies in the blood serum is a response to the administration of the *Aerovac* vaccine, which functions to agglutinate, neutralize, and kill compatible pathogens. In this study, the administration of the vaccine through feed (vaccinated feed) in gourami fish increased the antibody titer significantly compared to the control starting from day 14. Likewise, on days 21 and 28, the administration of the vaccine produced a significant antibody titer. Table 1 shows the levels of antibody titers given vaccinated feed with different volumes (mL) and duration of administration (days).

Table 1: Antibody titer of *Osphronemus gourami* given the *Aeromonas hydrophila* vaccinated feed

Treatment	Antibody titer on day				
	Day 0	Day 7	Day 14	Day 21	Day 28
T0	2,33[a]	2,00[a]	2,67[a]	1,67[a]	1,67[a]
T1	2,67[a]	3,33[a]	3,67[a]	2,33[a]	2,33[a]
T2	2,67[a]	3,33[a]	3,67[a]	3,00[b]	3,00[b]
T3	3,00[a]	3,01[a]	3,67[a]	3,00[b]	3,00[b]
T4	3,00[a]	3,00[a]	4,00[b]	3,00[b]	3,00[b]

The difference in letters means there are significant differences between groups at P<0.05. T0: unvaccinated feed, T1: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 10 days, T2: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 15 days, T3: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 10 days, T4: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 15 days.

Survival rate

Table 2 shows the survival of fish fed with vaccines and those that are not (T0, control) resulting in the highest survival when given 15 mL of vaccine for 15 days at 86.67% (T4).

Relative percent survival

Table 3 shows the level of relative protection (%) of gourami fish fed with vaccinated feed with different volumes (mL) and duration of vaccine administration (days). Based on the results of the study in Table 3, the provision of vaccinated feed with a dose of 15 mL for 15 days (T4) produced the best protection of 73.30%.

Mean time to mortality (MTM)

Table 4 shows the average time to mortality of gourami fish (days) fed with *Aerovac* vaccinated feed with different volumes (mL) and duration of vaccine administration (days) compared to the control. Based on the results of this study (Table 4), the optimal MTM value is given 15 mL of vaccine

for 10 days (T3) with an average time to death of 3.67 days, indicating that the number of deaths is getting lower.

Table 2: The survival rate of *Osphronemus gourami* fish vaccinated after being given *A. hydrophila* 10^4 cells mL^{-1} as a challenge test

Treatment	Replication			Survival rate (%)
	1	2	3	
T0	50	50	50	50.00[a]
T1	80	80	70	76.67[b]
T2	70	80	80	76.67[b]
T3	80	90	70	80.00[b]
T4	80	90	90	86.67[b]

The difference in letters means there are significant differences between groups at P<0.05. T0: unvaccinated feed, T1: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 10 days, T2: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 15 days, T3: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 10 days, T4: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 15 days.

Table 3: Relative percent survival (%) of *Osphronemus gourami* fed with *A. hydrophila* vaccinated feed

Treatment	Replication			Survival rate (%)
	1	2	3	
T1	60	60	40	53.33[a]
T2	40	60	60	53.33[a]
T3	60	80	40	60.00[a]
T4	60	80	80	73.30[a]

The difference in letters means there are significant differences between groups at P<0.05. T1: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 10 days, T2: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 15 days, T3: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 10 days, T4: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 15 days.

Table 4: Mean time to mortality of *Osphronemus gourami* fish fed with *A. hydrophila* vaccinated feed

Treatment	Replication			Mean Time to Death (days)
	1	2	3	
T0	3	2	3	2.67[a]
T1	3	2	3	2.67[a]
T2	3	3	4	3.33[a]
T3	2	5	4	3.67[a]
T4	3	1	1	1.67[b]

The difference in letters means there are significant differences between groups at P<0.05. T0: unvaccinated feed, T1: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 10 days, T2: vaccinated feed dose of 10 mL 100 g^{-1} feed given for 15 days, T3: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 10 days, T4: vaccinated feed dose of 15 mL 100 g^{-1} feed given for 15 days.

Discussion

Antibody titers

The results of the study showed that the provision of 10 mL of *Aerovac* vaccinated feed for 15 days in gourami fish was significantly different starting from day 21 (3.00) compared to the control (P <0.05). While the provision of 15 mL of *Aerovac* was significant starting from day 14 (4.00) on antibody titers (P <0.05). The provision of 15 mL of vaccinated feed for 15 days (T4) was the best treatment, more effective and efficient than the control and other treatments in producing significant antibody titers (P <0.05). The second-best treatment was the provision of 10 mL of *Aerovac* vaccine for 15 days, which produced significant (P <0.05) antibody titers starting from weeks 3 (3.00) and 4 (3.00). This study shows that vaccinated feed can induce antibody titer responses that can increase the immunity of gourami fish. The results of this study are in line with the report submitted (13), *A. hydrophila* vaccinated feed can protect African catfish, thereby reducing mortality rates. Therefore, vaccinated feed can be applied to fish farming at the farmer level.

Furthermore (14) stated that administering vaccines to catfish by intramuscular injection at a dose of 0.1 cells mL^{-1} of each fish can increase antibody titers in sampling weeks 1, and 2 (booster) and weeks 3 and 4 compared to controls. Research conducted by (9) proved that the use of the *A. hydrophila* GPl-04 vaccine mixed into feed can increase the response of African catfish in the formation of antibody levels, average MTD values, percentages of RPS, and SR (P<0.05). *Labeo rohita*, *Cirrhinus mrigala*, and *Ctenopharyngodon idella* fish injected with 0.1 mL *Aeromonas* vaccine and then challenged with *A. hydrophila* 10^8 CFU mL^{-1} by immersion were able to significantly increase antibody titers in all treatment groups compared to controls on the 28th day of observation (15).

The results of the study (16) stated that vaccination in African catfish significantly increased antibody titers compared to controls using attenuated *A. hydrophila* vaccine by heating at 100ºC for 60 minutes. Observations of antibody titers were carried out 2 weeks after booster vaccination with the best strains AGC-2 and AGC-8. Strains AGC-2 and AGC-8 were isolated from the gills of sick dumbo catfish, each from the River in Batang Village and Cindai Alus Village, Banjar Regency, South Kalimantan, Indonesia. Administration of attenuated *A. hydrophila* vaccine can increase antibody titers on the 28th day after vaccination in carp (*Cyprinus carpio* L.) (17). In tilapia, vaccination using *A. hydrophila* bacteria through intraperitoneal injection can increase antibody titers higher than controls (18).

Vaccination using bacteria other than *Aeromonas* has also been shown to increase immunity. *Streptococcus agalactiae* bacterin vaccine based on feed for 4 months can significantly increase antibody titer starting from week 1 and peaking in week 3 before gradually decreasing in week 6.

The increase in antibody titer is followed by increased immunity, resulting in greater survival than those not vaccinated (control). As reported by (19), vaccination can increase survival from 45.2 ± 2.45% in the control to 65.3 ± 4.8% in the single booster group, and 75.1 ± 2.1% in the double booster group. This proves and strengthens the role of vaccines, which can significantly increase immunity so that they can be applied to aquaculture cultivation.

Survival rate

Based on the research results in table 2, the administration of *A. hydrophila* whole cell *Aerovac* vaccine with different doses can significantly affect the survival of gourami fish with an optimal dose of 15 mL vaccine for 15 days of 86.67% (T4). This result is in line with the results of a study conducted by (20), the administration of *A. hydrophila* vaccine mixed into feed for 30 days can increase the survival of carp (*C. carpio*) to 97.33%. The same results were also found in Jambal Siam fish (*Pangasius hypophthalmus*) which were kept in cages and given the HydroVac vaccine mixed into the feed, which significantly increased the survival rate compared to the control with an optimal dose of 4 mL kg^{-1} feed (14). In African catfish, feeding with *A. hydrophila* vaccine for 15 days can increase the survival percentage better than feeding for 10 days (13). Increased survival can be caused by increased immunity due to increased total leukocytes. As reported by (5), administering a vaccine with a dose of 10^6 cells mL^{-1} can increase the total number of leukocytes and increase survival.

(17) reported that a live attenuated vaccine from a pathogenic *A. hydrophila* strain obtained through screening with rifampicin treatment is effective as a vaccine candidate against *A. hydrophila* in *C. carpio*. The live attenuated vaccine is thought to trigger innate and specific immune responses in fish, thereby increasing survival. Similarly, vaccination of tilapia using *A. hydrophila* intraperitoneally can increase resistance to bacterial infection, resulting in increased survival and a positive impact on the economy of fish farmers (18).

Relative percent survival

Research reported by (20) stated that administering the *A. hydrophila* vaccine through feed at a dose of 5 mL for 1 kg of feed for 30 days was able to provide a relative level of protection of 66.67% better than doses of 4 mL kg^{-1} (44.67%) and 3 mL kg^{-1} (44.67%). Added by (14), Jambal Siam fish (*P. hypophthalmus*) kept in cages given HydroVac vaccine through feed can significantly increase the relative protection rate by 56.25% compared to controls with an optimal 4 mL kg^{-1} feed dose.

(15) reported that *Labeo rohita*, *Cirrhinus mrigala*, and *Ctenopharyngodon idella* fish injected with 0.1 mL *Aeromonas* vaccine and then challenged with 10^8 CFU mL^{-1} *A. hydrophila* by immersion resulted in an RPS of 71% for the vaccinated group with 10^9 and 10^{10} CFU mL^{-1} and 86% for 10^8 CFU mL^{-1}. Furthermore, (17), administration of attenuated *A. hydrophila* vaccine can increase the RPS of common carp *C. carpio* L. by 83.7% on the 28th day after vaccination and tested against pathogenic bacteria. These results prove that vaccination can increase protection in vaccinated groups of common carp. Thus, vaccination can be applied to aquaculture systems.

(18) reported that the challenge test using *A. hydrophila* bacteria on vaccinated tilapia resulted in the highest relative protection level in week 6. This was due to the immune system in the vaccinated tilapia group increasing, which was indicated by a significant increase in the nitro blue tetrazolium value compared to the control group.

Mean time to mortality (MTM)

The results of the Aerovac vaccinated feed study produced an optimal MTM value in the T3 treatment of 3.67 days, which indicated a decrease in the number of deaths. While in the control (T0), treatments T1, T2, and T4 were not significantly different with an average time to death faster than T3, indicating a higher number of deaths compared to the T3 treatment. This shows that the provision of Aerovac vaccinated feed to gourami fish can protect against *A. hydrophila* infection, thereby reducing the number of deaths.

The results of this study are similar to the results of previous studies reported (9), vaccination of African catfish based on feed using *A. hydrophila* resulted in significant mean MTM between control and vaccinated treatment. Supplementation of 10 mL of *A. hydrophila* GPI-04 vaccine for 100 g of feed for 10 days is the best and most effective dose in protecting African catfish from *A. hydrophila* infection. This proves that the provision of vaccinated feed can be applied to cultivation because it can protect African catfish from *A. hydrophila* infection to the point of no death (zero mortality) at a dose of 10 mL/100 g^{-1} for 10 days of administration. While the feed-based vaccine was given at a dose of 10 mL for 100 g of feed for fifteen days and a dose of 15 mL for 100 g of feed for ten and fifteen days, there was no significant difference with the vaccine dose of 10 mL for 100 g of feed given for 10 days.

Other researchers (21) reported that administering the *A. hydrophila* vaccine was effective and significant against MTM of snakehead fish (*Ophiocephalus striatus*). Furthermore, (22) proved that administering the BmSL-02 type vaccine from *A. jandai* bacteria can significantly increase MTM in African catfish. The BmSL-02 type vaccine can increase the immunity of African catfish, and control aeromoniasis in North African catfish, with MTM values ranging from 1.8-2.7 days after exposure to active BmSL-02 *A. jandaei* bacteria. The mean MTM value in the groups vaccinated intramuscularly and intraperitoneally was faster than the MTM value in the groups vaccinated orally and by immersion. This proves that administering the

vaccine orally and by immersion is better than the intramuscular and intraperitoneal injection methods, which is indicated by a smaller number of deaths.

Meanwhile, according to (11), vaccination of African catfish using *A. veronii bv veronii* BmCL-03 produced MTM values varying between 1.17 – 1.94 days after being given a challenge test. The immersion vaccination method using the *A. veronii* vaccine is the optimal method that can reduce the average time to death with an MTM value of 1.94 days. Vaccination can control *A. veronii bv veronii* infection, reduce the number of fish mortality, and extend the mean time to death (days) or longer MTM value. Other researchers have proven (23), that the treatment of intra and extracellular *A. hydrophila* vaccine products in African catfish produces better efficacy than the control. The average time of death in the vaccine treatment was 1.57-1.67 days while the control was 1.09 days, indicating a lower number of African catfish deaths compared to no treatment (control). Thus, vaccines using intra and extra-cellular products can protect African catfish after being infected through a challenge test.

Conclusion

A. hydrophila GPL-04 vaccinated feed (Aerovac vaccine) in gourami fish can significantly increase antibody titer and survival, while the relative protection and meantime to-death values increase compared to controls but are not significant. The best dose of vaccinated feed in gourami fish is 15 mL 100 g^{-1} feed for 15 days of administration (T4) for 28 days of research. *A. hydrophila* GPL-04 vaccinated feed can be used for aquaculture cultivation at the farmer level, especially in gourami fish.

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Conflict of Interest

There is no conflict of interest in the writing of this article.

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دور لقاح *Aeromonas hydrophila* GPL-04 المستند على التغذية في التطعيم والحصانة لأسماك الجورامي (*Osphronemus gourami*)

كاهيونو بوربومارتونو[١]، أسري وولان إيريانا[٢]، عارف حسين[٢]، ديني سيسواني موليا[٢]

[١]قسم تربية الأحياء المائية، كلية الزراعة والثروة السمكية، [٢]قسم تربية الأحياء المائية، كلية تدريب المعلمين والتعليم، الجامعة المحمدية بوروكيرتو، بوروكيرتو، إندونيسيا

الخلاصة

تهدف هذه الدراسة إلى تحديد تأثير العلف الملقح باستخدام *Aeromonas hydrophila* GPL-04على المناعة في الأسماك الجورامي. استخدمت الدراسة طريقة معملية تجريبية، باستخدام تصميم عشوائي تماما مع أربعة علاجات وثلاثة تكرار، كل منها يحتوي على ١٥ سمكة. كانت العلاجات المقدمة عبارة عن علف تم تطعيمه بجرعات من T1 (١٠ مل ١٠٠ جم-١ علف لمدة ١٠ أيام)، T2 (١٠ مل ١٠٠ جم-١ علف لمدة ١٥ يوما)، T3 (١٥ مل ١٠٠ جم-١ علف لمدة ١٠ أيام)، و T4 (١٥ مل ١٠٠ جم-١ علف لمدة ١٥ يوما) والتحكم (تي٠). وكانت المعلمات الحصانة لوحظ عيار الأجسام المضادة، ومعدل البقاء على قيد الحياة (SR)، والبقاء في المئة النسبية (RPS)، ومتوسط الوقت حتى الموت (MTD). أجريت ملاحظات معلمات المناعة في الأيام ٠ و ٧ و ١٤ و ٢١ و ٢٨ عن طريق أخذ الدم عبر الوريد الأجوف المذنب. وأظهرت النتائج أن إعطاء العلف الذي تم تطعيمه لأسماك الجورامي لمدة ٢٨ يوما من الدراسة يمكن أن يزيد بشكل كبير من عيار الأجسام المضادة و سر ، ولكن ليس بشكل كبير ل رس و متد. كانت الجرعة المثلى من العلف الملقح لأسماك الجورامي في هذه الدراسة ١٥ مل/١٠٠ جم-١ علف لمدة ١٥ يوما من الإعطاء (T4).

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