RESEARCH ARTICLE

Transfer of maternal immunity using a polyvalent vaccine and offspring protection in Nile tilapia, *Oreochromis niloticus* [version 1; peer review: awaiting peer review]

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Abstract

Background: Vaccination is an effective and alternative means of disease prevention, however, it cannot be conducted on the offspring of fish. For this process to take place, the transfer of maternal immunity must be implemented. This study aims to determine the effectiveness of transferring immunity from the broodstock to the offspring using a polyvalent vaccine against *Aeromonas hydrophila, S treptococcus agalactiae,* and *Pseudomonas fluorescens* in Nile tilapia, *Oreochromis niloticus.*

Methods: Nile tilapia broodstock, with an average weight of 203g (±SD 23 g) was injected with a vaccine used as a treatment. Example include *A. hydrophila* monovalent (MA)*, S. agalactiae* monovalent (MS)*, P. fluorescens* monovalent (MP), *A. hydrophila* and *S. agalactiae* bivalent (BAS)*, A. hydrophila* and *P. fluorescens* bivalent (BAP), *P. fluorescens* and *S. agalactiae* bivalent (BPS), and *A. hydrophila, S. agalactiae,* and *P. fluorescens* polyvalent vaccines (PAPS). While the control was fish that were injected with a PBS solution. The broodstock's immune response was observed on the 7th, 14th, 21st, and 28th day, while the immune response and challenge test on the offspring was conducted on the 10 th, 20th, 30th, and 40th day during the post-hatching period.

Result: The application of PAPS in broodstock could significantly induce the best immune response and immunity to multiple diseases compared to other treatments. The RPS of the PAPS was also higher than the other types of vaccines. This showed that the transfer of immunity from the broodstock to the Nile tilapia offspring could protect it against bacterial diseases such as *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*.

Conclusion: The application of PAPS *A. hydrophila, S. agalactiae, P. fluorescens* vaccines increased the broodstock's immune response and it was transferred to their offsprings. They were able to produce tilapia seeds that are immune to diseases caused by *A. hydrophila, S.*

Open Peer Review

Reviewer Status *AWAITING PEER REVIEW*

Any reports and responses or comments on the article can be found at the end of the article.

agalactiae, and *P. fluorescens.*

Keywords
Aeromonas hydrophila, bivalent vaccine, monovalent vaccine, Pseudomonas fluorescens, Streptococcus agalactiae.

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Introduction

Tilapia was originally considered to be more resistant to bacterial, parasitic, mycological, and viral diseases than other species of cultivated fish. However, they are found to be susceptible to bacterial and parasitic diseases[1], particularly during the offspring phase[2]. Some of the diseases often found in tilapia in Indonesia include *S. agalactiae, A. hydrophila* and *P. fluorescens.*

Among the various methods of disease control, vaccination is one of the most effective ways, which is commonly used[3–6]. The administration of vaccines is meant to produce antibodies that could improve the immunity of tilapia. Unfortunately, they could not be administered to their offspring because the organs that form the immune response are not yet fully developed, therefore they are unable to produce antibodies[7].

An effective solution to the aforementioned issue is the application of maternal immunity transfer. This is the transfer of immunity from broodstock to offspring, by which immunoglobulins (Ig) are transferred through eggs[8]. Maternal immunity has been shown to improve the fish offspring's immunity against pathogens in the early phases of their life[9–12].

This process is usually carried out using monovalent vaccines[13–16]. However, a polyvalent vaccine would be more effective because it could control multiple diseases[17,18]. Though the effectiveness has been known, the application of polyvalent vaccines through maternal immunity has not been extensively investigated, particularly in Nile tilapia (*O. niloticus*).

The transfer of maternal immunity using PAPS for *S. agalactiae*, *Lactococcus garvieae*, and *Enterococcus faecalis* has been studied by Abu-elala *et al.*,[19] and three vaccine strains for *S. agalactiae* by Nurani *et al.*[20]. The types of bacterial diseases studied in the aforementioned studies are very limited even though Nile tilapia often suffer from them in fish farms and hatcheries[21]. Besides being infected by *S. agalactiae*[15,20–23], Nile tilapia are often infected by *A. hydrophila*[21,22,24] and *P. fluorescens*[24,25] leading to high mortality, including in Indonesia. Therefore, this study aims to examine maternal immunity transfer using the vaccines for *S. agalactiae*, *A. hydrophila,* and *P. fluorescens*. It was expected that the broodstock could pass their immunity to their offspring, making them resistant to the three ~~types of~~ diseases (*A. hydrophila, S. agalactiae*, and *P. fluorescens* bacteria), and also the production of tilapia offspring could also be increased. Furthermore, this study aims to determine the effectiveness of the transfer of immunity induced by PAPS against *A. hydrophila, S. agalactiae,* and *P. fluorescens* from the Nile tilapia (*O. niloticus*) broodstock to their offspring and the protection against *S. agalactiae, A. hydrophila,* and *P. fluorescens* ~~bacterial~~ infections.

Methods

Experimental animal

Nile tilapia broodstock, obtained from the Ompo Inland Hatchery, Soppeng, Indonesia, with an average weight of 203g (±SD 23 g) was used as experimental animals. They were kept in spawning ponds and fed with pellets that have a protein content of 30% ~~*ad libitum*~~ in the mornings and afternoons. Also, 25% of the water was replaced daily. One week after the fish spawned, they were harvested and a large number of Nile tilapia broodstock at gonad developmental stage 2 were obtained.

Vaccine production

Pure isolates of the *A. hydrophila*, *S. agalactiae,* and *P. fluorescens* bacteria were obtained from the Research and Development of Fish Disease Control Installation, Ministry of Marine Affairs and Fisheries, Depok, Indonesia. The vaccine tested was formalin-killed, whereby *S. agalactiae* and *P. fluorescens* were inactivated with 1% formalin while *A. hydrophila* was inactivated using 0.6% formalin.

Vaccine treatments and administration

The vaccine treatments consist of (1) a monovalent vaccine against *A. hydrophila* (MA), (2) a monovalent vaccine against *P. fluorescens* (MP), (3) a monovalent vaccine against *S. agalactiae* (MS), (4) a bivalent vaccine against *A. hydrophila, P. fluorescens* and (BAP), (5) a bivalent vaccine against *A. hydrophila* and *S. agalactiae* (BAS), (6) a bivalent vaccine against *P. fluorescens* and *S. agalactiae* (BPS), (7) a polyvalent vaccine against *A. hydrophila, P. fluorescens* and *S. agalactiae* (PAPS), and (8) the control, fish injected with PBS solution.

The vaccination method used was intramuscular (*i.m.*) and was administered at a dose of 0.4 mL/kg fish. After the fish were vaccinated, a booster with the same dose as the initial vaccination was later administered on the 7th day. However, before being injected with the vaccines, they were first anesthetized using MS-222, Sigma.

The gonad developmental stage 2 fish post-vaccination were reared using 3x3 m cages and installed in dirt ponds. Furthermore, 20 broodstock were reared per cage, consisting of 15 females and five males. The fish were fed with pellets at a dose of 4%/day in the morning, at midday, and in the afternoon. The water was replaced daily at a rate of 20%/day. The fish would spawn after being reared for approximately 4 weeks.

Broodstock and larvae immune response

Following vaccinations, the fish's immune response was observed on the 7th, 14th, 21st, and 28th day by collecting intramuscular blood samples. The immune response parameters were the antibody titer using the direct agglutination method[26], total leukocyte[20,22,27], phagocytic[28–30] and lysozyme activities[13,20,29,30].

Random blood sampling from the offspring was conducted on each treatment group on the 10th, 20th, 30th, and 40th day post-spawning period. Serum was collected by grinding the offspring in a tube with PBS-tween at a ratio of 4:1. It was then centrifuged at 6000 rpm for 5–10 minutes. Furthermore, the serum in the second layer of the centrifugation result was harvested and stored at 47°C for 30 minutes to inactivate the

complements[31]. It was then stored for agglutination titer and lysozyme activity.

Challenge procedures

The offspring challenge test was conducted on the 10, 20, 30, and 40 days old during the post-hatching period. It was carried out by dividing the fish into 7 groups based on the type of vaccine administered plus one unvaccinated. The control was challenged with the three types of pathogenic bacteria, namely *A. hydrophila*, *S. agalactiae*, and *P. fluorescens.*

This test was carried out by placing 20 offsprings into containers containing 4 liters of water and then they were immersed in water containing pathogenic bacteria at a dose of $2.1x10^8$ CFU/mL according to their relative treatments, each conducted triplicate. To observe the effectiveness of the vaccine, the relative percentage survival (RPS) was calculated[31,32] on the 14th day post-challenge test.

Data analysis

The data for the specific and non-specific immune response and RPS were analyzed statistically and with Duncan's test (IBM SPSS Statistic 21; Chicago, IL, USA).

Results

Broodstock total leukocyte dan phagocytic activity post-vaccination

In general, the different types of vaccines at each period of post-vaccination had a significant effect (P<0.05) on the broodstock's total leukocyte (Figure 1), and phagocytic activity (Figure 2). The follow-up test showed that the fish vaccinated with PAPS had the highest total leukocyte and phagocytic activity, followed by those vaccinated with bivalent and monovalent vaccines.

Broodstock and offspring agglutination titers

The broodstock's antibody (Table 1) increased, especially after the booster, except in the unvaccinated fish. After the peak, the broodstock's immune response remained high up to day 28 even though there was a tendency for it to decrease. All the types of vaccines at each point in time had a significant effect (P<0.05) on the agglutination titer in the broodstock. The Duncan's follow-up test showed that the vaccinated broodstock had a higher agglutination titer than the unvaccinated fishes. Also, the highest significant value was found in the vaccinated fishes with PAPS, followed by those vaccinated with the bivalent and monovalent vaccines.

Based on the effect of the vaccine on the broodstock's immune response, the agglutination titer in the offspring from the vaccinated broodstock at ages 10, 20, 30, and 40 days was higher than unvaccinated (P<0.05). The follow-up test showed that PAPS was more effective in increasing the agglutination titer in the offspring than the bivalent and monovalent vaccines. The results showed that the administration of vaccines in tilapia broodstock had a significant effect on the maternal immunity transfer to the offsprings that were up to 30 days old (Table 2).

Broodstock and offspring lysozyme activity

The lysozyme activity in the fishes from the vaccinated broodstock was higher than those unvaccinated ones (P<0.05) (Figure 3). Generally, the offspring from the broodstock that were vaccinated with PAPS had a higher lysozyme activity

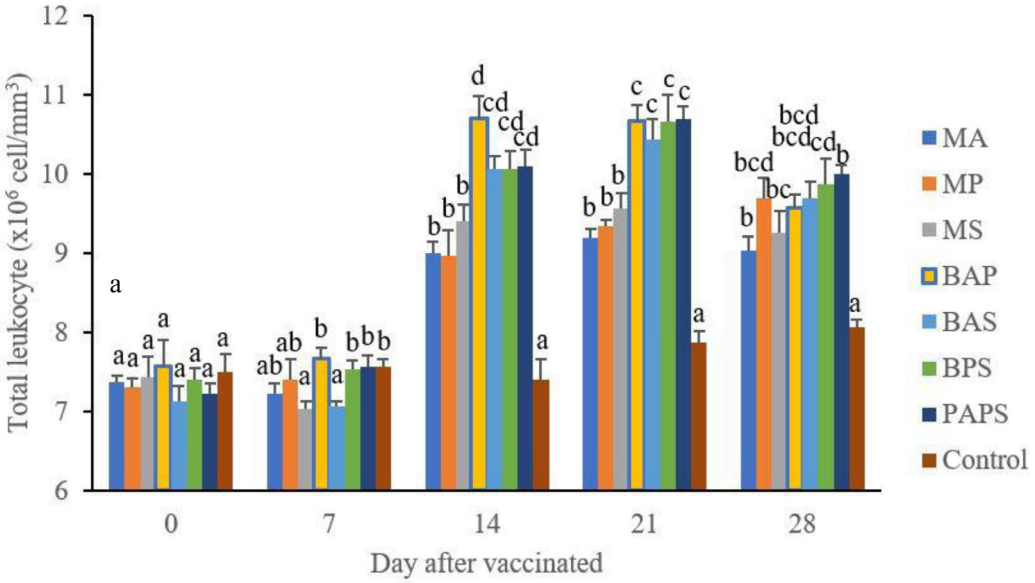

Figure 1. Total leukocyte of tilapia broodstock after the vaccination with various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae,* P: *P. fluorescens.* Values with different superscripts a,b indicate that their corresponding means are significantly different (P<0.05) according to one-way ANOVA followed by Duncan's test.

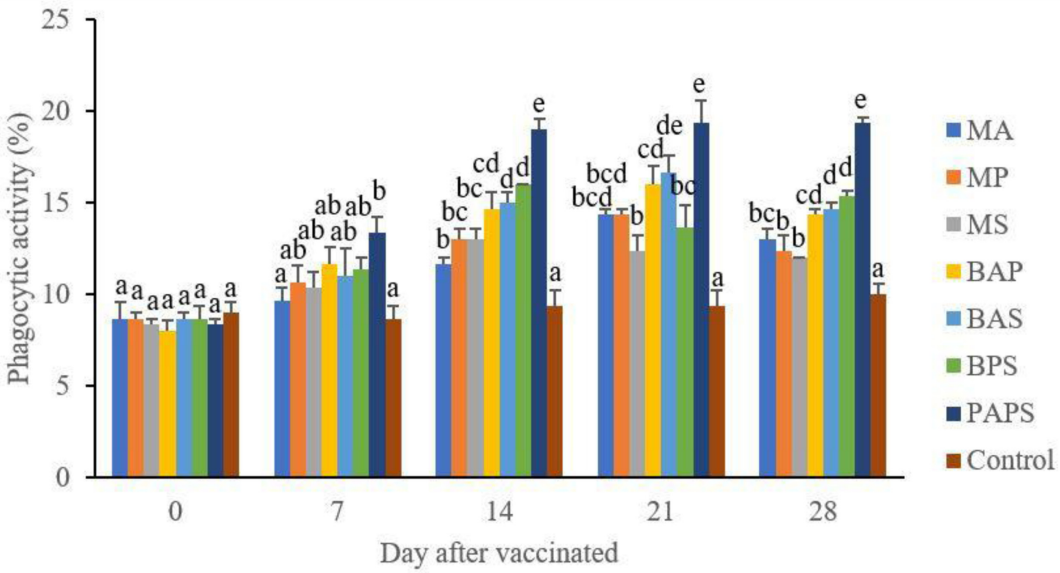

Figure 2. The phagocytic activity in the tilapia broodstock after being vaccinated with the various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae,* P: *P. fluorescens.* Values with different superscripts a,b indicate that their corresponding means are significantly different (P<0.05) according to one-way ANOVA followed by Duncan's test.

Table 1. The agglutination titer in Nile tilapia broodstock after being vaccinated with various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae,* P: *P. fluorescens.* Values with different superscripts a,b indicate that their corresponding means are significantly different (P<0.05) according to one-way ANOVA followed by Duncan's test.

Type of vaccine	Day after vaccinated (day)				
	0	7	14	21	28
MA	1.67±0.33^{a}	2.00±0.00^{a}	3.33±0.33^{a}	3.67±0.3^{bc}	3.67±0.33^{bc}
MP	1.67±0.33^{a}	2.67±0.33^{a}	3.67±0.33^{a}	3.33±0.33^{bc}	3.33±0.33^{b}
MS	1.33±0.33^{a}	2.33±0.33^{a}	3.33±0.33^{a}	3.00±0.00^{b}	3.33±0.33^{b}
BAP	2.00±0.58^{a}	2.33±0.33^{a}	4.33±0.33^{ab}	4.33±0.33^{c}	4.67±0.33^{bc}
BAS	1.67±0.33^{a}	2.33±0.33^{a}	4.33±0.33^{ab}	4.33±0.33^{c}	4.33±0.88^{bc}
BPS	1.67±0.67^{a}	2.33±0.33^{a}	4.33±0.33^{ab}	4.33±0.33^{c}	5.00±0.58^{c}
PAPS	1.67±0.33^{a}	3.67±0.33^{b}	5.33±0.33^{b}	6.67±0.33^{d}	6.67±0.33^{d}
Control	1.67±0.33^{a}	1.67±0.33^{a}	1.33±0.33^{a}	1.33±0.33^{a}	1.67±0.33^{a}

than those of other treatments (P<0.05) up to the 30th day. The results showed that the application of PAPS in tilapia broodstock could increase lysozyme activity transferred to the offsprings (Figure 4).

RPS of offspring post-challenge

Offsprings that were 10, 20, 30, and 40 days old from the vaccinated broodstock had higher RPS than those from the unvaccinated broodstock after being challenged with bacteria. The offsprings from the broodstock that were vaccinated with PAPS had the highest SR and RPS when challenged with 3 bacteria simultaneously (a combination between *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*) (Table 3) up to day 30.

Discussion

Efforts to produce seeds that are immune to several diseases was the best alternative to increasing Nile tilapia production. Furthermore, PAPSs for *A. hydrophila*, *S. agalactiae,* and

Table 2. The agglutination titer of tilapia offspring from maternal immunity produced by various types of vaccines at the ages of 10, 20, 30 and 40 days post-hatching (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila,* S: *S. agalactiae,* P: *P. fluorescens.* Values with different superscripts a,b indicate that their corresponding means are significantly different (P<0.05) according to one-way ANOVA followed by Duncan's test.

Type of vaccine	Day post-hatching (day)			
	10	20	30	40
MA	4.00±0.58^{ab}	3.67±0.33^{bc}	1.67±0.33^{a}	1.33±0.33^{a}
MP	4.00±0.00^{ab}	3.67±0.33^{bc}	1.67±0.33^{a}	1.33±0.33^{a}
MS	3.67±0.33^{b}	3.33±0.33^{b}	2.33±0.33^{ab}	1.33±0.33^{a}
BAP	4.67±0.33^{ab}	4.67±0.33^{c}	2.33±0.33^{ab}	1.67±0.33^{a}
BAS	5.00±0.58^{c}	4.33±0.33^{bc}	2.33±0.33^{ab}	1.67±0.33^{a}
BPS	4.33±0.33^{ab}	4.33±0.33^{bc}	2.33±0.33^{ab}	1.33±0.33^{a}
PAPS	6.33±0.33^{d}	5.67±0.33^{d}	3.00±0.33^{b}	1.67±0.33^{a}
Control	1.67±0.33^{a}	1.67±0.33^{a}	1.67±0.33^{a}	1.33±0.33^{a}

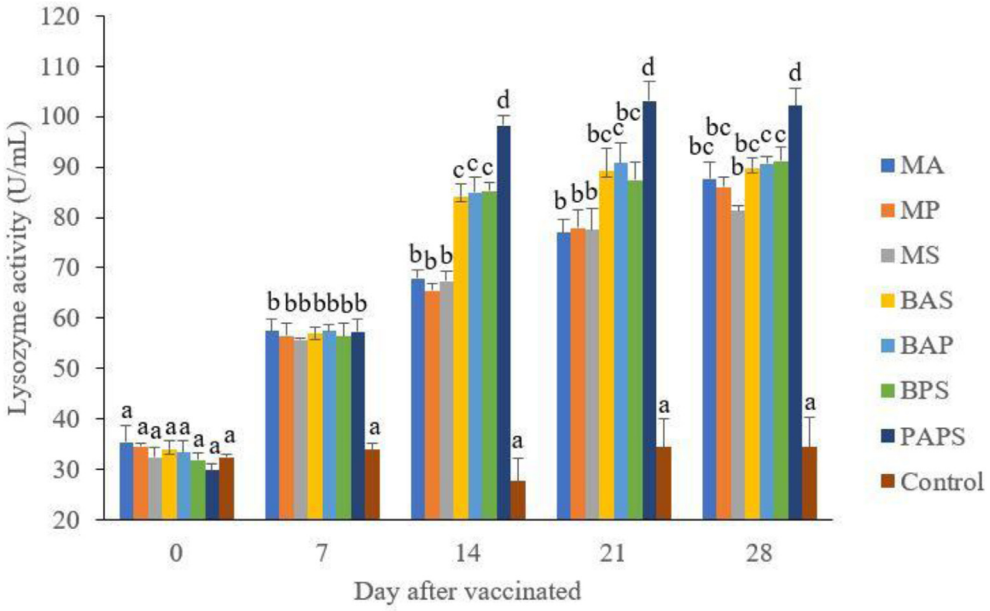

Figure 3. The lysozyme activity in the tilapia broodstock after being vaccinated with the various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae,* P: *P. fluorescens.* Values with different superscripts a,b indicate that their corresponding means are significantly different (P<0.05) according to one-way ANOVA followed by Duncan's test.

P. fluorescens was able to improve the broodstock's immune response which was then transferred to the seeds. This process was carried out in other to produce seeds that possess both lysozyme and antibodies and a high survival rate post-challenge test using pathogenic bacteria. This was better than the other treatments that made use of the bivalent and monovalent vaccines.

The results from the observation of the broodstock for 28 days showed that the total leukocyte (Figure 1), phagocytic (Figure 2), antibody titer (Table 1), and lysozyme activity (Figure 3), started to increase in week two post-vaccination. The broodstock vaccinated with PAPS showed a higher increase in the immune response compared to the others that were vaccinated with the bivalent, monovalent vaccines, and was the

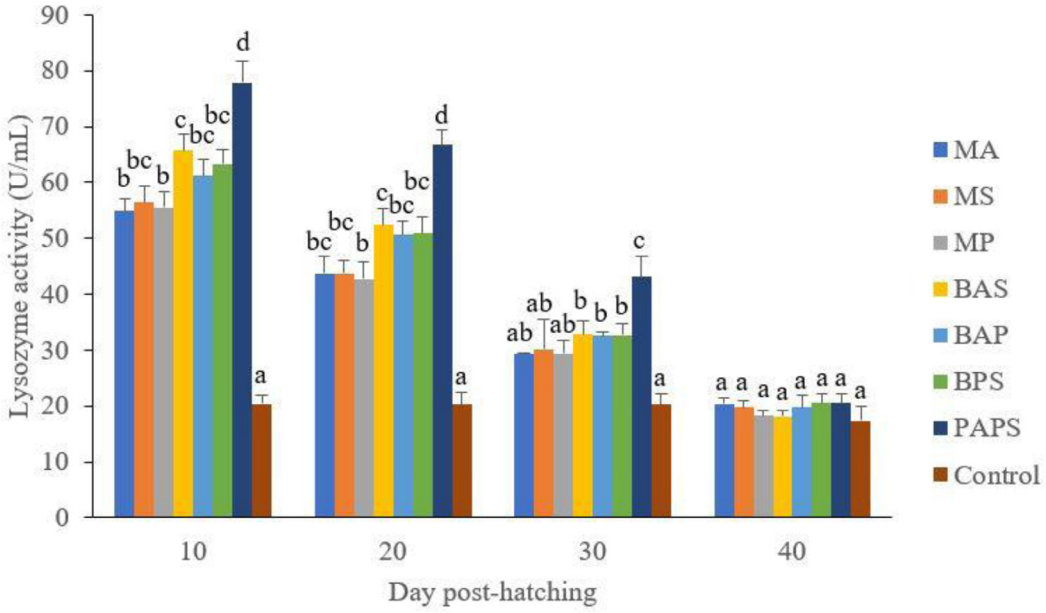

Figure 4. The lysozyme Activity of tilapia offspring from maternal immunity produced by various types of vaccines at the ages of 10, 20, 30 and 40 days post-hatching (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae,* P: *P. fluorescens.* Values with different superscripts a,b indicate that their corresponding means are significantly different (P<0.05) according to one-way ANOVA followed by Duncan's test.

Table 3. The Relative Percentage Survival (RPS) of tilapia offspring from maternal immunity produced by various types of vaccines at the ages of 10, 20, 30 and 40 days post-hatching. The offspring were produced by broodstock vaccinated with various types of vaccines through intramuscular (i.m.) injection (mean±SE).

Type of vaccine	Day post-hatching (day)			
	10	20	30	40
MA	66.67±4.81^{a}	55.26±5.26^{a}	41.03±2.56^{a}	14.29±4.96^{a}
MP	61.11±2.78^{a}	50.00±6.96^{a}	41.03±2.56^{a}	14.29±4.96^{a}
MS	63.89±2.78^{a}	52.63±4.56^{a}	43.59±2.56^{a}	17.14±2.86^{a}
BAP	72.22±2.78^{a}	60.53±4.56^{a}	46.15±4.44^{ab}	11.43±7.56^{a}
BAS	69.44±2.78^{a}	60.53±4.56^{a}	46.15±4.44^{ab}	14.29±4.95^{a}
BPS	69.44±7.35^{a}	57.89±6.96^{a}	43.59±2.56^{a}	11.43±2.86^{a}
PAPS	86.11±2.78^{b}	78.95±2.63^{b}	56.41±5.13^{b}	20.00±2.86^{a}

lowest in the unvaccinated broodstock[14,16,19,20,33]. This showed that PAPS could increase the Nile tilapia broodstock's immune response better than the other treatments.

The offspring produced from the broodstock that were vaccinated with PAPS had the highest antibodies (Table 2) and lysozyme activity (Figure 4) up to the 30th day post-hatching period and was the lowest in the offsprings from the unvaccinated broodstock (P<0.05). This demonstrated that their strong immune response was transferred to their offsprings[13–15,19,20,34] through the egg yolk[35].

The results from the challenge test using pathogenic bacteria (Table 3) showed that the offsprings that were produced using PAPS had a higher RPS compared to those from the offsprings produced from broodstocks that were treated using the monovalent and bivalent vaccines (P<0.05). This further showed that the vaccine treatment had adequately protected the fishes

from bacterial diseases with an RPS that was greater than 60% up to the 30th day post-hatching period[19,20,31]. The high RPS in the offspring during the challenge test using pathogenic bacteria in PAPS treatment was due to the broodstock's high number of leukocytes, phagocytic activity, the amount of antibody, and lysozyme activity transferred to the offsprings for protection against diseases.

The role of leukocytes which consist of neutrophils, lymphocytes, and monocytes, is to infiltrate the infected area for rapid protection[36], stimulating the production of antibodies through the recognition of foreign bodies, including vaccines and pathogens during the challenge test in this study. The phagocytic activity occurs during phagocytosis, which involves antibodies and complements during opsonization. Furthermore, the total leukocyte parameter increases in line with other immune responses, such as the antibacterial lysozyme, which triggers the complement system and phagocytic cells[36–38]. It encourages phagocytosis by activating leukocytes and polymorphonuclear macrophages or through opsonization[39]. The high number of leukocytes and a large amount of lysozyme in the treatment using PAPS which is similar to an infection by a pathogen indicated the success of PAPS in triggering the fish's immune system when developing an immune response.

The offsprings produced by the broodstock that were vaccinated with PAPS were protected from infections by *A. hydrophila*, *S. agalactiae,* and *P. fluorescens*. However, the monovalent vaccines only protected the offsprings from one type of bacteria. This is one of the advantages of applying PAPS. The results of this study revealed that the application of PAPS produced broodstock and offspring with better immune responses than the bivalent and monovalent vaccines. Therefore, the development of a polyvalent vaccine is more prudent than that of bivalent or monovalent because of its ability to target more than one species of bacteria[17,33,34,39–42]. The use of this type of vaccine caused the fish to respond to multiple antigens and form an immune response, thereby making it a strategic method in controlling bacterial diseases commonly found in culture and breeding environments[19,20,34,43]. Additionally, the application of polyvalent vaccines is more practical than the monovalent containing only one type of antigen. This showed that PAPS provided the most effective protection against diseases caused by pathogenic bacteria that often affect fishes, and thus is an ideal candidate for developing a polyvalent vaccine against bacterial infection.

Conclusion

The results show that the application of the polyvaccine against *A. hydrophila, S. agalactiae,* and *P. fluorescens* increased the antibody, lysozyme, total leukocytes, and phagocytic activity in Nile tilapa broodstock which was transferred to their offsprings, leading to a high RPS during the challenge test. Therefore, it is possible to produce seeds of Nile tilapia that are immune to diseases caused by *A. hydrophila, S. agalactiae,* and *P. fluorescens*. This process could be carried out through the vaccination of the broodstocks using a polyvalent vaccine against *A. hydrophila, S. agalactiae,* and *P. fluorescens*.

Data availability

Underlying data

OSF: Underlying data for '**Transfer of maternal immunity using a polyvalent vaccine and offspring protection in Nile tilapia, *Oreochromis niloticus***'. https://doi.org/10.31219/osf.io/cnqdg[44]

The project contains the following underlying data:

Data on broodstock immune response, offspring immune response, and offspring RPS in tilapia, *O. niloticus* can be accessed on OSF

Ethical statement

Research using fish in Indonesia has not been regulated and therefore it does not require animal ethics. However, this research has received approval from the Ministry of Education and Culture of the Republic of Indonesia (No.: 004/PL.22.7.1/SP-PG/2019). In addition, this study applies the principle of the International Animal Welfare standards including the assurance of fish welfare during maintenance and the use of drugs during sampling.

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