

Gonad Development and Blood Profile of Anti-KHV DNA Vaccinated Common Carp

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Received: 05 August 2020; Accepted: 24 August 2020

Abstract

Ardana Kurniaji, Sri Nuryati, Alimuddin, and Sri Murtini. 2020. Gonad development and blood profile of anti-KHV DNA vaccinated common carp. *Aquacultura Indonesiana*, 21 (2): 49-55. Koi herpesvirus (KHV) is one of pathogen that infects common carp. Alternative to prevent KHV infection is through vaccination. This study aimed to observe gonad development and blood profile on the common carp after being vaccinated by GP25 plasmid as DNA anti-KHV vaccine. Vaccination was performed on common carp broodstock at 30, 45 and 60 days before spawning. Three fishes were used for each treatment. Three fishes were injected with phosphate buffer saline as control. Blood collection for profile analysis was taken at 15 days pre-vaccination and 30, 45, 60 days post-vaccination. Egg diameter was observed during vaccination and, when spawning was performed. Gonad somatic index was measured in spawning time. The results showed that gonad somatic index in vaccinated broodstock was $13.75 \pm 1.57\%$ and broodstock control was $12.15 \pm 0.65\%$. Egg diameter were $0.57 \pm 0.06 - 0.97 \pm 0.13$ mm at vaccination and $1.47 \pm 0.23 - 1.53 \pm 0.12$ mm at spawning. There were differences morphological characteristic in fish at two different times. At vaccination time were abdomen still hard and genital papilla covered. While spawning time were abdomen appears rounded, enlarge and soft, papilla genital expands, widened anal and protruding. Erythrocyte decreased significantly on 30 days post-vaccination ie 1.05 ± 0.34 ($\times 10^6$ cells / mm³). Hemoglobin decreased on the 45 days ie 5.84 ± 0.72 g/dL and hematocrite decreased on 30 days ie $41.48 \pm 10.90\%$ post-vaccination. This study showed that anti-KHV GP25 DNA vaccine could reduce the erythrocytes, hemoglobin and hematocrite in normal level but not affecting in gonad development of common carp.

Keywords: Common carp; DNA vaccine; Gonad development; Koi herpesvirus

Introduction

One obstacle in rearing the common carp (*Cyprinus carpio*) is koi herpesvirus (KHV) outbreak. KHV is a very pathogenic virus and has caused many losses to farmers. KHV spreads widely in the world including in Indonesia (Sano *et al.*, 2004). The availability of vaccine as an alternative in preventing KHV infection is helpful. One of the vaccines that can prevent KHV infection is anti-KHV GP25 DNA vaccine, which was developed by Nuryati *et al.* (2010a). This vaccine is a DNA vaccine that has many advantages such as not posing a risk of infection and can induce cellular and humoral immunity simultaneously (Lorenzen and LaPatra 2005). The anti-KHV GP25 DNA vaccine has been shown to prevent KHV infection from various

research results either by injection, oral or immersion method (Nuswantoro *et al.*, 2012; Nuryati *et al.*, 2015; Aonulla *et al.*, 2016; Chairunnisa *et al.*, 2016). KHV infection can occur in all stadia including broodstock. This vaccine has been used in broodstock of common carp, and was able to improve immunity performance (Kurniaji *et al.*, 2018). Application of anti-KHV DNA vaccine to broodstock was expected to provide protection during the development of the gonads. Therefore, this study was aimed to observe the effect of anti-KHV GP25 DNA vaccine on development of gonad and the blood profile in common carp broodstock.

Materials and Method

Propagation of Vaccine

Vaccine that used in this study was anti-KHV GP25 DNA vaccine developed by Nuryati *et al.* (2010a). DNA vaccine was obtained from purification of bacterial plasmids *Escherichia coli* encoded vaccine genes which have been cultured following the method of Nuryati *et al.* (2010a). Plasmid was dissolved 100 µL ion exchange water, and DNA concentration was calculated using DNA/RNA calculator (Brown 1990). The vaccine plasmid was then ripped and verified by eletrophoresis method in agarose 1% to see the presence of GP-25 gene.

Broodstock of Common Carp

Broodstock used in this research was female common carp strain majalaya obtained from fish farmers in Bogor Regency, West Java, Indonesia. Broodstock was maintained in cage with size of 1×3×3 m³ and density of 1 fish/m³. Water quality during maintenance was suitable for fish growth (temperature 27-32°C, pH 7-8, DO >7 mg/L).

Research Design

This study used a completely randomized design with 3 treatments and 1 control. Each treatment was repeated 3 times. Treatment in this study was the difference in time of vaccination before spawning. Treatment A the broodstock was vaccinated 30 days before spawning, treatment B broodstock was vaccinated 45 days before spawning, treatment C broodstock was vaccinated 60 days before spawning, and control was broodstock without vaccination (injected with 1 mL PBS). Egg diameter was measuring in this stage. After vaccination, fish was fed with commercial diet (32-38% protein content), 2 times daily at satiation.

Fish Vaccination dan Gonad Observation

Broodstock of common carp were vaccinated intramuscularly injected with plasmid pmBA GP-25 with dose of 12.5 µg/100 grams of fish, dissolved in 1 mL of phosphate buffer saline (PBS). Before vaccination, fish was stunned with Ocean Free

Special Arowana Stabilizer (Qian Hu Corporation Ltd, Singapore) , 0.6-1.0 mL/L of water. Each treatment consisted of 3 female broodstocks. Observation of gonad maturity level was conducted by observing morphological characteristics refers to Jhingran and Pullin (1985). Gonad somatic index was calculated at spawning time by comparing the percentage of gonad weight and body weight (Effendie, 1979). Sample of fish egg for egg diameter measurement was taken by catheter (2-2.5 µm diameters) at vaccination time and spawning time. Observation was done under ocular microscope (0.1 mm precision).

Broodstock Hematology Analysis

Blood collection of broodstock was conducted 15 day before vaccination and 15, 30, 45, 60 days after vaccination. Blood was taken from caudal veins using a syringe rinsed with 3.8% Na-citric. Blood observation included total erythrocytes, hemoglobin, and hematocrite. The total calculation of erythrocytes refers to method of Blaxhall and Daisley (1973) ie the blood samples obtained from the sucked fish using pipettes containing red stirring sticks to a scale of 0.5. The sample was added with Hayem's solution (to lysis white blood cells) to scale 101. Blood in the pipette was stirred by swinging the pipette as forms an eight figure for 3-5 minutes until the blood can be mixed evenly. Two drops of blood solution in the pipette were removed, then droplets were deposited on the Neubauer type hemocytometer and covered with a glass cover to be observed under a microscope. The amount of erythrocytes was calculated using the following formula (Nabib and Pasaribu, 1989):

$$\Sigma \text{erythrocytes (cell/mm}^3\text{)} = \Sigma \text{cell count} \times \frac{1}{\text{vol. of big box}} \times \text{dilution factor}$$

Measurement of hemoglobin levels was done following Sahli method's (Wedemeyer and Yasutake, 1977) that convert the blood into hematinic acid after adding hydrochloric acid. The first step, blood was taken by sucking using Sahli pipette until scale of 0.02 mL and put into Sahli tube that had been filled with 0.1 N HCl solution until the red scale 10, then stand for 3 minutes. The sahlinometer tube was placed between 2 standard color tubes and aquadest was added

with dropper and stirred until the color changes exactly the same as the standard of Hb meter color. Hemoglobin levels were seen on gram% yellow scale that means the amount of Hb in grams per 100 mL of blood.

Measurement of hematocrite levels refers to method of Anderson and Siwicki (1993). First, blood was sucked with microhematocrite tube up to $\frac{3}{4}$ tube part. The tip of the tube was covered with crytoceal and centrifuged at 3000 rpm for 5 minutes. The value of the hematocrite level was known by comparing the length of the settling blood portion and the total length of all the blood in the tube. The calculation formula hematocrite levels is as follows:

$$\text{Hematocrite (\%)} = \frac{\text{volume red blood}}{\text{volume total}} \times 100$$

Statistical analysis

Obtained data include gonad maturity index, erythrocytes, hemoglobin and hematocrite were analyzed using ANOVA test at $p=0.05$ performed Duncan advanced test. The statistical test was done with of SPSS ver.16 program.

Results

Verification of anti-KHV GP25 DNA vaccine

Result of vaccine verification is shown in Figure 1, showed that sample in lane number 6 positively contains the anti-KHV GP25 DNA genome vaccine with a molecular weight of 8.0 kb, so it can be cultured and used for vaccination.

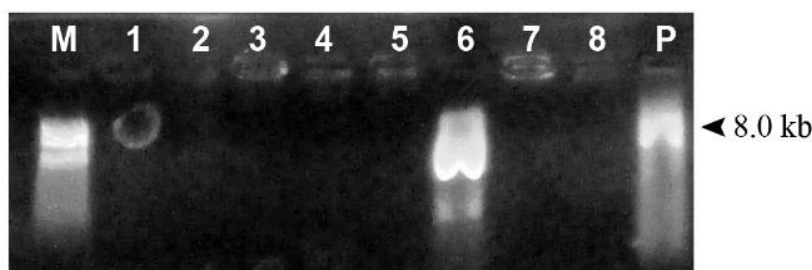


Figure 1. Electroforegram of DNA to verify the vaccine plasmid. DNA Marker (M): KAPA universal DNA ladder, samples from bacterial E. coli carrier stock of GP25 gene (No. 1-8), positive control of plasmid and DNA vaccine (P).

Gonad Development

The development of gonad was reflected by gonad somatic index

measurement and observation of morphological features, which can be seen in Table 1.

Table 1. The development of gonads includes gonad somatic index (GSI) and morphological characteristics

Treatments	Egg Diameter (mm)		GSI (%)	Morphological Characteristics	
	Vaccination	Spawning		Vaccination	Spawning
A	0.97±0.13	1.53±0.12	11.91±1.66	The abdomen was still hard,	Abdomen looks rounded,
B	0.76±0.06	1.47±0.23	12.99±2.17	not rounded	enlarged and soft, Genital
C	0.57±0.06	1.50±0.10	13.75±1.57	and the genital	papilla expands, slightly
K	1.57±0.12	1.57±0.12	12.15±0.65	papilla was covered	open and reddish, widened anal and protruding

Broodstock control injected PBS (Control)

Gonad development is an indicator that shows the maturity of fish reproduction before spawning. During the development of gonads, most of energy is directed to the development of gonads through vitelogenesis. Many factors that can affect gonad development include fish condition, nutrient intake and environmental condition. Fish vaccination conducted by injection method has the

potential to cause stress on fish and disturb gonad development. Moreover, during the developmental period of gonads, the body must respond to the presence of antigens from vaccine.

The results of this study showed that the administration of vaccine has no effect on gonad development. Gonad somatic index (GSI) show the percentage of gonad weight

of fish body. GSI in vaccinated broodstock showed not significant different ($P>0.05$) with control. This was supported by same morphological characteristics on vaccinated broodstock and control. Effendie (1979) stated that the maturity level of gonads can be determined by the morphology of gonad. The results of this study indicated that morphological characteristics of vaccinated fish and control showed abdomen still hard, not rounded and genital papilla was covered. At spawning time, morphological characteristics showed a rounded abdomen, enlarged and soft, red papilla genitalia and widened anal. Observation on gonad somatic index (GSI) fish also showed the value was in normal range. According to Yeganeh *et al.* (2012) the GAI of cultivated common carp is ranging from 4.3% to 15.2%. Egg diameters were $0.57\pm0.06 - 0.97\pm0.13$ mm at vaccination. It indicated the development of egg in stage of vitelogenesis (FAO, 2015). Different condition with egg diameter when broodstock spawning which was $1.47\pm0.23 - 1.53\pm0.12$ mm. It indicated that

developmental of egg in final stage or “spent” phases (FAO, 2015).

Total of Erythrocytes

The results showed that broodstock response to administration of the anti-KHV GP25 DNA vaccine. The response can be seen from difference of fluctuating values in each parameters that were significantly different from the control (fish without vaccination). Total erythrocytes in vaccinated broodstock were not significantly different from control pre-vaccination and 15 days after vaccination. Total erythrocytes in vaccinated broodstock was decreased on day 30, so that significantly different from control ($P<0.05$). On 45 days, total erythrocytes increased again but not significantly different with control ($P>0.05$). Total erythrocytes in vaccinated broodstock on 60 days continued to increase and significantly different with control, as shown in Fig. 2.

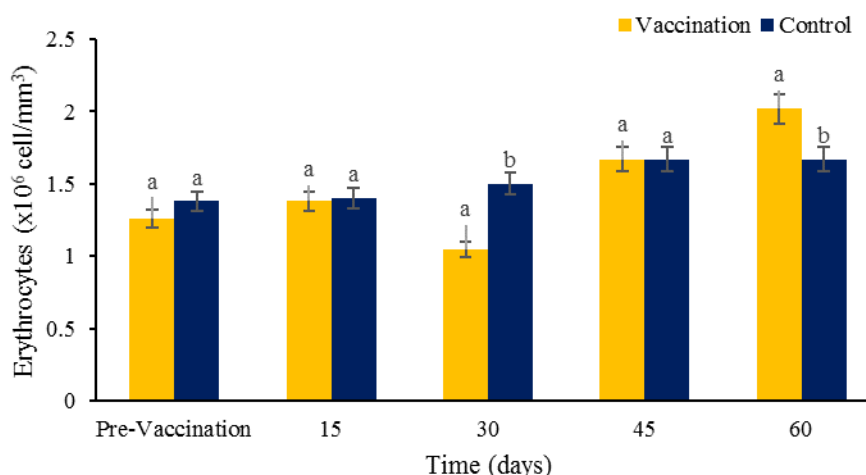


Figure 2. Total of erythrocytes in common carp broodstock

Blood profile is parameter that has been used as an indicator of health status in common carp well in vaccine efficacy (Nuryati *et al.*, 2010b), feeding test (Kumar *et al.* 2005) and hormonal (Ejraei *et al.*, 2015) in some other fish species (Adeyemo 2005; Mahasri *et al.*, 2011). The observations showed a decreasing total of erythrocytes in all treatments followed by a decreasing hemoglobin and hematocrite levels. According to Lagler *et al.* (1977) total of erythrocytes associated with level of hemoglobin that serves for binding Fe (iron) and transporting oxygen. Hematocrite shows the number of red blood cells to blood volume

in percent (Purves *et al.*, 2004), so the decreasing in total of erythrocyte will be followed by decreasing of hemoglobin and hematocrite levels. Although total of erythrocytes decreased significantly ($P>0.05$) was different with the control but still in the normal category (Svobodova and Vyukusova, 1991).

Hemoglobin Level

Hemoglobin levels showed a fluctuating value. Hemoglobin in vaccinated broodstock was not significantly different to control fish during pre-vaccination until 30 days after

vaccination. The hemoglobin level was significantly different on 45 days after vaccination and it showed that value of hemoglobin in vaccinated fish was lower than

control. On 60 days hemoglobin level that was increased again in vaccinated broodstock and was significantly different to control.

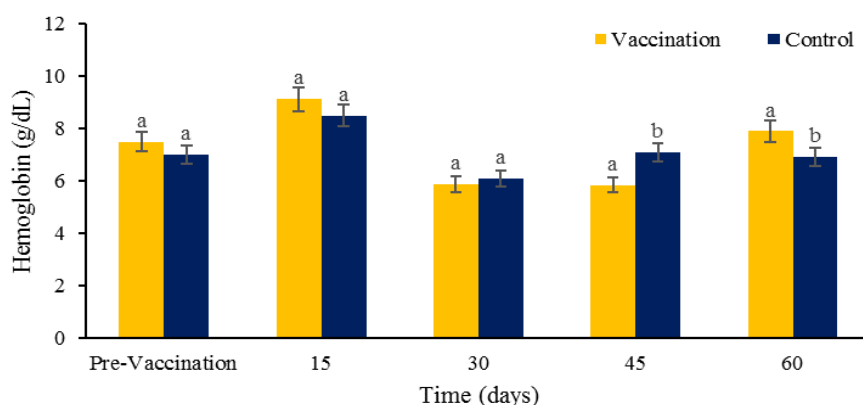


Figure 3. Hemoglobin levels in broodstock of common carp

Hematocrite Levels

Hematocrite levels before vaccination was showed no significant difference between vaccinated and control. Neither on the 15 days showed significantly different. Levels of hematocrite in vaccinated broodstock were

significantly different from control on 30 days after vaccination. On 45 days after vaccination, hematocrite levels was not significantly different with control up to 60 days.

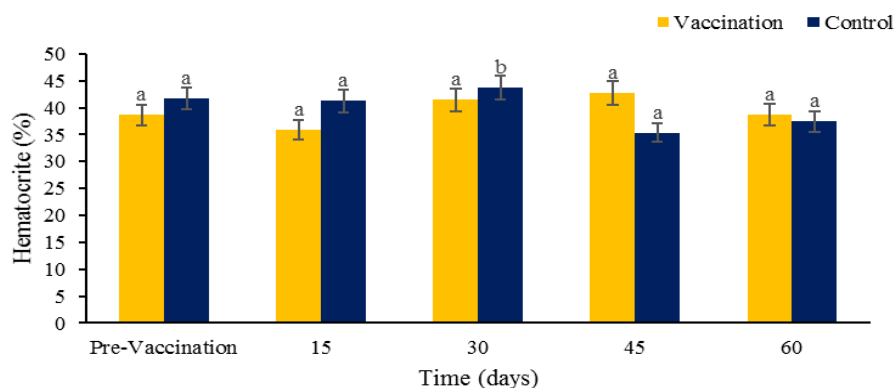


Figure 4. Hematocrite levels in broodstock of common carp

Total of erythrocytes was known decreased at 30 days post-vaccination ie $1.05 (\times 10^6 \text{ cells/mm}^3)$ and followed by hemoglobin decreasing at 45 days ie 5.84 g/dL. The decline in value was due to response to the presence of immune induction by the vaccine. For hematocrite, the increasing value occurred at 30 days post-vaccination and significantly different with control ($P < 0.05$) ie 41.48%. Dellman and Brown (1989) reported that low erythrocyte and hematocrite values indicating

a response to antigen. This can decrease fish appetite so the protein intake decrease in blood and also hemoglobin levels. In the case of microcytic anemia the reduced size of red blood cells causes the hematocritical level to be low (Jawad *et al.*, 2004). However, this decrease was temporary, so at 45 post-vaccination the erythrocyte value increased again followed by the increasing of hemoglobin and hematocrite at 60 days post vaccination.

Conclusion

Application of anti-KHV GP25 DNA vaccine caused no effect on the gonad development but has an effect on the blood profile. Decreasing of total erythrocyte occurred on 30 days post-vaccination followed by hemoglobin and hematocrite.

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