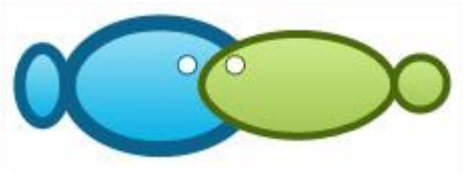

Effects of noni leaf (*Morinda citrifolia*) on hematologic and physiological profile of carp (*Cyprinus carpio*)

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Abstract. Increasing immunity is one way to prevent disease attacks in fish. This study determined the impact of noni leaf powder (*Morinda citrifolia*) on the hematological and physiological profile of carp (*Cyprinus carpio*). The research was conducted from August to October 2022. As an experimental research, a completely randomized design with one factor (noni leaf powder feed supplements) was applied. Consists of 2 levels, namely the experimental unit (a1, 1.5 g/100 g feed of Hi Provit 781-2) and the experimental control unit (a0, 0 g/g feed of Hi Provit 781-2). Feed was fed to fish reared in floating net cages (FNC) at the hydroelectric power reservoir (HPR) of Koto Panjang. The hematological (total erythrocytes, hemoglobin levels, and hematocrit levels) and physiological profiles (absolute weight, specific growth rate, survival rate, and blood glucose) of carp were examined. The hematological profile of carp in a1 was significantly different from the carp in a0. The total erythrocyte average of carp at a1 was higher ($1.61x10^6$ cells/mm^3) than at a0 ($1.44x10^6$ cells/mm^3). The average hemoglobin level in a1 was higher (7.93 g/dL) compared to 6.53 g/dL in a0. The hematocrit level in a1 was 31.67%, slightly higher than a0 (30.33%). Compared to the fish in a0, the carp in a1 had a better physiological profile. In the treatment which used noni leafs the fish survival rate was marginally higher at 94.67%, compared to 91% in a0, blood glucose was 81.67 mg/dL, compared to 57.67 mg/dL in a0, absolute weight was 25.61 g, compared to 16.69 g in a0, and specific growth rate was 1.68%/day, compared to 1.53%/day in a0. It can be concluded that adding noni leaf extract to fish feed can be used as an effort to control carp disease in the future.
Key Words: blood glucose, erythrocytes, hematocrit, hemoglobin, phytoimmunostimulants.

Introduction. Carp (*Cyprinus carpio*) has long been cultivated in Indonesia, has high economic value, is popular with the public, and is produced in large quantities. One of the areas where carp is grown is at the hydroelectric power reservoir (HPR) Koto Panjang, Kampar, Indonesia. Apart from being used as a power plant, the Koto Panjang HPR is also used by the local community as a carp-rearing area in a floating net cages (FNC). Carp cultivation has several advantages over other commodity cultivation. This fish is relatively easy to care for, grows fast, and is compatible with tropical climatic conditions such as in Indonesia. The high demand for carp is forcing people to increase the production of this fish (Rahman et al 2021; Woynarovich et al 2011; Effendi et al 2022a).

The problem that is often encountered in carp cultivation is the attack of pathogenic microbes. For example, bacterial, parasitic, fungal, and viral infections. This infectious disease can be transmitted from their parent or other fish. Cases of mass carp deaths occurred in West Java in 2001 when 80 tons of carp died within one month. Mass mortality of carp is mostly related to motile aeromonas septicemia (MAS) disease, a disease caused by *Aeromonas hydrophila* bacteria. The death of fish due to bacterial infection can be prevented by increasing the immune system of the carp itself (Sunarto et al 2005a; Sunarto et al 2005b; Effendi et al 2022b).

Fish body resistance can be stimulated using drugs or bioactive compounds that can modify the immune response by stimulating the fish's immune system. The immune system

can be improved by providing medicinal plant ingredients that contain bioactive compounds, which can increase the immune system in the fish's body. One way to introduce bioactive compounds into the fish's body is by mixing these compounds into fish feed. The feed is digested by the fish and the content of these bioactive compounds is expected to be absorbed by the fish's body. Bioactive compounds that can improve the immune system include flavonoids, saponins, steroids, terpenoids, and alkaloids (Bhimba et al 2010; Edu et al 2015; Middleton et al 2000).

These medicinal ingredients or bioactive compounds are immunostimulants, which may increase the immune response of fish both cellularly and humorally. These chemical compounds can be either animal or plant (called as phytoimmunostimulants). Phytoimmunostimulants are fed to fish through several methods, one of which is oral. These chemical compounds are mixed into the feed and the feed is consumed by the fish, the medicinal plants are absorbed by the fish's body (Dügenci et al 2003; Vallejos-Vidal et al 2016).

Noni (*Morinda citrifolia*) has been known for generations to have a myriad of benefits. Noni is often used as a medicinal plant material both from the leaves and fruit. Noni is traditionally used as a medicine to relieve pain and inflammation in the body (Sanni et al 2017). In some research in the field of fisheries, the provision of medicinal plant material from noni leaf powder mixed with feed is one of the efforts to overcome the problem of mass mortality of fish due to bacterial infection. Phytochemical analysis conducted by some authors (Wang et al 2002) revealed that noni leaves contain very strong steroid and triterpenoid compounds, flavonoids, phenols, tannins, and saponins. This study aimed to determine the effect of noni leaf powder as an immunostimulant on the hematological profile (total erythrocytes, hemoglobin levels, and hematocrit levels) and physiological profiles (absolute weight, specific growth rate, survival rate, and blood glucose) of carp.

Material and Method

Time, place, and materials. This research was conducted from August to October 2022 located at the hydroelectric power reservoir (HPR) Koto Panjang, Riau, Indonesia. Young noni (*Morinda citrifolia*) leaves were obtained by picking leaves that grow wild around the University of Riau campus. Carp, measuring 8-12 cm were obtained from the owner of a fish hatchery in Rao, Pasaman, West Sumatra, Indonesia. Pellet Hi Provit 781-2 is a commercial fish feed produced by PT. Central Pangan Pertiwi Animal Feedmill Co. Ltd., Karawang, West Java, Indonesia. The composition of this fish pellet is 31-33% protein, 4-6% fat, 3-5% fiber, and 9-10% water content.

Research methods. This research is an experimental study with a completely randomized design with one factor, namely the supplement of noni leaf powder. The experiment consisted of 2 levels, namely experimental unit a1 (1.5 g/100 g feed of Hi Provit 781-2) and experimental control a0 (0 g/100 g feed of Hi Provit 781-2). Feed was fed to fish reared in floating net cages (FNC) at the HPR Koto Panjang. The measured parameters were the hematological and physiological profiles of carp in the two ecosystems. The hematological profile included; total erythrocytes, hemoglobin levels, and hematocrit levels. The physiological parameters were; absolute weight, specific growth rate, survival rate, and blood glucose.

Feed preparation. The young and fresh noni leaves were picked and washed first, then dried under the hot sun. Once dry, leaves were cut into small pieces and ground until they were turned into a powder. The powder was filtered through a sieve with a size of 100-200 mesh (0.15-0.75 mm) and then mixed with finely ground Hi-Provit 781-2 fish feed pellets and re-milled to form new pellets (diameter of 0.8 mm) by using a Floating Fish Feed Pellet Making Machine, Type K50, Firman Ltd. Jakarta, Indonesia.

Rearing fish. Fish were reared in 6 FNC (floating net cage) units, sized 1m x 0.8m x 1m with a density of 25 fish per fish cage net. Each treatment (a1 and a0) used 3 FNC units. Fish were reared for one month and fed 3 times per day as much as 5% of body weight.

Fish hematological profile

Collecting fish blood. The fish (3 fish per FNC) were caught and anesthetized at 0, 30 and 60 days with clove oil at a dose of 0.05 ml/l. Blood was drawn from the caudal vein using 1-ml syringes that had been rinsed with 10% EDTA, collected in micro tubes (Axygon) and stored at room temperature until analysed.

Total erythrocyte calculation. The total number of erythrocytes was examined by referring to Blaxhall and Daisley (2006). Blood that had been mixed with 10% EDTA was sucked with a hemocytometer pipette (there were red spots for erythrocytes) up to the 0.5 mark. Then hayem solution was added (for erythrocytes) sucked up to the 101 mark. To mix the blood evenly, the pipette is stirred in the form of a figure eight for 3-5 minutes. Before counting erythrocytes, two drops of blood in the hemocytometer pipette were removed to remove air cavities, then the blood was dripped into the hemocytometer box and covered with a cover glass, then observed under a microscope with a magnification of 10x and 40x. The total number of erythrocytes was counted as 5 small boxes on the hemocytometer according to the formula:

$$\text{Erythrocyte count} = \Sigma \text{ N x } 10^4 \text{ cells/mm}^3$$

where N is the number of erythrocytes counted in 5 fields of view and 10^4 is the dilution factor.

Hemoglobin levels. The calculation of hemoglobin levels was carried out by referring to the Sahli method. The Sahlinometer tube is placed between 2 tubes of standard color. The tube was filled with 0.1 N HCl solution to 0 (the bottom line), then fish blood was taken from the microtube with a 0.02 mL Sahli pipette and inserted into the Sahlinometer tube. Then it was left to stand for 3 minutes, before cleaning the tip of the pipette first. Next, distilled water was added with a droplet dropper little by little while stirring with a glass stirrer until the color was the same as the standard color. Hemoglobin levels were expressed in g/dL or g%.

Hematocrit level. The procedure for measuring hematocrit levels was conducted by dipping the end of the microhematocrit tube into a tube containing blood. Three-quarters of the tube blood was taken. The end of the tube that already contains blood was closed with a crytoceal, namely by plugging the end of the tube into the crytoceal as deep as 1 mm to form a crytoceal plug.

The microhematocrit tube was centrifuged for 5 minutes at 500 rpm with the same volume tubes facing each other so that the centrifuge rotation was balanced. The sediment in the blood portion and the total length of the blood volume contained in the tube were measured using millimeter blocks. Blood hematocrit levels were calculated with the following formula:

$$\text{Hematocrit level} = \frac{\text{Length of the deposition of blood cells in the tube}}{\text{Length of the total volume of blood in the tube}} x\ 100\%$$

Fish physiological profile

Absolute weight growth. The calculation of absolute weight growth was the difference between the average weight of the fish at the end of the study and the average weight of the fish at the beginning of the study. The absolute weight growth was calculated based on the following formula:

$$W = W1 - W0$$

where:
W = absolute growth (g)
W1 = fish weight at the end of the study (g)
W0 = fish weight at the beginning of the study (g)

Specific growth rate. Specific growth rate (SGR) was the daily growth of fish during rearing, which was 30 days. The specific growth rate was calculated using the following formula:

$$SGR = \frac{\text{Ln Wt-Ln Wo}}{t} \text{ X } 100$$

where:
SGR = specific growth rate (% /day)
Wt = fish weight at the end of the study (g)
Wo = fish weight at the beginning of the study (g)
t = length of study (days)

Survival rate. Survival rate (SR) is a comparison of the number of fish that live from the beginning of the study to the end of the study. The survival rate was calculated by the following formula:

$$SR = \frac{Nt}{No} \, x \, 100\%$$

where:
SR = survival rate (%)
Nt = number of live fish at the end of the study
No = number of live fish at the beginning of the study

Blood glucose. The test was carried out in the morning before the fish were fed. Blood glucose calculations were carried out by referring to previous work (Eames et al 2010), namely checking the blood glucose of fish using a glucose test kit for humans (GlucoDR) with a range of 20-600 mg/dl. Fish blood was dripped onto a strip that had been attached to the GlucoDR device. The results of fish blood glucose levels were displayed on the GlucoDR screen.

Water quality analysis. Water quality measurements (temperature, pH, and ammonia levels) were carried out during maintenance, namely on the first day, the 15th day, and the 30th day.

Data analysis. Data were analyzed using an independent statistical analysis T-test using IBM SPSS 26 software. Water quality data were analyzed descriptively and referred to water quality standards for aquaculture.

Results

Fish hematological profile. The hematology profile of carp reared in a floating net cage (FNC) in HPR Koto Panjang was significantly different between carp that received noni powder in their diet (a1) when compared to the carp without the noni powder diet (a0). The average total carp erythrocytes in treatment a1 was higher ($1.61x10^6$ cells/mm^3) compared to the total erythrocytes of carp in experimental control a0 ($1.44x10^6$ cells/mm^3), significantly different, where (2-tailed) 0.012<0.050. The same thing was also found in hemoglobin levels, where the average hemoglobin level of carp in treatment of a1 (7.93 g/dL) was significantly higher than the carp in control treatment a0 (6.53 g/dL), where 0.000<0.050. The average hematocrit level was also the same, where the carp which received the immunostimulant in a1 treatment was significantly higher (31.67%)

compared to the carp hematocrit level in the experimental control a0 (30.33%), where (2-tailed) 0.047<0.050 (Table 1).

Fish physiological profile. In general, it can be seen that the physiology profile of carp reared in a floating net cage (FNC) in HPR Koto Panjang that received Hi Provit 781-2 diet enriched with noni leaf powder (a1) was better when compared to carp fed Hi Provit 781 -2 diet only (a0). The mean absolute weight of fish a1 was significantly better (25.61 g) when compared to fish in a0 (16.69 g), where (2-tailed) 0.002<0.050.

The average specific growth rate of fish fed Hi Provit 781-2 enriched with noni leaf powder (a1) was significantly better (1.68% day) when compared to carp fed Hi Provit 781-2 alone a0 (1.53% day), where (2-tailed) 0.002<0.050. The average survival rate of fish of a1 was slightly higher (94.67%) when compared to carp of a0 by 91.00%, but not significantly different, where (2-tailed) 0.093>0.050. The average blood glucose of fish that received a diet of a1 treatment was significantly better (81.67 mg/dL) when compared to carp in treatment of a0 (57.67 mg/dL), where (2-tailed) 0.000<0.050 (Table 2).

Table 1

Hematology of carp reared in the floating net cage (FNC) in HPR Koto Panjang

Experimental treatments	*Repetition*	*Total erythrocytes (10^6 cells/mm^3)*	*Hemoglobin levels (g/dL)*	*Hematocrit level (%)*
a1	1	1.68	8.00	32.00
	2	1.55	8.00	32.00
	3	1.60	7.80	31.00
Total		4.83	23.80	95.00
Average		1.61	7.93	31.67
a0	1	1.44	6.4	30
	2	1.45	6.6	30
	3	1.44	6.6	31
Total		4.33	19.60	91.00
Average		1.44	6.53	30.33

Table 2

Physiological profile of carp reared in the floating net cage (FNC) in HPR Koto Panjang

Experimental treatments	*Repetition*	*Absolute weight growth (g)*	*Specific growth rate (% day)*	*Survival rate (%)*	*Blood glucose (mg/dL)*
a1	1	24.52	1.64	96.00	81
	2	25.28	1.67	92.00	81
	3	27.02	1.74	96.00	83
Total		76.82	5.05	284.0	245.00
Average		25.61	1.68	94.67	81.67
a0	1	14.75	1.16	90.00	54
	2	17.17	1.29	93.00	61
	3	18.14	1.34	90.00	58
Total		50.6	4.59	273.0	173.00
Average		16.69	1.53	91.00	57.67

Water quality. The results of water quality measurements during carp rearing in HPR Koto Panjang obtained temperatures ranging from 28-31ºC. pH 6.4-6.9 and ammonia level ranged from 0.00084-0.0015 mg/dL. The water quality in this habitat was considered within normal limits.

Discussion. The hematological profile of the carp was significantly different for the fish that received the addition of noni powder in their diet (a1) when compared to the hematological profile of the fish that ate the feed without the addition of

phytoimmunostimulants (a0). Increased immunity of fish due to phytoimmunostimulants has been reported by some researchers previously. For example, Herlina (2017) reported that adding 5 and 10 g/kg of noni leaf powder diet increased total leukocytes and carp survival rate. Total leukocytes in treatment A (0 g/kg feed) was 6.65-8.77 x 10^5 cells/mm^3, treatment B (5 g/kg feed) was 8.16-13.3 x 10^5 cells/mm^3, and treatment C (10 g/kg feed) was of 16.18-19.30 x 10^5 cells/mm^3. The normal range of total carp erythrocytes ranges between 1.43-1.6 x 10^6 (Sezgin & Aydin 2021).

Other researchers (Setyaningsih et al 2019) also reported that the addition of noni leaf extract into feed has increased erytrocytes in comet goldfish (*Carassius auratus*) to 13.86 x 10^5 cells/mm^3, a nearly normal concentration. The extract also caused a decrease of leukocytes level down to normal levels (18.76 x 10^5 cells/mm^3) of heathy fish. Pratama (2019) reported that immersing tilapia (*Oreochromis niloticus*) fish in a noni fruit extract solution (concentration of 5400 ppm) for 96 hours increased the number of leukocytes from 8.06 ± 0.01 x10^4 cells/mm^3 to 10.65 ± 0.02 x10^4 cells/mm^3. Other researchers revealed that immersing gourami (*Osphronemus gouramy*) fish in a noni leaf extract solution (concentration 800 ppm) for 24 hours cured fish infected with *Aeromonas hydrophila*. Healing was characterized by mild histopathological damage, with a hyperplasia score of 1.9%, fusion of 1.6%, and necrosis of 1.2% (Pertiwi 2019). This shows that the content of active compounds, especially ascorbic acid, and flavonoids, and the content of amino acids in noni leaf extract play a role in stimulating leukocytes as a non-specific defense so that the active ingredient can function as an immunostimulant. Flavonoids can increase the production of leukocytes. Flavonoids also stimulate the immune system because leukocytes as foreign matter eaters are activated more quickly (Bovi et al 2023; Nayak & Mengi 2010).

One of the nutrients contained in noni leaves that plays an important role in increasing the production of erythrocytes is iron (Fe). Iron is very helpful in the process of making biconcave chips in the spinal cord which will then synthesize heme at the beginning of the formation of hemoglobin. Some authors mentioned that noni leaves powder contains Fe. Carp and other animal bodies that experience iron deficiency result in a decrease in the fish's immune system. Carp that has a low immune system will be very easily infected by pathogenic bacteria. Therefore, feeding with a mixture of noni leaf powder consumed by carp can increase the carp's body resistance (Sunder et al 2016; Effendi et al 2023).

Nayak and Mengi (2010), reported that the *Morinda citrifolia* plant has an immunostimulant effect on T and B lymphocytes due to immune-enhancing components in its fruit. Studies conducted in vivo found that IFN- production was up and IL-4 production was down. These findings suggested that noni juice regulates the immune system by activating CB2 receptors, inhibiting IL-4 production while boosting IFN- cytokine production (Palu et al 2008).

Examination of the physiology of carp in this study is intended to learn the growth and stress levels of carp that are kept in FNC. Analysis of the physiological profile of fish can be used as a reference to see the growth and stress levels of fish. Noni leaves contain saponin compounds which function as permeabilizing membranes and affect the growth and absorption of nutrients by fish (Das et al 2012). The positive impact of adding noni powder to feed can also be seen from the physiological profile, where carp that received an immunostimulant diet are healthier and grew better.

On Polynesian islands, the Noni plant's various parts have historically been utilized as herbal remedies for a variety of illnesses. Numerous scientific studies indicate that it contains numerous chemical components, including minerals, vitamins, micronutrients, anthraquinones, amino acids, fatty acids, iridoids, flavonoids, lignans, sterols, and polysaccharides, among others, that are beneficial in treating a variety of diseases (Ali et al 2016). Additionally, it has been claimed that ripe noni fruit contains volatile substances such as aldehydes, ketones, esters, alcohols, terpenes, and sulfur compounds. This implied that the flavor properties of noni juice may be influenced by the presence of sulfur. Other researchers (Wei et al 2011) mentioned the discovery of two iridoids and polyphenols from the coumarin, flavonoid, and phenolic acid families in noni juice.

Due to *M. citrifolia's* potent antioxidant activity and established health advantages, it is extensively used as a complementary and alternative therapy in many nations. It has

historically been used as a therapeutic medicine for some illnesses, acting as an antibiotic, anticancer, anthelmintic, analgesic, and immunostimulant. Additionally, it has been demonstrated to help treat ailments like gastritis, skin conditions, lung infections, menstruation and urinary tract problems, fever, diabetes, and venereal diseases (Ali et al 2016).

Various portions of the noni plant are said to contain more than 160 phytoconstituents, of which more than 120 have been linked to nutraceutical benefits and demonstrated biological activity (Nagalingam et al 2012). Micronutrients, non-volatile and volatile substances, ketones, lactones, beta-carotenoids, terpenoids, and proxeronine are all present in the fermented fruit extract (Sanni et al 2017). According to the findings, ripe noni fruit has the greatest antibacterial activity (21 mm) and greatest antifungal activity (19 mm) against *Klebsiella pneumonia* and *Aspergillus flavus*, respectively (Samiraj et al 2012).

Phytochemical qualitative analysis of noni leaves powder mentioned that the leaves contained very strong triterpenoids, and the compounds have the main function of stimulating red blood cells and the immune system (Deng et al 2012). Some authors (Asiseh et al 2020) reported that noni juice has an impact on the release of *Argulus* spp. from *Carassius auratus auratus*. *M. citrifolia* leaf extract significantly inhibited the growth of *Aeromonas hydrophilla* in vitro assay. Noni fruit juice with different dosages inhibited the infection of *Myxobolus* spp. on carp (Pongoh & Gemaputri 2018).

Carp that have a low immune system will be very easily infected by pathogenic bacteria. Therefore, feeding with a mixture of noni leaf powder consumed by carp can increase the carp's body resistance (Sunder et al 2016; Ali et al 2016; Moh et al 2021). It is also reported that noni leaf extracts powder inhibits the growth of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. The overall findings suggest that treating infectious diseases using solvent extracts of noni leaves is successful (Usha et al 2010). The maximum percentage of inhibition against *Trichophyton mentagrophytes* was shown by the dried noni fruit's methanolic extract (79.3%), but only about 50% activity was observed against *Penicillium*, *Fusarium*, and *Rhizopus* species (Jainkittivong et al 2009).

Conclusions. Carp fed with the noni powder-containing diet (a1) had a significantly different hematological profile from the carp fed with the control diet (a0). Carp had a higher average total erythrocyte count at a1 ($1.61x10^6$ cells/mm^3) than at a0 ($1.44x10^6$ cells/mm^3). Hematocrit levels were 31.67% > 30.33% and average hemoglobin levels were 7.93 g/dL > 6.53 g/dL. Similar to this, carp in treatment a1 had a better physiological profile than fish in treatment a0. Average blood glucose levels were higher (81.67 mg/dL than 57.67 mg/dL), average specific growth rate was higher (1.68%/day > 1.53%/day), and average absolute weight was higher on average (25.61 g > 16.69 g).

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