Phytoimmunostimulants increase of the immunity of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila* infection in brackish water

[1]Irwan Effendi, [1]Harum Farhana, [1]Mardalisa Mardalisa, [1]Dessy Yoswaty, [1]Henni Syawal, [2]Brian Austin, [3]Alastair R. Lyndon, [4]Sri Wahyuni, [1]Ronal Kurniawan

[1] Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia; [2] Institute of Aquaculture, Stirling University, Stirling, United Kingdom; [3] Institute for Life and Earth Sciences, Heriot-Watt University, Edinburgh, United Kingdom; [4] Faculty of Fisheries and Marine Sciences, University of Teuku Umar, Meulaboh, Indonesia. Corresponding author: R. Kurniawan, kurniawanronal09@gmail.com

Abstract. Diseases are a serious problem in common carp (*Cyprinus carpio*) farms, and using chemicals is hazardous. It is necessary to find solutions by utilizing natural ingredients such as phytoimmunostimulants. This study aimed to determine the effect of leaf powder of mango (*Mangifera indica*), guava (*Psidium guajava*), and noni (*Morinda citrifolia*) as phytoimmunostimulants on the hematological and physiological of common carp. The research was carried out from January to June 2023, using a completely randomized design (CRD) with 4 treatment levels and 3 replications. The treatment levels were P0 (experimental control unit or feeding the fish with commercial fish feed only), P1 (feeding the fish with commercial fish feed containing noni leaf powder of 1.5 g/100 g), P2 (feeding the fish with commercial fish feed containing guava leaf powder of 1.5 g/100 g), and P3 (feeding the fish with commercial fish feed containing mango leaf powder of 1.5 g/100 g). The fish were challenged with *Aeromonas hydrophila* bacteria on the 30th day. The hematological and physiological profiles of the fish were measured at 0, 30, and 45 days. The treatment using guava leaves showed the most promising results for total levels of erythrocites, hemoglobin, hematrocit, blood glucose and survival rate. The use of guava leaves provided the best immune response and could be developed as part of the diet in the future.
Key Words: fish immunity, fish pathogenic bacteria, hematological parameters, medicinal herbs, physiological parameters.

Introduction. Common carp (*Cyprinus carpio*) is a species of freshwater fish that is widely cultivated, for example in ponds, running water ponds, and in floating net cages. The main problem in common carp farming is the occurrence of bacterial infections disease which result in decreased fish production. The application of antibiotics during fish production can cause other problems, namely antibiotic-resistant bacteria and food safety because many antibiotics can be accumulated in the fish's body (Hai 2015; Effendi et al 2022a). Therefore, alternative treatments are needed that are more environmentally friendly and do not cause a resistant effect on bacteria (Song et al 2014). One of the diseases in common carp cultivation is motile *Aeromonas* septicemia (MAS), a bacterial disease caused by the bacterium *Aeromonas hydrophila*. This bacterium causes hemorrhagic septicemia, which is characterized by swelling of the stomach, intestines, lesions, skin abscesses, exophthalmia, and bleeding, especially in the gills and operculum (Pathania et al 2014).

Immunostimulants often referred to as immunostimulators, are substances (drugs and foods) that boost the activity of any component of the immune system, hence stimulating the immune system. Immune responses to certain immunostimulants, such as vaccinations or any antigen, are antigenic-specific. Non-specific immunostimulants, such as adjuvants and non-specific immunostimulators, work independently of antigenic specificity to enhance the immune response to other antigens or stimulate immune system components (Secombes & Fletcher 1992). Immunostimulants have been utilized as dietary supplements to increase weight gain, feed effectiveness, and/or disease resistance in farmed fish to preserve fish health and improve performance (Vallejos-Vidal et al 2016).

The use of immunostimulants is an alternative to the use of antibiotics and chemicals. According to Awad and Awad (2017), immunostimulants are materials that can improve the function of the immune system by using substances that stimulate the system. Medicinal plants have many benefits such as stimulation of appetite and growth, stimulation of the immune system, antimicrobial and antifungal activity, as well as anti-stress effects (Baghizadeh & Khara 2015). Some plants enhanced the immune system of fish, for example, mango leaves (*Mangifera indica*), noni leaves (*Morinda citrifolia*), and guava leaves (*Psidium guajava*) (Amulejoye et al 2020; Abdel-Tawwab & Hamed 2020). This study aimed to determine the effect of phytoimmunostimulants (mango, noni, and guava leaf powder) on the differentiation of common carp immunity challenged by the fish pathogenic bacteria *Aeromonas hydrophila*.

Material and Method

Research methods. This research was carried out from January to June 2023 at the Marine Microbiology Laboratory, University of Riau. The method used was an experimental method with a one-factor completely randomized design (CRD), namely dietary phytoimmunostimulant species, with 4 treatment levels and 3 repetitions. The treatments were P0 (experimental control unit or feeding the fish with commercial fish feed Hi Provit 781-2 only), P1 (feeding the fish with commercial fish feed Hi Provit 781-2 containing noni leaf powder of 1.5 g/100 g), P2 (feeding the fish with commercial fish feed Hi Provit 781-2 containing guava leaf powder of 1.5 g/100 g), and P3 (feeding the fish with commercial fish feed Hi Provit 781-2 containing mango leaf powder of 1.5 g/100 g).

Preparation of phytoimmunostimulant fish feed. The main ingredient of the feed was the commercial fish feed Hi-Pro-Vite 781-2, 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. The medicinal plants, namely noni leaves (*Morinda citrifolia*), guava leaves (*Psidium guajava*), and mango leaves (*Mangifera indica*) were collected from community plants around the University of Riau campus, Pekanbaru, Indonesia. The leaves were washed, air-dried, and dried in the sun. After drying, the leaves were chopped into small pieces, blended, and finely ground. The powder was sifted through a sieve with a size of 100-200 mesh and then mixed with finely ground Hi-Pro-Vite 781-2 fish feed pellets (1.5 g/100 g) and re-molded using a pellet grinding machine, making new pellets with a size of 2 mm. (Effendi et al 2022b).

Acclimatization and rearing of fish. Common carp, measuring 8-12 cm, was obtained from the owner of a fish hatchery in Rao, Pasaman, West Sumatra, Indonesia. In the laboratory, the common carp were acclimatized for 7 days in freshwater and then to brackish water (salinity 5 ppt) for another 7 days. On day 15, feeding with phytoimmunostimulant feed started. The containers were plastic drums with a capacity of 100 l, which were filled with 80 l of brackish water. The stocking density of each drum was 25 individuals. The fish were reared for the next 30 days.

Preparation of Aeromonas hydrophila isolates. The *Aeromonas hydrophila* isolate was obtained from a collection of virulent isolates at the Marine Microbiology Laboratory, University of Riau. The isolates were then cultured in nutrient agar media (Oxoid) and incubated in an incubator for 24 hours. The pathogen was then harvested from the bacterial lawn, suspended at 0.1 % saline (10^8 cells/ml), and used for fish challenge purpose.

Fish challenge. After being reared for 30 days (15 days of acclimatization and 15 days of being fed a diet containing phytoimmunostimulants), the fish were challenged with *Aeromonas hydrophila*. The bacterial solution density was 10^8 CFU/ml and injected 0.1 ml intramuscularly by using a 1 ml syringe. The fish was returned to the drum and reared for another 15 days.

Measurement of hematological and physiological parameters. Three samples of fish were used from each treatment. Blood was collected by anesthetizing the fish at cold temperature (±8°C) (Gül et al 2012; Witeska et al 2022). After that, blood was taken from the caudal vein using a syringe (1 mL). Furthermore, the blood was stored in Eppendorf tubes with anticoagulant for hematology observation. The hematological parameters measured were total erythrocytes, hemoglobin level, and hematocrit level. Meanwhile, blood glucose content and survival rate were evaluated. Measurements (Blaxhall & Daisley 1973) were carried out at the beginning of the experiment, on day 30, and on day 45 (14 days after the challenge with *Aeromonas hydrophila*).

Water quality. Water quality parameters measured were temperature, dissolved oxygen (DO), salinity, and pH. Temperature measurements were carried out using a thermometer, dissolved oxygen using a DO-meter, pH using a pH-meter, and salinity using a hand-refractometer. Water quality measurements were carried out on days 1, 30, and 45.

Data analysis. All data (total erythrocytes, hemoglobin level, hematocrit level, blood glucose content, and survival rate) were tabulated and then analyzed by using analysis of variance (ANOVA). Water quality parameters were analyzed descriptively.

Results

Total erythrocytes. The results of the study showed that the addition of phytoimmunostimulants to carp feed which was reared for 30 days then challenged with pathogenic bacteria and then was reared for another 14 days had a significant effect on total erythrocytes (p<0.05). On day 30, the highest total erythrocytes were recorded in treatment P2 (1.77 x 10^6 cells/mm^3), followed by P1 (1.36 x 10^6 cells/mm^3), P3 (1.28 x 10^6 cells/mm^3), and P0 (1.28 x 10^6cells/mm^3). While on day 45, the total erythrocytes tended to increase, except in the experimental control, where the highest total erythrocytes were still recorded at P2 (1.88 x 10^6 cells/mm^3) and then followed by P1, P3, and P0 (Table 1). ANOVA results showed that feeding containing phytoimmunostimulants had a significant effect on total common carp erythrocytes 30 days and 15 days after the challenge (p<0.05). P2 was the best treatment and had significantly different results from all other treatments.

Table 1

Total erythrocyte profile of common carp on days 30 and 45 of the experiment

No	*Treatment*	*30 days (x 10^6 cells/mm^3)*	*45 days (x 10^6 cells/mm^3)*
1	P0 (experimental control)	1.28±0.08	1.16±0.02
2	P1 (mango)	1.36±0.60	1.57±0.10
3	P2 (guava)	1.77±0.04	1.88±0.02
4	P3 (noni)	1.28±0.30	1.48±0.18

Phytoimmunostimulants enhanced common carp immunity, such as total erythrocytes ranging from 1.28 to 1.88 x 10^6 cells/mm^3. The total erythrocytes of common carp that were fed a diet containing phytoimmunostimulants, namely P1, P2, and P3, were within the normal range. The highest total erythrocytes were found in treatment P2, which ranged from 1.77 to 1.88 x 10^6 cells/mm^3. When compared to fish fed with commercial pellets alone, the total erythrocytes ranged between 1.16-1.28 x 10^6 cells/mm^3. Here, it is clear that the total erythrocytes of the common carp in the experimental control were lower than those of fish fed with phytoimmunostimulants.

Hemoglobin level. Common carp hemoglobin levels during the study period ranged between 4.52-7.49 g/dL. The highest hemoglobin level was found in the P2 treatment, which ranged from 7.73-7.49 g/dL. This figure is far above when compared to fish in treatment P0 which were only fed with commercial pellet feed, where hemoglobin levels ranged from 4.52-5.83 g/dL. Fish hemoglobin levels in treatment P1 ranged from 6.45-6.73 g/dL, and P3 ranged from 6.15-6.55 g/dL (Table 2). However, this range of hemoglobin levels is still within the normal range. The analysis of variance (ANOVA) showed that feeding containing phytoimmunostimulants had a significant effect on common carp hemoglobin levels on days 30 and 45 (15 days after the challenge test) where $p<0.05$). The P2 treatment was the best and significantly different from all other treatments.

Table 2

Total hemoglobin profile of common carp on days 30 and 45 of the experiment.

No	*Treatment*	*30 days (g/dL)*	*45 days (g/dL)*
1	P0 (experimental control)	5.40±1.78	4.52±0.28
2	P1 (mango)	6.73±0.50	6.45±0.08
3	P2 (guava)	7.37±0.45	7.49±0.18
4	P3 (noni)	6.55 ±0.48	6.15±0.09

Hematocrit level. The hematocrit level is the percentage of the volume of red blood cells in the blood. Common carp hematocrit levels during the study ranged from 19.67-36.33%. The highest hematocrit level was found in the P2 treatment, which ranged from 33.00-36.33%. The hematocrit levels at and P0 were lower, ranging from 19.67 to 27.67% (Table 3). The results of the analysis of variance showed that feeding containing phytoimmunostimulants had a significant effect on common carp hemoglobin levels on day 30 and 45 experiments ($p<0.05$). The P2 treatment was significantly different from all other treatments.

Table 3

Hematocrit level profile of common carp on days 30 and 45 of the experiment

No	*Treatment*	*30 days (%)*	*45 days (%)*
1	P0 (experimental control)	23.00±1.73	19.67±0.58
2	P1 (mango)	30.33±3.51	32.33±3.06
3	P2 (guava)	33.00±3.00	36.33±2.08
4	P3 (noni)	28.67±4.51	27.67±2.08

Blood glucose level. The addition of phytoimmunostimulants to the feed affects the blood glucose levels of common carp. At the beginning of the study, it was noted that the blood glucose levels were relatively normal. This level changed slightly on day 30, where the fish in treatment P1, P2, and P3 received feed containing phytoimmunostimulants. This change was more visible on day 45 or 15 after the fish were challenged with *Aeromonas hydrophila*. Common carp glucose levels ranged from 65.67-86.33 mg/dL. However, the number in the experimental control unit (without the addition of phytoimmunostimulants) reached 147.33 mg/dL (Table 4). Feed containing phytoimmunostimulants had a significant effect on common carp blood glucose levelsbetween treatments ($p<0.05$).

Table 4

Common carp blood glucose levels (mg/dL) during the study

No	*Treatment*	*30 days*	*45 days*
1	P0 (experimental control)	53.67±4.04	147.33±5.03
2	P1 (mango)	70.67±1.53	75.33±2.52
3	P2 (guava)	73.00±3.00	86.33±2.52
4	P3 (noni)	65.67±2.09	75.00±1.73

Survival rate. The survival rate of fish after being challenged with pathogenic bacteria *Aeromonas hydrophila* is used as an indicator of whether the administration of phytoimmunostimulants affects fish health. The fish survival rate calculated at the end of the study showed that the survival rate ranged from 73.33 to 94.67% (Table 5). The lowest percentage was recorded in treatment P0 (experimental control) and the highest in treatment P2 (the fish fed with feed containing guava leaf powder). The results of the analysis of variance (ANOVA) showed that phytoimmunostimulants had a significant effect on the survival rate of fish (p<0.05), and the highest survival rate was scored in P2 (%) treatment.

Table 5

Survival rate of common carp during the study (%)

No	*Treatment*	*0 day*	*30 days*	*45 days*
1	P0 (experimental control)	100	100	73.33 ± 2.31
2	P1 (mango)	100	100	90.67 ± 2.31
3	P2 (guava)	100	100	94.67 ± 2.31
4	P3 (noni)	100	100	78.67 ± 4.62

Water quality. The water quality during the study showed that the temperature ranged from 28.0-29ºC, salinity 5 ppt, pH 6.6-6.8, and ammonia 0.00096-0.0027 mg/L. It is considered that the water quality supported common carp growth.

Discussion. The increase in the number of common carp erythrocytes in the P2 treatment is thought to be caused by the content of secondary metabolites from guava, such as flavonoids, which can increase the work of blood-producing organs (lymphomyeloid) so that blood production can increase (Omitoyin et al 2019). Of the three leaves used as phytoimmunostimulants, the highest flavonoid content was in guava leaves at 0.86-7.41%, followed by mango leaves at 0.26-2.35% (Ayodele & Dolapo 2015) and noni leaves of 0.21-0.75% (Rohman et al 2007). Sezgin and Aydin (2021) reported normal common carp erythrocyte totals ranging from 1.03-2.14 x 10^6 cells/mm^3.

Hemoglobin levels in the P0, P1, and P3 treatments decreased after the challenge test. According to Rajabiesterabadi et al (2019), the normal range for common carp hemoglobin levels ranges from 4.9-9 g/dL. Fish that have hemoglobin levels below the normal range will experience anemia. Fish that experience anemia will disrupt their metabolic processes and will lack energy, which means fish will be susceptible to disease. Decreased oxygen levels in the blood cause decreased hemoglobin levels. Infection with *Aeromonas hydrophila* bacteria can interfere with the function of the gill lamellae. It can reduce the oxygen content in the blood. The increase in hemoglobin levels in the P2 treatment was thought to be due to the content of guava leaves, which could inhibit bacterial growth. Flavonoids function as antibacterial substances and inhibit enterotoxins so that they can produce blood cells again under normal conditions (Amelia et al 2021).

Normal common carp hematocrit levels range from 21-44%. The hematocrit level is influenced by several other factors such as erythrocytes (number, size, shape, the ratio of anticoagulant to blood, storage area, and homogeneity), environment, sex, species and age of fish when blood is drawn (Shabirah et al 2019). Treatment of feed containing phytoimmunostimulants affected common carp hematocrit levels. The highest hematocrit levels (P2) ranged between 33.00-36.33%, followed by P1 (30.00-32.33%) and P3 (28.67-27.67%). However, the hematocrit level is still within the normal range. The common carp hematocrit level after the challenge in this study showed better results than the hematocrit level of carp reported by Hoseinifar et al (2014).

An increase in blood glucose levels indicates the fish is experiencing stress, leading to requiring a lot of energy and causing glucocorticoid increase. Common carp blood glucose level after challenge test in treatment P0 was very abnormal. It is suspected that fish experience stress due to infection attacks by *Aeromonas hydrophila*. Jiang et al (2017) mentioned that fish in stressful conditions require a lot of energy to adapt to existing conditions in keeping themselves alive. As a result, glucose will move into the blood, increasing blood glucose levels.

On the 45th day, blood glucose levels of common carp in the feed treatment containing phytoimmunostimulants were all within normal limits. Whereas in the experimental control unit, this figure is far above normal. According to Tavabe et al (2020), normal common carp glucose ranges between 58-120 mg/dL. P2 glucose levels ranged between 73.00-86.33 mg/dL, P1 ranged between 70.67-75.33 mg/dL, and P3 ranged between 65.7-75.00 mg/dL. The increase in blood glucose levels, although still within normal levels, in treatments P1, P2, and P3 was due to the presence of secondary metabolites from phytoimmunostimulants contained in the feed. Flavonoids are also one of the natural antioxidants that can change or reduce free radicals and are anti-free radicals (Abdel-Tawwab & Hamed 2020).

The fish survival rate at the end of the study was quite remarkable compared to the experimental control (P0). This shows that the immune response generated through a mixture of Hi Provit 781-2 with noni leaves, guava leaves, and mango leaves can suppress fish mortality. A similar result was reported by Nie (2015), who investigated the effects of *Rehmannia glutinosa* on growth performance, immunological parameters, and disease resistance to *Aeromonas hydrophila* in common carp.

Ferdous et al (2017) also explained that guava leaf extract increased non-specific immunity against foreign objects in the body. It is suspected that the increase in total leukocytes that occurred on day 15 after the challenge test using *Aeromonas hydrophila* was due to the carp's body response to the entry of foreign objects into their body. This opinion is supported by the report of Amelia et al (2021), which stated that guava leaf extract is an immunostimulant of common carp infected by motile *Aeromonas* septicemia.

The increase of fish immunity in the treatment of guava leaves is thought to be because guava leaves contain active compounds in the form of flavonoids, alkaloids, tannins, and saponins, which can improve growth performance and the carp immune system (Das et al 2012; Effendi et al 2022b). Guava leaves have the highest flavonoids compared to noni leaves and mango leaves (Ayodele & Dolapo 2015; Zhang et al 2020).

Conclusions. It is concluded that the administration of phytoimmunostimulants has a significant effect on the hematology and physiology of fish. The use of guava leaves as a phytoimmunostimulant significantly improves the health and immunity of common carp. Guava leaves (P2) showed the best results, with total erythrocytes at $1.77–1.88 \times 10^6$ cells/mm^3, hemoglobin at 7.37–7.49 g/dL, hematocrit at 34.00–36.33%, glucose at 73.00–86.33 mg/dL, and a survival rate of 94.67%. These findings support guava leaves as a promising option for enhancing fish health and disease resistance in aquaculture.

Acknowledgements. The authors would like to thank the Institute for Research and Community Service, University of Riau, for funding this research. For their participation, thanks are also conveyed to: 1) the Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, 2) Institute of Aquaculture, Stirling University, Stirling, UK, 3) Institute for Life and Earth Sciences, Heriot-Watt University, Edinburgh, UK and 4) Faculty of Fisheries and Marine Sciences, University of Teuku Umar, Meulaboh, Indonesia.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Abdel-Tawwab M., Hamed H. S., 2020 Antagonistic effects of dietary guava (*Psidium guajava*) leaves extract on growth, hemato-biochemical, and immunity response of cypermethrin-intoxicated Nile tilapia, *Oreochromis niloticus*, fingerlings. Aquaculture. 529:735668. doi: 10.1016/j.aquaculture.2020.735668.

Amelia R., Fidyandini H. P., Harpeni E., 2021 The application of guava leaf extract (*Psidium guajava* Linnaeus) as immunostimulant of common carp (*Cyprinus carpio* Linnaeus) infected by motile *Aeromonas* septicemia. Journal of Aquatropica Asia 6(2):48-59.

Amulejoye F. D., Adebayo O. T., Dada A. A., 2020 Reproductive impact of aqueous extract of *Mangifera indica* leaves on female African catfish *Clarias gariepinus* broodstock. International Journal of Fisheries and Aquatic Studies 8(3):657-662.
Awad E., Awad A., 2017 Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunology 67:40-54.
Ayodele O. D., Dolapo E. O., 2015 Total antioxidant activity, total phenolic and total flavonoid content of some plant leaves in South-West Nigeria. International Journal of Scientific & Engineering Research 6(8):418-427.
Baghizadeh E., Khara H., 2015 Variability in hematology and plasma indices of common carp *Cyprinus carpio*, associated with age, sex, and hormonal treatment. Iranian Journal of Fisheries Sciences 14(1):99-111.
Blaxhall P. C., Daisley K. W., 1973 Routine methods for use with fish blood. Journal of Fish Biology 5(6):771–781.
Das T. K., Banerjee D., Chakraborty D., Pakhira M. C., Shrivastava B., Kuhad R. C., 2012 Saponin: role in animal system. Veterinary World 5(4):248-254.
Effendi I., Yoswaty D., Syawal H. Austin B., Lyndon A. R., Kurniawan R., Wahyuni S., Al-Harbi A., 2022a The use of medicinal herbs in aquaculture industry: a review. Current Aspects in Pharmaceutical Research and Development 7:7–20.
Effendi I., Yoswaty D., Syawal H., Austin B., Lyndon A. R., Kurniawan R., Wahyuni S., 2022b Effects of herbal growth promoters on common carp (*Cyprinus carpio* L). AACL Bioflux 15(3):1096-1103.
Ferdous J., Hossain M. M. M., Jaman M. H. U., Rupom A. H., Tonny N. I., Jaman A., 2017 *Psidium guajava* leaf extracts fed to mono-sex Nile tilapia *Oreochromis niloticus* enhance immune response against *Pseudomonas fluorescens*. European Journal of Clinical and Biomedical Sciences 3(1):34-42.
Gül A., Benli C. K., Ayhan A., Memmi B. K., Selvi M., Sepici Dinçel A., Cakirogullari G. C., Erkoc F., 2012 Sublethal propoxur oxicity to juvenile common carp (*Cyprinus carpio* L., 1758): biochemical, hematological, histopathological, and genotoxicity effect. Environmental Toxicology and Chemistry 31(9):2085-2092.
Hai N. V., 2015 The use of medicinal plants as immunostimulants in aquaculture: a review. Aquaculture 446(1):88-96.
Hoseinifar S., Soleimani N., Ringø E., 2014 Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (*Cyprinus carpio*) fry. British Journal of Nutrition 112(8):1296-1302.
Jiang D., Wu Y., Huang D., Ren X., Wang Y., 2017 Effect of blood glucose level on acute stress response of grass carp *Ctenopharyngodon idella*. Fish Physiology and Biochemistry 43(5):1433–1442.
Nie G.-X., 2015 Effects of *Rehmannia glutinosa* on growth performance, immunological parameters, and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.). Aquaculture 435: 293-300.
Omitoyin B. O., Anjani E. K., Orisasona O., Bassey H. E., Kareem K. O., Osho, F. E., 2019 Effect of guava (*Psidium guava* L.) aqueous extract diet on growth performance, intestinal morphology, immune response and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. Aquaculture Research 2019(00):1-11. doi: 10.1111/are.14068.
Pathania D., Katoch R., Mahajan A., Sharma M., Paul R., 2014 Etiopathological investigations on clinical dropsy in carps (*Cyprinus carpio*) due to *Aeromonas hydrophila* in Himachal Pradesh. The Indian Journal of Animal Sciences 72(2):197-219.
Rajabiesterabadi H., Yousefi M., Hoseini S. M., 2019 Enhanced haematological and immune responses in common carp *Cyprinus carpio* fed with olive leaf extract-supplemented diets and subjected to ambient ammonia. Aquaculture Nutrition 26(3):763-771.
Rohman A., Riyanto S., Hidayati N. K., 2007 [Antioxidant activity, total phenolic content, and total flavonoids of noni leaves (*Morinda citrifolia* L.)]. Agritech. 27(4):147-151. [In Indonesian].

Secombes C. J., Fletcher T. C., 1992 The role of phagocytes in the protective mechanisms of fish. Annual Review of Fish Diseases 2(1992):53-71.
Sezgin A., Aydin B., 2021 Effect of replacing dietary soybean meal with pumpkin (*Cucurbita pepo*) seed cake on growth, feed utilization, hematological parameters, and fatty acid composition of mirror carp (*Cyprinus carpio*). Aquaculture Research 52(11):5870-5881.
Shabirah A., Rosidah, Mulyani Y., Lili W., 2019 Effect of types isolated lactic acid bacteria on hematocrit and differential leukocytes fingerling common carp (*Cyprinus carpio* L.) infected with *Aeromonas hydrophila* bacteria. World News of Natural Sciences 24:22-35.
Song S. K., Beck B. R., Kim D., Park J., Kim J., Kim H. D., Ringø E., 2014 Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunology 40(1):40-48.
Tavabe K. R., Yavar M., Kabir S., Akbary P., Aminikhoei Z., 2020 Toxicity effects of multi-walled carbon nanotubes (MWCNTs) nanomaterial on the common carp (*Cyprinus carpio* L. 1758) in laboratory conditions. Comp Biochem Physiol C Toxicol Pharmacol 237:108831. doi: 10.1016/j.cbpc.2020.108832.
Vallejos-Vidal E., Reyes-López F., Teles M., MacKenzie S., 2016 The response of fish to immunostimulant diets. Fish Shellfish Immunology 56:34–69.
Witeska M., Kondera E., Ługowska K., Bojarski B., 2022 Hematological methods in fish - not only for beginners. Aquaculture 547:737498. https://doi.org/10.1016/j.aquaculture.2021.737498.
Zhang W., Wang J., Chen Y., Zheng H., Xie B., Sun Z., 2020 Flavonoid compounds and antibacterial mechanisms of different parts of white guava (*Psidium guajava* L. cv. Pearl). Natural Product Research 34(11):1621–1625.

Received: 27 February 2024. Accepted: 14 June 2024. Published online: 03 February 2025.
Authors:
Irwan Effendi, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28293, Indonesia, e-mail: helpingirwan@gmail.com
Harum Farhana, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28293, Indonesia, e-mail: harum.farhana0583@student.unri.ac.id
Mardalisa Mardalisa, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28293, Indonesia, e-mail: mardalisa@lecturer.unri.ac.id
Dessy Yoswaty, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28293, Indonesia, e-mail: dyoswaty13@gmail.com
Henni Syawal, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28293, Indonesia, e-mail: hennirizal@gmail.com
Brian Austin, Institute of Aquaculture, Stirling University, Stirling FK9 4LJ, Scotland, United Kingdom, e-mail: baustin5851@gmail.com
Alastair Robert Lyndon, Life and Earth Sciences, Heriot-Watt University, Edinburgh EH14 4AS, United Kingdom, e-mail: a.r.lyndon@hw.ac.uk
Sri Wahyuni, Faculty of Fisheries and Marine Science, Teuku Umar University, Kampus Alue Peuyareng Street, 23615 West Aceh, Indonesia, e-mail: sri.wahyuni@utu.ac.id
Ronal Kurniawan, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28293, Indonesia, e-mail: kurniawanronal09@gmail.com

How to cite this article:
Effendi I., Farhana H., Mardalisa M., Yoswaty D., Syawal H., Austin B., Lyndon A. R., Wahyuni S., Kurniawan R., 2025 Phytoimmunostimulants increase of the immunity of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila* infection in brackish water. AACL Bioflux 18(1):277-284.