The Israeli Journal of Aquaculture – Bamidgeh • ISSN 0792-156X • IJA.74.2022.1823524, 9 pages
CCBY-NC-ND-4.0 • https://doi.org/10.46989/001c.57781

Hematological parameters of red tilapia (*Oreochromis* sp.) fed lemongrass essential oil (*Cymbopogon citratus*) after challenge with *Streptococcus agalactiae*

Huyen Nguyen Thi Thuong, Thi-Lan-Nhi Tran, Thi-Nhu-Trang Le, Van-Thanh Vo *

Department of Human and Animal Physiology, Biology Faculty, Ho Chi Minh City University of Education, 280 An Duong Vuong Str., Ward 4, Dist. 5, Ho Chi Minh City, Vietnam

(Received Nov 24, 2022; Accepted Dec 9, 2022; Published Dec 22, 2022)

Keywords: Hematological, red blood cells, white blood cells, lemongrass, essential oil

Abstract

The study involved feeding lemongrass essential oil (LEO) supplements to red tilapia (*Oreochromis* sp.) at concentrations including Control - 0 mg, T1 – 200 mg, T2 – 300 mg, and T3 – 400 mg per kg of feed. The research investigated changes in hematological (HCT, Hb, RBC, WBC & thrombocytes) and erythrocyte's morphological (major/minor axis; perimeter, and area of erythrocyte) parameters before infection, 5- and 10-days post-infection (DPI). According to analytical findings, a diet containing LEO enhanced the synthesis of both erythrocytes and leukocytes in the peripheral blood of red tilapia after 20 days of being used. Therefore, the indicators of this group of fish showed better performance than those that did not use LEO supplement five days after bacterial infection. Fish fed 200 mg/kg of LEO after being challenged with *S. agalactiae* for ten days showed an improved effect on red blood cell production. White blood cells decreased at all concentrations because of citral's immunomodulatory properties.

* Corresponding author. Van-Thanh Vo, thanhvv@hcmue.edu.vn

Introduction

Tilapia is one of the potential commodities on the worldwide market since it meets the standard requirements, including having flesh that is moderately flavored, white, odorless, and easy to fillet (FAO, 2022). Furthermore, due to its adaptability to various farming systems and settings (FAO, 2022), cultivating and producing red tilapia is relatively easy, requires little expense, and requires no specific seed production technology (Nandlal & Pickering, 2004). This freshwater fish is also produced in large quantities, with global production in 2020 totaling more than 6 million tons, accounting for 4.98% of the total production of all aquatic species and placing it 6th among the top ten species of world aquaculture (FAO, 2022). However, the development and expansion of tilapia production are facing many difficulties due to disease due to infections caused by bacteria and parasites (Amal & Zamri-Saad, 2011).

Streptococcus agalactiae is one of the common pathogens which causes hemorrhagic disease in red tilapia. This streptococcal disease in tilapia has negatively affected fish farms and is a serious economic problem as it significantly reduces worldwide tilapia production (Amal & Zamri-Saad, 2011).

Tilapia is one of the potential commodities on the worldwide market since it meets the standard requirements, including having flesh that is moderately flavored, white, odorless, and easy to fillet (FAO, 2022). Furthermore, due to its adaptability to various farming systems and settings (FAO, 2022), cultivating and producing red tilapia is relatively easy, requires little expense, and requires no specific seed production technology (Nandlal & Pickering, 2004). This freshwater fish is also produced in large quantities, with global production in 2020 totaling more than 6 million tons, accounting for 4.98% of the total production of all aquatic species and placing it 6th among the top ten species of world aquaculture (FAO, 2022). However, the development and expansion of tilapia production are facing many difficulties due to disease due to infections caused by bacteria and parasites (Amal & Zamri-Saad, 2011).

Antibiotics are the primary tool humans employ to prevent and control disease because they work quickly and effectively against various disease-causing bacteria. However, due to major concerns, including the development of bacterial strains that are resistant to antibiotics and the impact of antibiotic residues on consumer and environmental health (Smith et al., 1994; Cabello, 2006), it is believed that not a viable strategy to adopt this method of disease prevention. As a result, many researchers are becoming increasingly interested in and taking advantage of the plants extracted to replace antibiotics due to their exceptional benefit of reducing drug-resistant bacteria conditions and being non-toxic to both people and the environment.

Many studies on the effects of plant extracts have been published, including knowledge of their ability to protect shrimp and fish from a variety of harmful bacterial species (Tan & Vanitha, 2004; Alsaid et al., 2010; Kareem et al., 2016). The mechanisms of antibacterial activity of plant extracts were researched and discovered, including increased cell permeability, cytotoxicity, gene silencing, cell filamentation, membrane disruption, genomic DNA binding, etc. (Nafiqoh et al., 2020). Considering how frequently it is used to treat illnesses, lemongrass is sometimes referred to as a herb. Naik et al., 2010) reported that low LEO can still impact antibiotic-resistant bacteria. The authors also concluded that LEO might be used instead of antibiotics to treat some resistant bacteria. The effects of LEO on animals' hematological parameters change, particularly aquatic species, and its antibacterial efficacy against *S. agalactiae* has only been the subject of a small number of experimental research (Citarasu, 2010).

The study aimed to evaluate the effects of a diet containing LEO of red tilapia through hematological indicators in the challenge with *S. agalactiae* causing hemorrhagic disease.

The results support information and a base for expanding plant extracts as an antibiotic alternative in seafood production.

Materials and Methods

Experimental animals

At the Southern Freshwater Aquaculture Breeding Center in Tien Giang province, 160 healthy red tilapia (approximately 2.5 months old) of unknown gender were acquired. The fish were kept in tanks measuring 200 × 80 × 60 cm (length × width × height). The experimental tanks used water that had been dechlorinated and aerated 24 hours every a-day.

Bacteria S. agalactiae

S. agalactiae strain was provided by the Department of Fisheries, University of Agriculture and Forestry, Ho Chi Minh City. Bacteria were proliferated in Tryptone Soya Broth (TSB) medium at 28℃ for 24-48 hours.

Feeds preparation

The LEO used in the experiment was purchased at Heber Vietnam Co., Ltd. The results of chemical composition analysis by Gas chromatography/Mass spectrometry (GC/MS) from the LEO manufacturer included α-Citral and β-Citral, accounting for 55.2% and 44.8% of the total essential oil content, respectively. The food preparation method in this study was carried out as described by Silva et al. (2019). The desired LEO concentrations (200, 300, and 400 mg) were dissolved in 100 mL of cereal alcohol before the mixture was sprayed on one kilogram of feed. Then, they were dried for 24 hours at 25ºC and preserved at –18ºC until the feeding time.

Experimental design

The experiment was randomly arranged with four tanks (n=40 fish/tank) corresponding to the concentrations of essential oils used in the diet (Control, 200 – T1, 300 – T2, and 400 – T3 mg per one kg of feed). Red tilapias were grown steadily for 30 days, and after that, the experimental groups were fed a diet containing LEO for 20 days. Finally, all fish groups were infected with *S. agalactiae*. Fish blood samples were collected through 3 stages: stage 1, blood collection was performed after 20 days of feeding with LEO supplemented (before infection); stage 2, blood samples were conducted after five days of *S. agalactiae* infection; and stage 3 was in the next five days of stage 2.

Challenge with S. agalactiae

Fish were infected by immersion in a 20L tank with a concentration of *S. agalactiae* of 10^6 CFU/mL. Twenty fish were released into the tank for an hour, then removed and raised to the old tank and; replicated with the next twenty fish (Pirarat et al., 2015).

Hematological analysis and erythrocytes size determination

For hematological analysis, ten individuals were randomly selected from each treatment, and blood was collected from the caudal vein using a syringe containing an anticoagulant solution. The number of red blood cells was determined using a Neubauer counting chamber. HCT value was measured using the EZ Reader Microhematocrit card of LW Scientific after blood was centrifuged at 1200 rpm for 5 min. Hb was determined by the Sahli hemoglobin method. The white blood cell count was performed by Giemsa staining of blood smears, and the count was calculated following the equation of Hrubec et al. (2000).

RBC (cells/mm^3) = the number of RBC in the five-count zones ×10000;
WBC & thrombocytes (cells/mm^3) = (number of WBC & thrombocytes of 1500 cells × number of RBC in 1 mm^3 of blood)/1500.

The dimension of erythrocytes (including the length of the major and minor axis) was determined by ImageJ 1.53a software on Giemsa staining slides. The area and perimeter of erythrocytes were determined using the formula for the area and perimeter of the ellipse (Chernyavskikh et al., 2018): Perimeter $= 2\pi\sqrt{\frac{\left(\frac{a}{2}\right)^2+\left(\frac{b}{2}\right)^2}{2}}$ and $area = \pi ab$. Where a is the length of the major axis and b is the length of the minor axis.

Statistical analysis

One-way ANOVA processed the investigated value on Minitab 18 software. The Turkey test performed differences between treatments and stages. The results are presented as Mean ± SD.

Results

The dimension parameters of erythrocytes

Table 1 displays the change in erythrocyte size after feeding a diet with LEO, as determined by the major axis, minor axis, perimeter, and area of red blood cells. Fish fed 400 g/kg of LEO were found to have the greatest value for the dimension parameters of erythrocytes. Therein, the minor axis value at T3 was not statistically significant ($p>0.05$) higher than the control, leading to an identical perimeter and area of erythrocyte in T3 and control. Compared to fish that did not consume a diet containing LEO, fish in T1 and T2 had mean values of the major axis, perimeter, and area of red blood cells that were statistically substantially smaller. Additionally, there was no difference between these two groups of fish.

Table 1 The dimension parameters in erythrocyte cells of red tilapia after being fed LEO supplements in the diet

Parameters	Control	T1	T2	T3
Major axis (µm)	10.18±1.16^b	9.75±0.89^c	9.82±0.82^c	10.35±1.00^a
Minor axis (µm)	7.97±3.52^a	7.68±0.81^b	7.87±1.11^{ab}	8.08±0.88^a
Perimeter (µm)	28.87±7.72^a	27.62±1.96^b	28.01±2.48^b	29.23±2.25^a
Area (µm^2)	258.33±140.80^a	235.23±34.31^b	243.82±4.21^b	262.90±40.45^a

$^{a, b, c}$ *shows the significant difference in rows ($p<0.05$)*

Following *S. agalactiae* infection on red tilapia, the morphological indices of erythrocytes in fish that did not consume the LEO-containing feed are recorded in **Table 2**. After five days of *S. agalactiae* challenge in fish not on the LEO diet, the size indices of the large and small shafts decreased by 11% and 15.81%, respectively, in comparison to the fish before infection (10.18 and 7.97 µm), which resulted in a corresponding decrease in the perimeter and area of red blood cells at this time. In contrast, red tilapia infected at ten days showed a significant increase compared to the uninfected time for all the dimension parameters of erythrocytes.

Table 2 The dimension parameters in erythrocyte cells after five days and ten days of challenge with *S. agalactiae* (three-stage on Control groups)

Parameters	Before infection	5 DPI	10 DPI
Major axis (μm)	10.18±1,16[b]	9.06±0,75[c]	10.84±0,80[a]
Minor axis (μm)	7.97±3,52[b]	6.71±0,82[c]	9.02±0,93[a]
Perimeter (μm)	28.87±7,72[b]	25.09±1,90[c]	31.35±2,35[a]
Area (μm^2)	258.33±140,80[b]	191.37±31,13[c]	308.20±48,73[a]

[a, b, c] shows the significant difference in rows ($p<0.05$); DPI: day post-infection

The hematological parameters

Table 3 shows the hematological parameters of red tilapia fed a diet supplemented with different concentrations of LEO after challenge with *S. agalactiae*. After 20 days of a diet, there was a rapid increase in HCT, WBC & thrombocytes in all treatments T1, T2, and T3 ($p<0.05$) compared with the control. The highest increase is in the treatment supplemented with 400 mg/kg of LEO (T3). Compared to fish in a control condition that eats ordinary food, the levels of Hb and RBC at T3 indicate a statistically significant improvement ($p<0.05$). In contrast, fish in T1 and T2 have no change ($p>0.05$) during the same period.

Table 3 Hematological parameters of red tilapia fed LEO after five and ten days of challenge with *S. agalactiae*

Parameters	Stage	Control	T1	T2	T3
HCT (%)	Before infection	32.62±3.36[B, c]	36.60±5.14[A, b]	37.15±6.23[A, b]	39,82±6.90[A, a]
	5 DPI	28.53±6.47[C, c]	30.04±6.91[B, c]	34.84±3.43[B, b]	37.28±6.59[B, a]
	10 DPI	36.52±7.90[A, a]	28.46±5.12[B, b]	27.63±5.63[C, b]	29.45±4.43[C, b]
Hb (g%)	Before infection	8.05±1.19[A, b]	858±1.25[A, ab]	7.95±1.44[A, b]	8.86±0.89[A, a]
	5 DPI	5.48±1.12[C, c]	7.11±1.42[B, b]	7.01±1.48[B, b]	8.51±1.25[A, a]
	10 DPI	7.15±1.69[B, a]	6.41±1.63[B, ab]	5.82±1.09[C, b]	6.30±1.16[B, ab]
RBC $\times 10^6$ (cell/mm^3)	Before infection	1.38±0.29[A, b]	1.53±0.27[A, ab]	1.54±0.54[A, ab]	1.57±0.38[A, a]
	5 DPI	0.98±0.36[B, c]	1.25±0.36[B, b]	1.22±0.25[B, b]	1.37±0.36[B, a]
	10 DPI	1.28±0.57[A, b]	1.68±0.52[A, a]	1.12±0.39[B, bc]	0.97±0.22[C, c]
Total WBC & thrombocytes $\times 10^4$ (cell/mm^3)	Before infection	4.06±1.28[B, b]	6.83±1.58[A, a]	6.58±3.69[A, a]	7.12±3.97[A, a]
	5 DPI	4.93±2.44[A, b]	5.69±2.05[B, b]	5.31±1.29[B, b]	7.14±2.68[A, a]
	10 DPI	3.66±1.44[B, c]	5.39±1.54[B, a]	4.77±1.89[B, ab]	4.30±1.15[B, bc]

[a, b, c, and d] show a significant difference in rows ($p<0.05$). [A, B, and C] show a significant difference in columns in the same groups ($p<0.05$); HCT: hematocrit; Hb: hemoglobin; RBC: red blood cell; WBC: white blood cell; DPI: day post infection

Fish fed a diet without LEO after being challenged to *S. agalactiae* for five days demonstrated statistically significant decreases in HCT, Hb, and RBC parameters but increased WBC & thrombocyte count ($p<0.05$). All hematological parameters in red tilapia given both 200 and 300 mg of LEO tended to decline, and the difference was statistically significant. While there was a modest decline in the HCT and RBC indices in fish fed 400 mg of LEO in the feed, there was no impressive change in the Hb, WBC & thrombocyte parameters. ($p>0.05$).

After ten days of *S. agalactiae* challenge in the control group, the mean values of HCT, Hb, and RBC increased, while the WBC & thrombocyte count declined significantly compared to the period after five days of infection. Red tilapia in the treatment fed 200 mg of LEO in the feed ten days after infection exhibited no change in HCT, Hb, and WBC & thrombocyte indices

compared to the period five days after infection. Still, RBC grew considerably (p<0.05). In addition, the hematological parameters for the other two treatments all showed a decline, except for the RBC index, WBC & thrombocyte of T2, and Hb index of T3, which did not differ significantly from the period after five days.

Discussion

Hematological parameters, a crucial diagnostic tool for fish health, might fluctuate depending on the stressors, the treatment, and any parasite or viral infections present in (Fazio, 2019; Lourenço et al., 2014). The circulatory system responds fast to foreign things because it is sensitive to them, which in this study are bacteria and chemical constituents of essential oils. The current study's hematological parameters of red tilapia (*Oreochromis* sp.) reacted positively to the addition of LEO to their diet. Fish fed with 200 and 300 mg promoted erythropoiesis, as shown by a substantial rise in HCT, but did not accelerate the maturation of erythrocytes, which shows why erythrocyte morphological indices at both concentrations were lower than the control. Furthermore, Härdig and Höglund claimed that the manufacture of hemoglobin within the cells causes immature red blood cells that enter the circulation to gradually enlarge in size throughout their maturation (Härdig & Höglund, 1983). While feeding fish with a concentration of 400 mg was effective with all hematological indices, the erythrocyte morphology index also reached the highest value. Some studies also gave similar results when supplementing with different plant-based substances on the same fish, such as ginseng (Ginsana® G115) (Goda, 2008); cinnamon (Ahmad et al., 2011); thyme, rosemary, and fenugreek (Gültepe et al., 2014); mint (Vo et al., 2022).

Hemorrhagic illness in red tilapia is brought on by the *S. agalactiae* bacterium, which can release hemolysin. It breaks down erythrocytes and is known as complete hemolysis (β-hemolysis) (Amal & Zamri-Saad, 2011), (Evans et al., 2002). In 2014, Alsaid et al. observed that *S. agalactiae* cocci attacked red blood cells by attaching to the cell wall; the authors also hypothesized that a drop in RBC, HCT, and Hb would signal the presence of hypochromic microcytic anemia (Alsaid et al., 2014). The capacity of bacteria to cling to the surface of red blood cells when investigated on tilapia was also highlighted in the report of Su et al. (2017). The control fish in the current study showed considerably smaller erythrocytes and fewer RBC count after five days of the challenge. This was followed by a decline in HCT and Hb index, which may have been caused by bacteria that may alter or even destroy red blood cells. McNulty et al.'s viewpoint is also a reasonable explanation for this event for juvenile and adult Tilapia individuals after seven days (McNulty et al., 2003). When mature erythrocytes have died due to infection with *S. iniae*, immature erythrocytes (smaller than mature erythrocytes) are released into the peripheral circulation (McNulty et al., 2003). Meanwhile, Bailone et al. (2010) suggested that red blood cells were destroyed by leukocyte activity. After five days of challenge with bacteria of fish supplemented with LEO at all concentrations, hematological indices (HCT, Hb, RBC, and WBC & thrombocytes) were higher than those of fish not using this diet. This shows that LEO has contributed to reducing hemolysis due to bacteria's destruction of red blood cells and its ability to stimulate the immune system. Nafiqoh et al. (2020) also held the same opinion that extracts from the leaf of *Piper betle*, *Psidium guajava*, and *Tithonia diversifolia* could negatively affect *A. hydrophila* activity or prevent erythrocytes hemolysis. The report of Sheeja et al. (2006) also agrees with the above view and shows that the hemolysis rate is reduced thanks to the antioxidants present in the plant extracts.

However, the increased RBC, Hb, and HCT values, as well as the erythrocyte morphology indicators in fish that did not feed the LEO diet after ten days of the *S. agalactiae* challenge, may explain that it is because of a decrease in the amount of RBC, Hb, and HCT at five days, which led to a decrease in the transport of oxygen and nutrients to the tissues. As a result, erythropoiesis has been boosted to satisfy the need for bodily

red blood cells (Fánge, 1992). Zanjani et al*.* (1969) also proposed that hemoglobin concentration may represent the body's oxygen requirement, controlling erythropoiesis as a regulatory mechanism in fish, which is corroborated by the current study's findings. For fish with LEO added to the diet at ten days of infection, T1 significantly improved RBC and had a higher mean value than the other treatments. Regarding WBC & thrombocytes, both T1 and T2 showed no statistical significance when compared with the 5-day post-infection time showing the enhancement of the hematopoietic activity of 200 mg of LEO and is still promoted after two stages of bacterial infection.

In contrast, in fish fed a diet supplemented with 400 mg of LEO ten days after infection, the number of WBC & thrombocytes decreased significantly compared to the time of 5 days of infection. The chemical component citral found in LEO can inhibit the synthesis of macrophage cytokines, or to put it another way, they function as an immune modulator, according to the research by Bachiega and Sforcin (2011). This is also completely coincidental, and it would be a good way to explain the findings mentioned above, given that the primary components of the LEO utilized in the study, α-Citral and β-Citral, are both Citral isomers.

In conclusion, when fish were fed LEO before infection, hematological parameters were improved in detail erythrocyte-related indices (HCT, Hb, RBC), and the immune system was also sharply increased (WBC & thrombocytes). Fish erythrocytes are believed to play an important role in immune system participation and phagocytosis. The increase or decrease in hematological parameters is sometimes uneven - all this is to help the fish body find ways to cope and adapt to environmental conditions (new food sources, as well as infectious factors). They might inhibit the action of the bacteria in the body or aid in reducing the hemolysis of red blood cells in the challenge experiment with *S. agalactiae* bacteria. Before and after infection, fish administered at a concentration of 200 mg could stimulate the synthesis of blood cells (red blood cells, white blood cells, and thrombocytes). During the pre-infection and early phases of the sickness, the 400 mg content in meals contributes significantly to stimulating red blood cell synthesis and strengthening the immune system. However, fish at T3 were immunomodulated in the latter stages of hemorrhagic illness due to citral in the essential oil.

Acknowledgments

For providing the facilities and equipment for this work, the authors are very grateful to the Department of Animal and Human Physiology, Biology Faculty, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

References

Ahmad, M. H., El Mesallamy, A. M. D., Samir, F., & Zahran, F. (2011). Effect of Cinnamon (*Cinnamomum zeylanicum*) on Growth Performance, Feed Utilization, Whole-Body Composition, and Resistance to *Aeromonas hydrophila* in Nile Tilapia. *Journal of Applied Aquaculture*, *23*(4), 289–298. https://doi.org/10.1080/10454438.2011.626350

Alsaid, M., Daud, H., Bejo, S. K., & Abuseliana, A. (2010). Antimicrobial activities of some culinary spice extracts against *Streptococcus agalactiae* and its prophylactic uses to prevent Streptococcal infection in Red Hybrid Tilapia (*Oreochromis* sp.). *World Journal of Fish and Marine Sciences*, *2*(6), Article 6.

Alsaid, M., F. Abuseliana, A., H. Daud, H., M. Mustapha, N., Bejo, S. K., M. Abdelhadi, Y., & H. Hamdan, R. (2014). Haematological, biochemical and clinical signs changes following experimental infection of *Streptococcus agalactiae* in red hybrid tilapia (*Oreochromis* sp.). *Aquacultura Indonesiana*, *15*(2), 86–93. https://doi.org/10.21534/ai.v15i2.36

Amal, M. N. A., & Zamri-Saad, M. (2011). Streptococcosis in Tilapia *Oreochromis niloticus*: A Review. *Pertanika Journal of Tropical Agricultural Science*, *34*(2), 195–206.

Bachiega, T. F., & Sforcin, J. M. (2011). Lemongrass and citral effect on cytokines production by murine macrophages. *Journal of Ethnopharmacology*, *137*(1), 909–913. https://doi.org/10.1016/j.jep.2011.07.021

Bailone, R., Martins, M., Mouriño, J., Vieira, F., Pedrotti, F., Nunes, G., & Silva, B. (2010). Hematology and agglutination titer after polyvalent immunization and subsequent challenge with *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). *Archivos de Medicina Veterinaria*, *42*(3). https://doi.org/10.4067/S0301-732X2010000300015

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, *8*(7), 1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Chernyavskikh, S. D., Quyet, D. H., & Van Thanh, V. (2018). Effect of Temperature on the Morphometrical and Physical Parameters of Erythrocytes and Polymorphonuclear Leucocytes in *Carassius gibelio* (Bloch). *Inland Water Biology*, *11*(1), 92–96. https://doi.org/10.1134/S1995082918010030

Citarasu, T. (2010). Herbal biomedicines: A new opportunity for aquaculture industry. *Aquaculture International*, *18*(3), 403–414. https://doi.org/10.1007/s10499-009-9253-7

Evans, J. J., Klesius, P. H., Gilbert, P. M., Shoemaker, C. A., Al Sarawi, M. A., Landsberg, J., Duremdez, R., Al Marzouk, A., & Al Zenki, S. (2002). Characterization of beta-haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *Journal of Fish Diseases*, *25*(9), 505–513. https://doi.org/10.1046/j.1365-2761.2002.00392.x

Fánge, R. (1992). Fish Blood Cells. In W. Hoar, D. Randall, & A. Farell (Eds.), *Fish Physiology* (Vol. 12B, pp. 1–54). Elsevier. https://doi.org/10.1016/S1546-5098(08)60008-4

FAO. (2022). *Benchmarking species diversification in global aquaculture*. FAO; https://doi.org/10.4060/cb8335en

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, *500*, 237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030

Goda, A. M. A. S. (2008). Effect of Dietary Ginseng Herb (Ginsana ® G115) Supplementation on Growth, Feed Utilization, and Hematological Indices of Nile Tilapia, *Oreochromis niloticus* (L.), Fingerlings. *Journal of the World Aquaculture Society*, *39*(2), 205–214. https://doi.org/10.1111/j.1749-7345.2008.00153.x

Gültepe, N., Bilen, S., Yılmaz, S., Güroy, D., & Aydın, S. (2014). Effects of herbs and spice on health status of tilapia (*Oreochromis mossambicus*) challenged with *Streptococcus iniae*. *Acta Veterinaria Brno*, *83*(2), 125–131. https://doi.org/10.2754/avb201483020125

Härdig, J., & Höglund, L. B. (1983). On accuracy in estimating fish blood variables. *Comparative Biochemistry and Physiology Part A: Physiology*, *75*(1), 35–40. https://doi.org/10.1016/0300-9629(83)90040-3

Hrubec, T. C., Cardinale, J. L., & Smith, S. A. (2000). Hematology and pasma chemistry reference intervals for cultured Tilapia (*Oreochromis hybrid*). *Veterinary Clinical Pathology*, *29*(1), Article 1. https://doi.org/10.1111/j.1939-165X.2000.tb00389.x

Kareem, Z. H., Abdelhadi, Y. M., Christianus, A., Karim, M., & Romano, N. (2016). Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (*Oreochromis niloticus*) and their resistance to *Streptococcus agalactiae* infection. *Fish Physiology and Biochemistry*, *42*(2), Article 2. https://doi.org/10.1007/s10695-015-0173-3

Lourenço, K. G., Claudiano, G. S., Eto, S. F., Aguinaga, J. Y., Marcusso, P. F., Salvador, R., de Moraes, J. R. E., & de Moraes, F. R. (2014). Hemoparasite and hematological parameters in Nile tilapia. *Comparative Clinical Pathology*, *23*(2), 437–441. https://doi.org/10.1007/s00580-012-1638-8

McNulty, S. T., Klesius, P. H., Shoemaker, C. A., & Evans, J. J. (2003). Hematological Changes in Nile Tilapia (*Oreochromis niloticus*) Infected with *Streptococcus iniae* by Nare Inoculation. *Journal of the World Aquaculture Society*, *34*(3), Article 3. https://doi.org/10.1111/j.1749-7345.2003.tb00080.x

Nafiqoh, N., Sukenda, Zairin, M., Alimuddin, Lusiastuti, A., Sarter, S., Caruso, D., & Avarre, J.-C. (2020). Antimicrobial properties against *Aeromonas hydrophila* and immunostimulant effect on Clarias gariepinus of *Piper betle, Psidium guajava*, and *Tithonia diversifolia* plants. *Aquaculture International*, *28*(1), Article 1. https://doi.org/10.1007/s10499-019-00439-6

Naik, M. I., Fomda, B. A., Jaykumar, E., & Bhat, J. A. (2010). Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacterias. *Asian Pacific Journal of Tropical Medicine*, *3*(7), 535–538. https://doi.org/10.1016/S1995-7645(10)60129-0

Nandlal, S., & Pickering, T. (2004). *Tilapia fish farming in Pacific Island countries* (Vol. 1). Secretariat of the Pacific Community.

Pirarat, N., Pinpimai, K., Rodkhum, C., Chansue, N., Ooi, E. L., Katagiri, T., & Maita, M. (2015). Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*. *Animal Feed Science and Technology*, *207*, 93–103. https://doi.org/10.1016/j.anifeedsci.2015.03.002

Sheeja, K., Shihab, P. K., & Kuttan, G. (2006). Antioxidant and Anti-Inflammatory Activities of the Plant *Andrographis Paniculata* Nees. *Immunopharmacology and Immunotoxicology*, *28*(1), 129–140. https://doi.org/10.1080/08923970600626007

Silva, L. T. de S., Pereira, U. de P., Oliveira, H. M., Brasil, E. M., Pereira, S. A., Chagas, E. C., Jesus, G. F. A., Cardoso, L., Mouriño, J. L. P., & Martins, M. L. (2019). Hemato-immunological and zootechnical parameters of Nile tilapia fed essential oil of Mentha piperita after challenge with *Streptococcus agalactiae*. *Aquaculture*, *506*, 205–211. https://doi.org/10.1016/j.aquaculture.2019.03.035

Smith, P., Hiney, M. P., & Samuelsen, O. B. (1994). Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. *Annual Review of Fish Diseases*, *4*, 273–313. https://doi.org/10.1016/0959-8030(94)90032-9

Su, Y., Feng, J., Liu, C., Li, W., Xie, Y., & Li, A. (2017). Dynamic bacterial colonization and microscopic lesions in multiple organs of tilapia infected with low and high pathogenic *Streptococcus agalactiae* strains. *Aquaculture*, *471*, 190–203. https://doi.org/10.1016/j.aquaculture.2017.01.013

Tan, B., & Vanitha, J. (2004). Immunomodulatory and Antimicrobial Effects of Some Traditional Chinese Medicinal Herbs: A Review. *Current Medicinal Chemistry*, *11*(11), 1423–1430. https://doi.org/10.2174/0929867043365161

Vo, V. T., Tran, T. H., Nguyen, T. T. T., Truong, V. T., Pham, C. T., Pham, T. M., & Thuong, H. N. T. (2022). Hematological Parameters of Red Tilapia (*Oreochromis* sp.) Fed Essential Oils of *Mentha piperita* after Challenge with *Streptococcus agalactiae*. *Pakistan Journal of Zoology*, 1–8. https://doi.org/10.17582/journal.pjz/20211106031127

Zanjani, E. D., Yu, M.-L., Perlmutter, A., & Gordon, A. S. (1969). Humoral Factors Influencing Erythropoiesis in the Fish (Blue Gourami—Trichogaster trichopterus). *Blood*, *33*(4), 573–581. https://doi.org/10.1182/blood.V33.4.573.573