INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 2, p. 352-362, 2022

RESEARCH PAPER OPEN ACCESS

Hematological Parameters of Red Tilapia (*Oreochromis* sp.) Fed Powder of *Cinnamomum Cassia* after Challenge with *Streptococcus agalactiae*

Thi-Lan-Nhi Tran[1], Van-Thanh Vo[1], Thuy-Linh Nguyen[1], Nguyen-Tien-Nghia Le[1], Thi-Hieu Tran[1], Huyen Nguyen Thi Thuong[1*]

Department of Human and Animal Physiology, Ho Chi Minh City University of Education, Ho Chi Minh City, Viet Nam

Key words: Hematological parameters, Red Tilapia, Cinnamon powder, *Streptococcus agalactiae*.

http://dx.doi.org/10.12692/ijb/21.2.352-362 Article published on August 20, 2022

Abstract

The study investigated the effects of supplementing cinnamon powder (*C. cassia*) to red Tilapia (*Oreochromis* sp.) diets at various rates (control, C1 - 0.5%, C2 - 1.0%, and C3 - 1.5%) on hematological parameters at three time periods before infected, five and ten days post-infected (DPI) *S. agalactiae* at a density of 10^6 CFU/mL. The hematological parameters and dimensions of erythrocyte examined in this study include hematocrit (HCT); hemoglobin (Hb); red blood cell counts (RBC); total white blood cells and thrombocytes (WBC & thrombocytes); major/minor axis; perimeter, and area of the erythrocyte. The result shows that, after twenty days of feeding fish cinnamon powder, it stimulated the erythropoietic and leukopoietic in the fish at all rates examined. Addition of a 1.5% concentration of cinnamon powder containing contributes to stimulating the immune system of fish and improving the hematological parameters against *S. agalactiae* with a density of 10^6 CFU/mL after both 5 and 10 days of *S. agalactiae* challenge while the addition of 1.0% has effect after 5 days.

* **Corresponding Author:** Huyen Nguyen Thi Thuong ✉ huyenntth@hcmue.edu.vn

Introduction

Red Tilapia (*Oreochromis* sp.) – a hybrid between *Oreochromis mossambicus* and *O. niloticus* (Romana-Eguia *et al.*, 2004) is gradually becoming the main export object in aquaculture worldwide. In 2018, the production of red tilapia was over one million tones, accounting for 1.9% of the world's total aquaculture production (FAO, 2020). Although the world's aquaculture has been affected by the Covid-19 pandemic recently, red tilapia production has gradually recovered as global tilapia production reaches nearly 7 million tons in 2020 (FAO, 2021).

Disease outbreaks are always a huge challenge for aquaculture because of heavy losses to the national economy. In the tilapia genus, the common pathogens include *Aeromonas hydrophila, Ichthyophitirius multifillis, Tricodhina* sp.*, Flavobacterium columnare, Edwardsiella tarda, Gyrodactylus niloticus* and *Streptococcus* sp. (Klesius *et al.*, 2008). Among them, the disease caused by *Streptococcus agalactiae* is the most common pathogen in red Tilapia with a high mortality rate and short mortality time at all stages of fish development (Amal and Zamri-Saad, 2011). Nowadays, the improvement in medicine and technology gives farmers more options to resolve disease outbreaks. However, the tendency of using antibiotics is frequently found because of the advantages of impact duration, diversity in the destination object, price, and availability (easy to find) (Cabello, 2006). The overuse of antibiotics when knowledge is still limited leads to many serious consequences, such as antibiotic resistance and negative effects on consumer health due to antibiotic residues in final products. For that reason, it is essential to find new approaches that both have several advantages and reverse antibiotic resistance.
Plant extracts, especially herbs, have been used in medicine for centuries. Up to now, besides herbs, several plant species have also been studied in aquaculture as an alternative to antibiotics, proven by their ability to resist many pathogens on different species of shrimp and fish (Agarwal and Singh, 1999; Salisbury *et al.*, 2002; Immanuel *et al.*, 2004; Tan and Vanitha, 2004; Citarasu, 2010; Kareem *et al.*, 2016). Cinnamon (*Cinnamomum cassia*) – a popular spice in Chinese cuisine, has been demonstrated that positive effect on human health (Ravindran *et al.*, 2004). Currently, cinnamon is also an interesting object in aquaculture as an improving immune system substance for aquatic animals because of its antibacterial, anti-stress and anti-inflammatory properties (Tung *et al.*, 2010; Ahmad *et al.*, 2011; Kareem *et al.*, 2016; Santos *et al.*, 2016; Huang *et al.*, 2020). In recent years, there has been no published report on the use of cinnamon powder for red Tilapia infected with hemorrhagic bacteria *S. agalactiae* around the world (Yin *et al.*, 2008).

The study was conducted to evaluate the antibacterial ability of cinnamon powder (*C. cassia*) as a supplemental diet of red tilapia (*Oreochromis* sp.) infected with *S. agalactiae*.

The research results provide scientific information for promoting the replacement of antibiotics by using medicinal products derived from plants in the aquaculture process.

Material and methods

Experimental animals

One hundred healthy red tilapia (about 2.5 months old), gender unidentified, were purchased at the Southern Freshwater Aquaculture Breeding Center, Tien Giang province. The fish were kept in tanks measuring 200 × 80 × 60 cm (length x width x height).

The water used is tap water that has been dechlorinated. The experimental tanks were aerated 24 every a-day. Water quality and temperature were monitored daily to avoid poor water quality that causes stress to the fish.

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°C for 24-48 hours.

Feeds preparation

The cinnamon powder used in the experiment was purchased at the local market. The main composition referenced by the research of Vernin *et al.* (1994) using GC/MS includes (E)-cinnamaldehyde (39.50%); Benzaldehyde (23.80%); Borneol and α-terpineol (3.80%); Salicylaldehyde (1.27%) (Vernin *et al.*, 1994). First, the commercial feed was mixed with cinnamon powder corresponding to each concentration (0.5%, 1.0% and 1.5%). Second, the wet blend was passed through a pellet machine to produce the feed pellets corresponding to each concentration. Finally, the final product was dried and stored in an airtight container at 4°C until use.

Experimental design

The experiment was randomly arranged with five tanks (n=40 fish/tank), including two control and three experiments treatment (C1, C2, C3) with the addition of cinnamon powder in the diet. Specifically, Control 1: commercial feed pallets and *S. agalactiae* non-infection; Control 2: commercial feed pallets and infection; C1, C2, C3 correspond to the concentration of cinnamon powder added 0.5%, 1.0%, 1.5% and *S. agalactiae* infection, respectively.

Firstly, fish were raised stably for 30 days with commercial feeds. Secondly, the fish were fed with the supplemented diet with the arranged treatments corresponding to each rate. The fish were fed at 5% of body weight, twice a day for 20 days. Thirdly, the fish in the C1, C2, C3 treatments were infected with *S. agalactiae*. Finally, fish blood samples were collected through 3 stages: stage 1, blood collection was performed after 20 days of feeding with cinnamon powder supplemented and no *S. agalactiae* infection; stage 2, blood samples were conducted after 5 days of *S. agalactiae* infection; and stage 3 were in the next 5 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 anticoagulant solution. The number of red blood cells was determined using a Neubauer counting chamber and was calculated according to the formula of Natt and Herick (1952) (Natt and Herrick, 1952). HCT value was measured using the Microhematocrit Reader card of LW Scientific after blood was centrifuged at 1200 rpm for 5 min. Hb was determined by the Sahli hemoglobin method (Huyen and Thanh, 2019). The white blood cell count was performed by Giemsa staining of blood smears and the count was calculated following the equation (Hrubec *et al.*, 2000).

The dimension of erythrocytes (including the length of the major and minor axis) was determined by Image J 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; Frolova *et al.*, 2017).

RBC (cells/mm^3) = the number of RBC in the five-count zones x 10000

WBC & thrombocytes (cells/mm^3) = (number of WBC of 1500 cells × number of RBC in 1 mm^3 of blood)/1500

$$\text{Perimeter} = 2\pi.\sqrt{\frac{\left(\frac{a}{2}\right)^2+\left(\frac{b}{2}\right)^2}{2}}$$

Area = π.a.b

Where, a: length of major axis

b: length of minor axis

Statistical analysis

The investigated value was processed by one-way ANOVA on Minitab 18 software. Differences between treatments and stages were performed by the Turkey test. The results are presented as Mean±SD.

Result

The dimension parameters of erythrocytes

The dimension change in erythrocytes after twenty days of dietary supplementation with different cinnamon powder concentrations is shown in Table 1.

These markers were highly elevated in all experimental groups (C1; C2; C3) when compared with the control ($p<0.05$). The mean major axis (µm) value was most extensive at C1 (20.26), C2 and C3 were lower and there was no significant difference between the two treatments ($p>0.05$). Regarding the mean value of the minor axis (µm), C2 had the highest value (15.85), followed by C1 and C3 at 15.15 and 15.31, respectively with no significant difference ($p>0.05$). The highest perimeter values were founded in C1 and C2 ($p>0.05$), C3 was lower than control and a similar pattern was observed in the area values.

Table 1. The dimension parameters in erythrocyte cells of red tilapia after fed powder cinnamon supplements in diet.

Parameters	Control 1	C1	C2	C3
Major axis (µm)	18.73±2.40[c]	20.26±2.09[a]	19.79±2.09[b]	19.89±2.09[b]
Minor axis (µm)	14.47±2.01[c]	15.31±1.77[b]	15.85±1.72[a]	15.15±1.96[b]
Perimeter (µm)	52.75±5.49[c]	56.55±4.61[a]	56.45±4.81[a]	55.68±4.88[b]
Area (µm²)	854.24±174.95[c]	975.63±158.49[a]	988.62±169.54[a]	948.32±171.18[b]

a, b, c: shows the significant difference in rows ($p<0.05$).

The result of dimension in erythrocyte after five days and ten days of challenge with *S. agalactiae* is detailed in Table 2. The major axis length increased significantly after five days of infection (about an 8% increase) compared with fishes that were not infected. Therefore, the values of perimeter and area also increased at the same time, although the length of the minor axis insignificantly changed. In contrast, all of these indices were significantly reduced at 10 days of infection when compared with the control.

The hematological parameters

Table 3 investigates the hematological parameters of red Tilapia-fed cinnamon powder in previous and post-infected with *S. agalactiae* experiments. Twenty days after the introduction of cinnamon powder diets, the mean RBC and HCT levels of fish fed with 0.5% cinnamon powder exhibited the greatest improvement. Compared to fish in control group, fish fed at 1.0% showed no change, and when 1.5% of cinnamon powder was added to the feed, these indices decreased significantly. Although no significant differences were observed in Hb levels, they increased slightly during the use of powder cinnamon on a diet. Furthermore, after the fish were fed the food supplemented with powder cinnamon, the total amount of BC and TC steadily increased and reached 9.57×10^4 cells/mm^3 (C3), which was higher than roughly 83.7% of the control.

Fish in the control treatment (without adding cinnamon powder) showed that HCT, Hb, RBC and total WBC & thrombocyte levels were significantly increased after being infected by *S. agalactiae* for five days. The mean values of HCT for fish fed with a cinnamon powder diet were lower than that of the control fish. Similarly, the RBC numbers of those experiments that fed cinnamon powder also tended to drop but C1 did not differ from the control. Fish in C3 treatment was observed as high as the control in terms of total WBC & thrombocytes, in contrast to C1 and C2, which were much lower.

At ten days after infection with *S. agalactiae*, the treatment that did not use the cinnamon-supplemented diet had a higher Hb indice and reached an average value of 10.28 g%. On the other hand, the HCT and RBC indices exhibited stability between the two time periods, five and ten days following the bacterial challenge. The number of

WBC & thrombocytes after ten days of *S. agalactiae* challenge tended to be as high as that of the time after five days, which was 12.5%, and the period without infection, which was 75.6%. Regarding the fish-fed cinnamon powder supplemented diets, 1.5% cinnamon powder showed no significant differences in all hematological parameters compared to the fish fed without supplements. The HCT, Hb and RBC of C2 were lower, while the total WBC & thrombocytes did not differ from the control. Fish fed with 0.5% cinnamon powder had the lowest total WBC & thrombocytes values, which were similar to C2.

Table 2. The dimension parameters in erythrocyte cells after five days and ten days of challenge with *S. agalactiae.*

Parameters	Control 1	Control 2	
		5 DPI	10 DPI
Major axis (µm)	18.73±2.40^{b}	20.23±2.55^{a}	18.29±2.35^{c}
Minor axis (µm)	14.47±2.01^{a}	14.50±2.04^{a}	13.71±2.36^{b}
Perimeter (µm)	52.75±5.49^{b}	55.48±5.57^{a}	51.01±5.46^{c}
Area (µm^2)	854.24±174.95^{b}	923.10±183.41^{a}	788.45±180.20^{c}

a, b, c: shows the significant difference in rows ($p<0.05$).

Discussion

The hematological parameters change such as red blood cell count, erythrocyte-related indices (Hb and HCT) and white blood cell counts may implicate the abnormal status of fish. Evaluation of hematological indices is useful for the detection of pathogens or intrinsic and extrinsic factors (Campbell, 2015), such as the presence of several chemical compounds in certain concentrations. The positive or negative effects of the use of plant extracts in the diet on fish health are also evaluated by investigating hematological parameters. There have been many studies showing the positive effects of this supplement through a variety of different extracts. In the current study, the hematological parameters and size of erythrocytes of red Tilapia fed with 0.5% cinnamon powder after twenty days were significantly increased compared to the control. In the 1.0% and 1.5% supplementation of cinnamon powder, although there were no improvements in HCT, Hb and RBC levels, both the dimension parameters and the mean values of total WBC & thrombocytes raised above those of regular diet. Hence, it was found that including cinnamon powder in the diet of red Tilapia resulted in improved values of hematological parameters and erythrocyte dimensions. Research by Ahmad *et al.* (Ahmad *et al.*, 2011) reported that the mean values of erythrocyte count, hemoglobin, and hematocrit increased significantly in all fish fed cinnamon-supplemented diets for 12 weeks and the highest results were found in 1.0% cinnamon concentration. Moreover, hematological parameters of red tilapia (HCT, Hb, RBC, and total WBC & thrombocytes) after adding essential oils of *Mentha piperita* at a concentration of 0.5% for 15 days showed a considerable improvement, which was described in a recent study by Vo *et al* in 2022 (Vo *et al.*, 2022). The previous findings are comparable to several of the same research on tilapia that use other plant extracts for dietary supplements such as thyme, rosemary, and fenugreek (Gültepe *et al.*, 2014); ginseng (Ginsana G115) (Goda, 2008), guava leaf (Kamble *et al.*, 2018). According to Pratheepa *et al.* (Pratheepa *et al.*, 2010), the increase in erythrocyte count, HCT and WBC were attributed to the *Aegle marmelos* leaf extract that stimulated the production of red blood cells and lymphocytes in the blood of *Cyprinus carpio.*

S. agalactiae is the main cause of hemorrhagic disease in red tilapia (Amal and Zamri-Saad, 2011), (Bowater *et al.*, 2012), (Musa *et al.*, 2009), which is also regarded to be one of the extrinsic factors that stress fish (Francis-Floyd, 2018; Yanong and Francis-Floyd, 2019). The majority of vertebrate species, including fish, have a stress response that involves a rapid release of catecholamines followed by the release of corticosteroids (Clauss *et al.*, 2008). When the blood contains high levels of catecholamines, an ion exchange occurs in the red blood cell membrane that increased osmotic pressure and causes the red blood cells to enlarge when water enters the cells (erythrocyte swelling) (Railo *et al.*, 1985). This explains why the size of erythrocyte cells in fish five days post-infected expanded more than the control fish. Accompanied by an increased HCT levels, it can be a sign of macrocytic anemia when fish are in a stressed state (Clauss *et al.*, 2008), which leads to low oxygen exchange rate in the body (Bailone *et al.*, 2010), (Martins *et al.*, 2008) due to the non-guaranteed functions of the erythrocyte. According to Ragnar Fange, the levels of oxygen in the blood dropped, which will activate the erythropoietic in response to the needs of the body (Ragnar, 1992).

This is evidenced in our results that the mean value of RBC and Hb significantly increased compared to non-infected fish. In addition, fish-fed cinnamon powder showed significantly higher total leukocyte and thrombocyte counts after 5 days of bacterial infection, similar to the results reported by Alsaid *et al.* (Alsaid *et al.*, 2014), and they also believed that this was a natural response of the fish non-specific immune system to defend against bacteria.

Table 3. Hematological parameters of red Tilapia fed cinnamon powder after five and ten days of challenge with *S. agalactiae*.

Parameters	Stage	Control	C1	C2	C3
HCT (%)	Before infection	$38.61\pm2.89^{B,b}$	$46.38\pm4.55^{A,a}$	$39.14\pm4.49^{A,b}$	$36.58\pm3.23^{C,c}$
	5 DPI	$41.30\pm4.28^{A,a}$	$37.92\pm3.21^{B,b}$	$38.82\pm3.92^{A,b}$	$38.68\pm4.12^{B,b}$
	10 DPI	$41.55\pm3.47^{A,a}$	$35.91\pm5.61^{C,b}$	$34.16\pm6.84^{B,b}$	$40.40\pm4.39^{A,a}$
Hb (g%)	Before infection	$8.23\pm0.69^{C,a}$	$8.83\pm1.34^{A,a}$	$8.44\pm1.61^{B,a}$	$8.41\pm1.10^{B,a}$
	5 DPI	$9.16\pm0.95^{B,b}$	$8.66\pm1.33^{A,c}$	$10.54\pm1.23^{A,a}$	$9.63\pm1.34^{A,ab}$
	10 DPI	$10.28\pm1.0^{A,a}$	$9.32\pm1.85^{A,ab}$	$8.68\pm1.08^{B,b}$	$10.25\pm1.31^{A,a}$
RBC $\times10^6$ (cell/mm^3)	Before infection	$1.70\pm0.36^{B,c}$	$2.17\pm0.46^{A,b}$	$1.87\pm0.28^{A,bc}$	$1.94\pm0.30^{A,b}$
	5 DPI	$1.92\pm0.30^{A,a}$	$1.84\pm0.32^{B,a}$	$1.53\pm0.35^{B,b}$	$1.59\pm0.18^{B,b}$
	10 DPI	$1.95\pm0.33^{A,a}$	$1.55\pm0.37^{C,b}$	$1.44\pm0.30^{B,b}$	$1.81\pm0.37^{A,a}$
Total WBC & thrombocytes $\times10^4$ (cell/mm^3)	Before infection	$5.21\pm1.71^{C,c}$	$8.40\pm2.30^{A,ab}$	$8.14\pm1.80^{A,b}$	$9.57\pm2.77^{AB,a}$
	5 DPI	$8.13\pm1.84^{B,a}$	$6.38\pm1.99^{B,b}$	$6.00\pm2.70^{B,b}$	$8.20\pm1.98^{B,a}$
	10 DPI	$9.15\pm1.97^{A,ab}$	$7.13\pm1.44^{B,c}$	$8.02\pm2.33^{A,bc}$	$10.29\pm3.42^{A,a}$

a, b, c, d shows the significant difference in rows ($p<0.05$). A, B, C shows the significant difference in columns in the same groups ($p<0.05$).

Control 1: control of before infection stage; control 2: control of 5 DPI and 10 DPI infection stage.

Clauss *et al.* claimed that lymphopenia and granulocytosis, in relative terms, reflected the manifestation of the stress response on a leukogram (Clauss *et al.*, 2008), which raised the total white blood cell indice since fish had a high proportion of lymphocytes in their total white blood cells (Campbell, 2015). After three days of being infected with *Mycobacterium marinum*, Ranzani-Paiva *et al.* similarly observed a significant rise in lymphocyte counts and a reduction in neutrophils leading to severe inflammation in tilapia (Ranzani-Paiva *et al.*, 2004). In our research, the numbers of total WBC & thrombocytes increased may be caused by an enhancement of thrombocyte counts in the peripheral because of their important role in the immune system (Tavares-Dias and Moraes, 2007), coagulation, and inflammatory (Tavares-Dias, 2003). While RBC, total WBC & thrombocytes of fish were substantially lower than in the period of non-infection fish, experimental fish at C3 at the time five days after infection showed a considerable improvement in HCT and Hb levels. The drop in the numbers of RBC might be explained by a decrease in the requirement for erythrocyte production in the peripheral circulation, which demonstrated that a concentration of 1.5% cinnamon powder aided in reducing the effect of pathogens on blood cells or protected red blood cells from hemolysis. Similar to this, (Nafiqoh *et al.*, 2020) discovered that the *Piper betle* could prevent hemolysis of erythrocytes or reduce hemolytic activity *A. hydrophila*. Beside, (Rattanachaikunsopon and Phumkhachorn, 2010) demonstrated that cinnamaldehyde was the only compound present in the composition of cinnamon that could resist *S. iniae* with a MIC of 20 µg/mL. In this study, the WBC numbers decreased after the challenge with *S. agalactiae*, which can be explained by a greater concentration of leukocytes at infection sites. Agree, Brum *et al* observed that fed with ginger powder (1.0% and 1.5%) and clove basil (0.5%) stimulated the leukocytes' migration to the infection sites (Brum *et al.*, 2017).

The total WBC & thrombocytes index of control fish 2 increased considerably after ten days of *S. agalactiae* challenge compared to the period after five days of infection, and even if the stress has been removed, the tendency is likely to be maintained for some time (Houston, 1990; Wedemeyer *et al.*, 1990). Red Tilapia fed a diet supplemented with 1.5% cinnamon powder experienced a rise in all hematological indicators (HCT, Hb, RBC, and total WBC & thrombocytes) compared to the period following 5 days of infection and had noticeably higher values when compared to feeding cinnamon powder at the other concentrations. These results demonstrate the greater efficacy of cinnamon bark powder at a concentration of 1.5% in the feed of red Tilapia during *S. agalactiae* challenge. Following other research, dietary guava leaf extracts were performed as an immunostimulant, and their effects weren't seen until after a bacterial challenge (Kamble *et al.*, 2018).

Conclusion

Adding cinnamon powder to fish diets can exhibit greater hematological parameters. Moreover, the addition of a 1.5% concentration of cinnamon powder containing contributes to stimulating the immune system of fish and improving the hematological parameters against *S. agalactiae* with a density of 10^6 CFU/mL after both 5 and 10 days of *S. agalactiae* challenge while the addition 1.0% has effect after 5 days. Our results indicate the positive effects of cinnamon powder on its ability against pathogenic *S. agalactiae* in red Tilapia, it can support the development of trends in the use of medicinal plants in the aquaculture industry.

Acknowledgements

The authors especially thank the Department of Animal and Human Physiology, Biology Faculty, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam for supporting facilities and equipment for this study.

References

Agarwal S, Singh VK. 1999. Immunomodulators: A Review of Studies on Indian Medicinal Plants and Synthetic Peptides. Indian National Science Academy **65(3),** 179–204.

Ahmad MH, El Mesallamy AMD, 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 MF, Abuseliana AH, Daud HM, Mustapha N, Bejo SK, Abdelhadi MY, Hamdan HR. 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 MNA, Zamri-Saad M. 2011. Streptococcosis in Tilapia *Oreochromis niloticus*: A Review. Pertanika Journal of Tropical Agricultural Science **34(2),** 195–206.

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/S0301732X2010000300015

Bowater RO, Forbes-Faulkner J, Anderson IG, Condon K, Robinson B, Kong F, Gilbert GL, Reynolds A, Hyland S, McPherson G, Brien JO, Blyde D. 2012. Natural outbreak of *Streptococcus agalactiae* (GBS) infection in wild giant Queensland grouper, *Epinephelus lanceolatus* (Bloch), and other wild fish in northern Queensland, Australia. Journal of Fish Diseases **35(3),** 173–186. https://doi.org/10.1111/j.1365-2761.2011.01332.x

Brum A, Pereira SA, Owatari MS, Chagas EC, Chaves FCM, Mouriño JLP, Martins ML. 2017. Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. Aquaculture **468,** 235–243. https://doi.org/10.1016/j.aquaculture.2016.10.020

Cabello FC. 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

Campbell TW. 2015. Exotic Animal Hematology and Cytology (Fourth Edition). John Wiley & Sons, Inc.

Chernyavskikh SD, Quyet DH, Thanh VV. 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

Clauss TM, Dove ADM, Arnold JE. 2008. Hematologic Disorders of Fish. Veterinary Clinics of North America: Exotic Animal Practice **11(3),** 445–462. https://doi.org/10.1016/j.cvex.2008.03.007

FAO. 2020. Sustainability in action. https://doi.org/10.4060/ca9229en

FAO. 2021. July 16. Tilapia sector growth to resume after shaking off pandemic effects. Food and Agriculture Organization of the United Nations. https://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1416636/

Francis-Floyd R. 2018. Stress—Its Role in Fish Disease. University of Florida/Institute of Food and Agricultural Sciences. http://www.edis.ifas.ufl.edu/fa005

Frolova AY, Chernyavskikh SD, Quyet HD, Thanh VV. 2017. The Effect of the Incubation Temperature on Morphological and Physical Parameters of *Hypophthalmichthys molitrix* Erythrocytes and Polymorphonuclear Leukocytes. Research Journal of Pharmacy and Technology **10**(3), 795–798. https://doi.org/10.5958/0974-360X.2017.00150.0

Goda AMAS. 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

Houston A. 1990. Blood and Circulation. In Methods for fish biology. American Fisheries Society, 273–334.

Hrubec TC, Cardinale JL, Smith SA. 2000. Hematology and pasma chemistry reference intervals for cultured Tilapia (*Oreochromis hybrid*). Veterinary Clinical Pathology **29(1),** 7–12. https://doi.org/10.1111/j.1939-165X.2000.tb00389.x

Huang Y, Liu H, Liu S, Li S. 2020. *Cinnamon Cassia* Oil Emulsions Stabilized by Chitin Nanofibrils: Physicochemical Properties and Antibacterial Activities. Journal of Agricultural and Food Chemistry **68(49),** 14620–14631. https://doi.org/10.1021/acs.jafc.0c03971

Huyen NTT, Thanh VV. 2019. Laboratory practice human and animal physiology. HCMC University of Education.

Immanuel G, Vincybai VC, Sivaram V, Palavesam A, Marian MP. 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquaculture **236(2004),** 53–65. https://doi.org/10.1016/j.aquaculture.2003.11.033

Kamble MT, Yakupitiyage A, Salin R, Chavan BR. 2018. Effect of *Psidium guajava* and *Phyllanthus acidus* Leaf Extract on Immunostimulant Response of Nile Tilapia Against *Streptococcus agalactiae* Infection. The Israeli Journal of Aquaculture - Bamidgeh **70**, 1–9.

Kareem ZH, Abdelhadi YM, 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),** 757–769. https://doi.org/10.1007/s10695-015-0173-3

Klesius P, Shoemaker C, Evans J. 2008. Streptococcus: a worldwide fish health problem. Proceedings from the 8th International Symposium on Talipia in Aquaculture **1,** 83–107.

Martins Ml, Mouriño Jlp, Amaral Gv, Vieira Fn, Dotta G, Jatobá Amb, Pedrotti Fs, Jerônimo Gt, Buglione-Neto Cc, Pereira-Jr. G. 2008. Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. Brazilian Journal of Biology **68(3),** 657–661. https://doi.org/10.1590/S1519698420080003000025

Musa N, Wei LS, Musa N, Hamdan RH, Leong LK, Wee W, Amal MN, Kutty BM, and Abdullah SZ. 2009. Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. Aquaculture Research **40(5),** 630–632. https://doi.org/10.1111/j.1365-2109.2008.02142.x

Nafiqoh N, Sukenda, Zairin M, Alimuddin, Lusiastuti A, Sarter S, Caruso D, Avarre JC. 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),** 1–13. https://doi.org/10.1007/s10499-019-00439-6

Natt MP, Herrick CA. 1952. A New Blood Diluent for Counting the Erythrocytes and Leucocytes of the Chicken. Poultry Science **31(4),** 735–738. https://doi.org/10.3382/ps.0310735

Pirarat N, Pinpimai K, Rodkhum C, Chansue N, Ooi EL, 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

Pratheepa V, Ramesh S, Sukumaran N. 2010. Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila*. Pharmaceutical Biology **48(11),** 1224–1239. https://doi.org/10.3109/13880201003713598

Ragnar F. 1992. Fish blood cells. In Fish Physiology, Vol. 12B. Academic press, Inc, 1–54.

Railo E, Nikinmaa M, Soivio A. 1985. Effects of sampling on blood parameters in the rainbow trout, Salmo gairdneri Richardson. Journal of Fish Biology **26**(**6**), 725–732. https://doi.org/10.1111/j.1095-8649.1985.tb04312.x

Ranzani-Paiva MJT, Ishikawa CM, Eiras AC das, Silveira VR da. 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757). Brazilian Archives of Biology and Technology **47**(**6**), 945–953. https://doi.org/10.1590/S1516-89132004000600014

Rattanachaikunsopon P, Phumkhachorn P. 2010. Potential of cinnamon (*Cinnamomum verum*) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*). Fisheries Science **76(2),** 287–293. https://doi.org/10.1007/s12562-010-0218-6

Ravindran PN, Nirmal Babu K, Shylaja M. 2004. Cinnamon and cassia: the genus Cinnamomum 36, CRC Press.

Romana-Eguia MRR, Ikeda M, Basiao ZU, and Taniguchi N. 2004. Genetic diversity in farmed Asian Nile and red hybrid tilapia stocks evaluated from microsatellite and mitochondrial DNA analysis. Aquaculture **236(1–4),** 131–150. https://doi.org/10.1016/j.aquaculture.2004.01.026

Salisbury JG, Nicholls TJ, Lammerding AM, Turnidge J, Nunn MJ. 2002. A risk analysis framework for the long-term management of antibiotic resistance in food-producing animals. International Journal of Antimicrobial Agents **20(3),** 153–164. https://doi.org/10.1016/S0924-8579(02)00169-3

Santos WM dos, Brito TS de, Prado SA de, Oliveira CG de, Paula AC de, Hoyos DC de M, Ribeiro PAP. 2016. Cinnamon (*Cinnamomum* sp.) inclusion in diets for Nile tilapia submitted to acute hypoxic stress. Fish & Shellfish Immunology **54,** 551–555. https://doi.org/10.1016/j.fsi.2016.04.135

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

Tavares-Dias M. 2003. *Variáveis hematológicas de teleósteos brasileiros de importância zootécnica*. Universidade Estadual Paulista, Centro de Aquicultura. https://books.google.com.vn/books?id=lGb2kQEACAAJ

Tavares-Dias M, Moraes FR de. 2007. Leukocyte and thrombocyte reference values for channel catfish (Ictalurus punctatus Raf), with an assessment of morphologic, cytochemical, and ultrastructural features. Veterinary Clinical Pathology **36**(**1**), 49–54. https://doi.org/10.1111/j.1939-165X.2007.tb00181.x

Tung YT, Yen PL, Lin CY, Chang ST. 2010. Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*) leaves. Pharmaceutical Biology **48(10),** 1130–1136. https://doi.org/10.3109/13880200903527728

Vernin G, Vernin C, Metzger J, Pujol L, Parkanyi C. 1994. GC/MS analysis of cinnamon and cassia essential oils: a comparative study. Developments in Food Science **34**, 411–424.

Vo VT, Tran TH, Nguyen TTT, Truong VT, Pham CT, Pham TM, Thuong HNT. 2022. Hematological Parameters of Red Tilapia (*Oreochromis* sp.) Fed Essential Oils of *Mentha piperita* after Challenge with *Streptococcus agalactiae*. Pakistan Journal of Zoology. https://doi.org/10.17582/journal.pjz/20211106031127

Wedemeyer G, Barton B, McLeay D. 1990. Stress and acclimation. In Methods in fish biology. American Fisheries Society, 451–489.

Yanong RPE, Francis-Floyd R. 2019. Streptococcal Infections of Fish. University of Florida/Institute of Food and Agricultural Sciences.

Yin G, Ardó L, Jeney Z, Xu P, Jeney G. 2008. Chinese Herbs (*Lonicera japonica* and *Ganoderma lucidum*) Enhance Non-Specific Immune Response of Tilapia, *Oreochromis niloticus*, and Protection Against *Aeromonas hydrophila*. In Diseases in Asian Aquaculture, Vol. VI. Asian Fisheries Society, 269–282.