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A Mini Review : Effect of *Nigella sativa* L on Haematological Parameters of Cultured Fish

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ABSTRACT

Disease attacks on fish will have an impact on changes in various components in the body, one of which is hematological conditions. Therefore, the hematological index can be used as an important diagnostic tool to evaluate the health status of fish. In addition, the hematological index can also be used as an indication of the condition of the body's resistance to disease. Several publications show the hematological parameters of fish that can be used to diagnose fish health status, including the number of erythrocytes, the number of leukocytes, the hematocrit value and the hemoglobin value. The use of herbal ingredients can be used to help improve the condition of the hematological parameters of fish. *Nigela sativa* is one of the herbal ingredients that can induce several hematological parameters of fish, as an indication of an increase in specific and non-specific immunity of the fish body including increasing lysozyme and phagocytic activity, expression of various cytokines, so that fish resistance to disease and other environmental disturbances, such as stress. physiology can improve. So the purpose of this article is to describe the effect of *N. sativa* administration on hematological parameters in various types of cultured fish. Based on evidence from various scientific works, it gives an illustration, that the use of *N. sativa* seeds in the form of flour or oil can be used as a supplement to improve hematological conditions, based on the parameters of the number of erythrocytes, leukocytes, hematocrit and hemoglobin levels in various types of cultured fish.

Keywords: *Nigella sativa*, hematology, fish, immunity.

1. INTRODUCTION

The aquaculture industry is an important sector to meet the needs of animal protein for humans. In line with the development of the industry, diseases and stress factors have also increased [1]. To produce healthy and efficient fish production, the problems of disease and stress must be controlled, one way is to improve fish health. Efforts to improve fish health can be done by providing supplements from herbal ingredients added to the feed. The use of herbal ingredients as alternative supplements in feed has several advantages, including being bio-degradable and environmentally friendly, easy to find, safe for consumers, and the individual fish themselves [2]. One of the herbal ingredients that can be used is *Nigella sativa* seeds. The active ingredients contained in Nigella sativa L seeds include thymoquinone, dithymoquinone, thymohydroquinone,

p-cymene, and thymol which include components of fatty oils (stable) and essential oils (essential/volatile) [3]. However, the most important bioactive is thymoquinone [4, 5]. Based on the active ingredients it contains, *N. sativa* has several pharmacological activities, including antibacterial [6, 7], antioxidant, anticancer and anticholesterol [8] and also as an immunostimulant that can improve the fish's immune system, so that fish health improves. and avoid disease attack [9]. A significant supporting aspect in determining the health status of fish is to look at the hematological profile [10]. Blood is one component of defense against disease that enters the body of fish [11]. Hematological profiles can also provide important information about the physiological status of fish, both influenced by processes inside the fish body and from the external environment [12]. The hematological profile plays a very important role in the body's metabolic and physiological activities, as well as components of immunity to defend against diseases that infect the fish body [13]. Changes in hematological parameters are considered as a prognostic tool as well as the main signal indicating a disturbance in the homeostatic defense ability of the fish body [14], so that the hematological profile can be used as an important diagnostic tool to assess the health status of fish [15]. Information on fish health status and fish physiology is very important, because it is significantly closely related to the economic conditions of industry players in the fisheries sector [15]. Many studies have shown that administration of N. sativa affects the quality and quantity of hematological parameters such as white blood cells (WBC), red blood cell count (RBC), hematocrit (Hct) and hemoglobin (Hb), in humans and several types of experimental animals [16]. . The purpose of this article is to review the effect of N. sativa on the hematological parameters of several types of cultured fish.

2. HEMATOLOGICAL FUNCTION

Hematology is a branch of science that deals with blood cells and blood-forming organs [17,18]. Hematological conditions are influenced by the environment and have an impact on physiological and metabolic processes of the body, so that the interest in hematology studies, among others, helps in observing the interaction between blood characteristics and environmental characteristics as well as physiological conditions [19]. Hematology can act as a pathological reflector of the status of animals exposed to toxins and other conditions [20], serves to diagnose a disease, determine pregnosis, determine the effect of a treatment, observe the immune system and in general the function in measuring hematology is to knowing the health status of fish [21; 22]. Hematology can be used as a method that is quite good for observing changes that occur in the fish's body, indicated by changes in blood profiles. Changes that occur, among others, are due to attacks by germs or due to an unfavorable environment, metabolic disorders, damage to structures and/or organ functions, the influence of agents/drugs, and stress [23]. Hematological observations are quite important, because in the circulatory system, blood is an important component functioning in the transportation of nutrients and oxygen [24], circulating substances that enter the body as well as those produced by the body from metabolic processes.

Hematological components consist of red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (platelets), blood plasma, hemoglobin. This blood component is very helpful in monitoring toxicity from feed or other environments that will affect the health status of animals

[25]. This blood component profile also plays a role in diagnosing disease in animals [26 Merck Manual, 2012] and the level of blood damage [27]. Good/normal blood composition tends to indicate that the animal is in a healthy condition, metabolic and physiological processes can work well [22]. If there is a change in the blood profile, the physiological condition and health of the animal will be affected [27, 28]. The main function of erythrocytes is to transport oxygen which is bound by hemoglobin present in erythrocytes to form oxyhemoglobin which is used in the respiratory process [29]. So the function of hemoglobin is to transport oxygen to all body tissues for the oxidation process of digested food, and to release energy for other body functions and to transport carbon dioxide out of the body [30, 22]. Erythrocytes also function in the transport of carbon dioxide (CO2) [31]. A decrease in the number of erythrocytes indicates a decrease in oxygen levels to be carried to the tissues and carbon dioxide levels returned to the lungs [32; 33; 34].

Leukocytes are non-specific body defenses that function against infectious diseases by localizing and eliminating the invasion of pathogenic organisms through the process of phagocytosis. Another function is to produce, transport and distribute antibodies as an immune response. So leukocytes can be used as an indicator of infection in the body. The body will produce more leukocytes when foreign objects/disease germs enter the body [35]. If the leukocyte cell level is low, the fish will be easily exposed and have a high risk of disease infection. White blood cells in high numbers are able to produce antibodies for the phagocytosis process and have high resistance to disease [33] and can increase the ability to adapt to an inappropriate environment [36]. The hematocrit is (Hct), the percentage of the blood column occupied by red blood cells is the hematocrit, while the percentage of the blood column occupied by leukocytes is the leukocyte (Lct). Usually, fish blood consists of 60-80% plasma, 20-40% red blood cells, and 0.5-2.0% leukocytes [24].

3. CLASSIFICATION AND MORPHOLOGY OF *Nigella sativa* L.

Nigella sativa L. (Picture 1) is known as black cumin (English), habbatussauda (Arabic), kalonji (India). The Middle East and some Asian countries Nigella sativa seeds have been used for hundreds of years to treat various diseases in humans [37, 38]. The classification of *N. sativa* L. plants is as follows:

Kingdom : Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Ranunculales
Family: Ranunculaceae
Genus: Nigella
Species : *Nigella sativa* L [39]

Figure 1. Black Cumin (*N. sativa* L.)
(Source: 40)

N. sativa L. is native to West Asia and the Mediterranean with a subtropical climate. The morphological characteristics of this plant are erect stems, green color, soft, grooved, ribbed, and coarse hair. The green leaves are oval, about 1.5-2 cm long, are single leaves with a pointed base and tip. The flower petals are bluish-white, usually five in number. Seeds of *N. sativa* are blackish brown, oval in shape [41]. *N. sativa* L forms a fruit capsule consisting of several white trigonal seeds. ripe fruit capsules, marked by an open capsule and the seeds inside will pop out and turn black [42].

4. THE CONTENT OF *Nigella sativa* L.

N. sativa L. seeds contain various constituents including water, fatty oil 22-38%, essential oil (essential) 0.40-1.50%, protein (eight of the nine essential amino acids) 20.8-31.2% , carbohydrates 24.9-40%, minerals 3.7-7%, saponins 0.013%, alkaloids 0.01% and vitamins 1-4%. However, the percentage of compounds contained in *N. sativa* seeds is determined by geographical location, harvest time, and cultivation method. The composition of the fatty oil is linoleic acid (Omega-6), oleic acid, palmitoleic acid, linolenic acid (Omega-3), myritoleic acid, dihomolionenic acid, stearic acid, eicosadienoic acid, myristic acid, arachidic acid, behanic acid, sterols, tocopherols (α, , and) timoquinone, retinol (vitamin A), carotenoids (β-carotene). The composition of the essential oil (essential) consists of thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, thymol carvakrol, -Pinene, -Pinene, longifolenen and t-Anethole. Protein contains compounds of glutamic acid, arginine, aspartic acid, leucine, glycine, valine, lysine, threonine, phenylalanine, isoleucine, histidine, methionine. The composition of carbohydrates consists of glucose, rhamnose, xylose, arabinose. Mineral content consists of calcium, phosphorus, iron, potassium, sodium, zinc, magnesium, manganese, copper, selenium. Saponin compounds consist of Hederagenin (melanthigenin), -Hederin (melanthin) and alkaloids consisting of Nigelicine, Nigellimine, Nigellidine, while the vitamin content consists of vitamin A, Thiamin, Riboflavin, Pyridoxine, Niacin, Folacin, Vitamin C [43, 3, 44, 45, 46, 47]. Compounds that have pharmacological activity contained in *N. sativa* L seeds are mostly contributed by thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, and thymol which includes components of fatty (stable) and essential oils (essential/volatile) [3]. However, the most important bioactive is thymoquinone [4, 5]. The chemical structure of the active compound is presented in Figure 2.

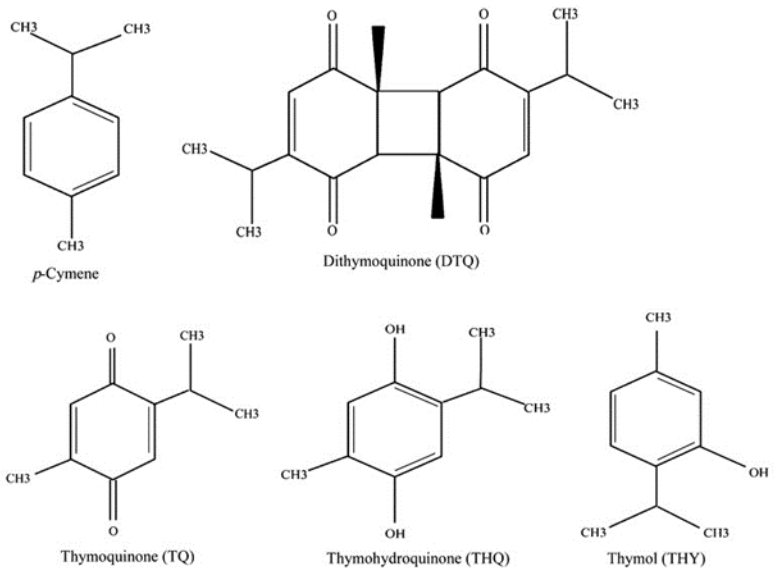

Figure 2. Chemical structures of major compounds from seeds of *N. sativa* L. [3]

5. THE BENEFITS OF *Nigella sativa* L

Nations in Arab countries, Far East Asia, Europe, and Africa have long used *N. sativa* as a traditional medicine to treat various diseases [48], among others, to treat respiratory disorders, chronic headaches, back pain, diabetes, paralysis , infection, inflammation and hypertension. In addition, it is also used to treat nasal inflammation, orchitis, eczema and joint swelling [49]. Consumption of *N. sativa* is important to maintain kidney, bile, liver function, and also to improve the immune system [37, 38]. In addition to medicine, the seeds of this plant are used as a spice for various processed foods, such as cakes, yogurt, pickles, sauces, and salads [50]. The pharmacological activity of *N. sativa* is an effective antioxidant both in vitro and in vivo [51], antidiabetic shown histologically in the pancreas of rats experienced an increase in pancreatic cell degeneration after being given Nigella sativa [52], antihypertensive [53], has a neuroprotective effect. proven to manage depression [54], and other neurological disorders including Alzheimer's disease [55], Parkinson's disease [56] and Epilepsy [57], anti-Infammatory and Analgesic, wherein the essential oil and thymoquinone at various doses had dose-dependent anti-inflammatory activity [58] and demonstrated substantial pain-relieving effects in wriggling acetic acid, formalin, and tail fick test [59], antimicrobial which include antibacterial [60], antifungal [61, 62], antiviral [63,64] and antiparasitic [65, 66]. *N. sativa* L. works as an immunomodulator by stimulating and strengthening the body's immune system by increasing the number, quality, and activity of the body's immune cells and also by modulating (repairing) the immune system [9], especially the content of thymoquinone is able to increase the proliferation and differentiation of lymphocyte cells which are immune cells [67]. Increased proliferation of lymphocyte cells as an indicator of increased activity of the immune system against infection [68] Consumption of 1 gram of *N. sativa* seed twice a day can increase human immune function significantly [69].

6. EFFECTS OF *N. sativa* SEEDS ON FISH HEMATOLOGY

A. Effect on erythrocyte cell count

Erythrocyte cells in fish are generally elliptical or ovoid in shape, but each species has different dimensions and lengths of erythrocyte cells about 10 - 20 mm and 6-10 mm wide [24]. Based on the results of the study, fish fed with *N. sativa* seed supplements experienced an increase in the number of erythrocytes compared to control fish (diet without supplements) [70]. African catfish fed diet supplementation with *N sativa* seeds produced a higher number of erythrocytes compared to fish, a dose of 9% N sativa seeds produced the highest erythrocyte levels, which was 6.29 cells/mm3, while the erythrocyte levels in control fish was 6 ,15 cells/mm3. Rainbouw trout *Oncorhynchus mykiss* with an average weight of 90±5 g, after being fed with *N. sativa* essential oil added to the feed at the rate of 1g/100g feed for 21 days, produced an erythrocyte cell count of $(3.5\pm0.4)x10^6$ µL, while the number of red blood cells was $(3.5\pm0.4)x10^6$µL. control fish erythrocytes of (3.3 ± 0.4) $x10^6$ µL [71]. The study on the same fish, namely rainbouw trout *Oncorhynchus mykiss* of average weight 108.7±17.0 g, but the diet added with *N. sativa* seeds in the form of flour produced a different number of erythrocytes when compared to using oil. Doses of 0.1 – 20 g/kg feed N. sativa seed powder additive given for 60 days did not affect the increase in the number of erythrocytes [72].

B. Effect on leukocyte cell count

In general, leukocytes have a larger size than erythrocytes, are colorless and can move in the presence of pseudopods (Pseudopodia) with a life span of 13-20 days [73]. It has been stated above that leukocytes have an important function for non-specific defense of the body, it can protect the fish body from pathogen attack. Leukocytes consist of granular and non-granulated cells. The granular leukocytes consist of eosinophils, basophils, and neutrophils, while the non-granulated cells consist of T and B lymphocytes, monocytes and macrophages. Several studies reported that N sativa seeds can increase the number of leukocytes in fish. *Lates calcallifer* whose feed was added with N. sativa seed supplements experienced an increase in the number of leukocytes. The highest increase occurred at a dose of 7.5%/kg of feed, the leukocyte count reached about 80,000 cells/mm^3, while the leukocyte count of fish without supplements was below 40,000 cells/mm3 [74]. African catfish (*Clarias gariepinus*) after being given black cumin experienced a higher increase compared to control fish. The highest increase was achieved in fish that were given black cumin supplements by 9%/kg of feed, the number of leukocytes obtained was 25516, 67 cells/mL, while in control fish it was 16143.33 cells/mL. [75]. Rainbouw trout *Oncorhynchus mykiss* with average weight of 90±5 g, after being fed with *N. sativa* essential oil added to the feed at the rate of 1g/100g feed for 21 days, produced leukocyte count $(16.2\pm0.1)x10^3$ µL, while fish leukocyte cell count control (16.3 ± 9.5) $x10^3$ µL [71]. Here it is seen that *N. sativa* has no effect on increasing the number of leukocytes in rainbouw trout. However, in another study, rainbouw trout *Oncorhynchus mykiss* of average weight 108.7±17.0 g, whose feed was added with N sativa seeds in the form of flour, had an effect on increasing the number of leukocytes. Doses of 1 – 10 g/kg feed *N. sativa* seed powder additive given for 60 days have an effect on increasing the number of leukocytes. The values obtained ranged from 98.1 ± 6.6 to 104.1 ± 5.1[72].

C. Effect on hematocrit (hct) levels

Hematocrit (hct) is a measure of the capacity of blood to carry oxygen, the higher the hct value, the higher the blood's ability to carry oxygen [76]. The Hct value of teleost fish ranges from 0% to 70%. Hematocrit values in Lates calcarifers with The addition of different black cumin (*N. sativa*) resulted in different hematocrit values.The highest hematocrit value was in fish whose feed

was added to *N. sativa* seeds at a dose of 7.5%/kg, which was 55.58%, while in control fish the hematocrit value was added. the lowest, namely 36.38% [74]. This indicates that N. sativa seeds can increase erythrocyte levels, because hematocrit is the percentage of the blood column occupied by erythrocytes [24]. The results of another study showed tilapia (*Oreochromis niloticus*) measuring 8.5±0.5 cm which was given black cumin supplement at a dose of 3.5%/kg of feed showed a faster increase in hematocrit levels than compared to other treatments after being challenged with *Streptococcus agalactiae* bacteria. The resulting hematocrit level was originally 19% to 32% on the 8th day after the challenge test [77]. Tilapia (*Oreochromis niloticus*) with a hematocrit content of 32% was categorized as healthy fish, as according to Hardi et al. (2011) [78] the average hematocrit levels of healthy tilapia ranged from 27.3 to 37.8%. The results of another study showed that tilapia Average body weight were 101 ± 1g fed with *N. sativa* added by 2% resulting in a higher hematocrit level compared to control, which was 29.50 ± 1.44%, while the hematocrit level in control fish was 28.75 ± 1.04 % [79]. Fish with high hct values show a healthier condition and can also show a response to increasing stress so that it functions as an anti-stress response to compensate for the increased demand for oxygen for metabolic energy [76].

D. Effects on hemoglobin (Hb) levels

The average amount of hemoglobin (Hb) of African catfish after being given N. sativa seeds was higher than the control. The dose of 9%/kg of feed produced the highest hemoglobin level, which was 7.55 g/dl, while the hemoglobin level of control fish was 5.00 g/dl [75]. Rainbouw trout Oncorhynchus mykiss with average weight of 90±5 g, after being fed with *N. sativa* essential oil added to the feed at a rate of 1g/100g feed for 21 days, resulted in Hb levels of 10.3±0.4 g dL-1, this value was higher compared to the Hb value of control fish, which was 10.03±0.7 g dL-1 [71]. The results of another study showed that tilapia (*Oreochromis niloticus*) average body weight were 101 ± 1g fed with N. sativa added 1% and 2% resulted in an increase in Hb levels compared to control, respectively 8.81±0.37 g/dl and 8.88± 0.47 g/dl, while the Hb content of control fish was 7.63±0.43 g/dl [79]. Rainbouw trout *Oncorhynchus mykiss* of average weight 108.7±17.0 g, whose feed was added with *N. sativa* seeds in the form of flour, had an effect on increasing hemoglobin levels. The dose of 20 g/kg feed *N. sativa* seed powder additive given for 60 days resulted in the highest hemoglobin levels, compared to control and other treatments. The hemoglobin value obtained was 10.2 ± 0.9 g/dL [72].

7. CONCLUSION

Based on evidence from various scientific works, as stated above, it describes the use of N sativa seeds in the form of flour or oil, which can be used as supplements to improve hematological conditions, based on the parameters of the number of erythrocytes, leukocytes, hematocrit and hemoglobin levels in various types of cultured fish.

Competing Interests

Author has declared that no competing interests exist.

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