ค่าโลหิตวิทยาบางประการและอัตราการรอดของปลานิลที่เลี้ยงด้วยอาหารผสมกระเทียมหลังการกระตุ้นด้วยเชื้อ *Aeromonas hydrophila*

Some Hematological Parameter and Survival Rate of Nile tilapia (*Oreochromis niloticus*) Fed with Dietary Garlic Cloves (*Allium sativum*) in Post-Challenge with *Aeromonas hydrophila*

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Received: 6 May 2021, Revised: 23 August 2021, Accepted: 13 September 2021

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมกระเทียมในอาหารต่อค่าโลหิตวิทยาบางประการ และความต้านทานโรคต่อเชื้อ *Aeromonas hydrophila* ในปลานิล (*Oreochromis niloticus*) โดยทำการเลี้ยงปลานิลขนาด 10.00±0.05 กรัมต่อตัว ด้วยอาหารผสมกระเทียมที่ระดับความเข้มข้น 0 (ชุดควบคุม) 1 3 5 และ 7 กรัมต่ออาหาร 1 กิโลกรัม เป็นเวลานาน 45 วัน จากนั้นทำการทดสอบความต้านทานเชื้อโดยการฉีดเชื้อ *A. hydrophila* บันทึกอัตราการตาย ผลการทดลองพบว่า ปลาที่ได้รับอาหารผสมกระเทียมในทุกระดับมีอัตราการตายต่ำกว่าชุดควบคุมอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ ผลการศึกษาค่าโลหิตวิทยาบางประการในปลาที่ได้รับอาหารผสมกระเทียมก่อนและหลังการกระตุ้นด้วยการฉีดด้วยเชื้อ *A. hydrophila* เปรียบเทียบกับปลากลุ่มควบคุม พบว่า ปลาที่ได้รับอาหารผสมกระเทียมทุกระดับมีปริมาณเม็ดเลือดแดงและเม็ดเลือดขาวสูงกว่าปลากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ จากผลการศึกษาสรุปได้ว่าการให้อาหารผสมกระเทียมสามารถส่งเสริมการตอบสนองทางภูมิคุ้มกัน และความต้านทานโรคต่อเชื้อ *A. hydrophila* ในปลานิลได้

คำสำคัญ: ปลานิล, กระเทียม, *Aeromonas hydrophila*, ค่าโลหิตวิทยา

สาขาเทคโนโลยีการผลิตสัตว์น้ำ คณะสหวิทยาการ มหาวิทยาลัยขอนแก่น วิทยาเขตหนองคาย อำเภอเมือง จังหวัดหนองคาย 43000
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ABSTRACT

The aim of this research was to study the effect of dietary garlic (*Allium sativum*) supplementation on some hematological parameters and disease resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). The Nile tilapia with 10.00±0.05 g/fish initial body weight were fed with diet supplemented with 0, 1, 3, 5 and 7 g/ kg of feed for 45 days. After that, challenged with *A. hydrophila* was performed and mortality rate was recorded. The challenge test with *A. hydrophila* showed that fish fed diets supplemented with garlic in all levels had statistically significant lower mortality rate than the control group (p<0.05). Moreover, some hematological studies in fish fed dietary garlic at pre- and post-challenged with *A. hydrophila* were compared with the control fish. The results revealed that fish fed with garlic at all levels had significantly higher red blood cell and white blood cell counts than the control group (p<0.05). From the results of the study, it was concluded that the use of dietary garlic can promote immune responses and disease resistance against *A. hydrophila* in tilapia.

Key words: Nile tilapia, garlic, *Aeromonas hydrophila*, hematology

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*, Linn) is an important freshwater fish in Thailand with great economic value. The properties of Nile tilapia are easy to raise, fast growing, can eat a variety of foods and popular with consumers. In 2019, the total production of freshwater animals in Thailand is 427,330 tons, worth 25,977.04 million baht, tilapia has the highest production volume of 228,982 tons or 53.58% of the total output, worth 11,434.46 million baht (Department of Fisheries, 2021). The Nile tilapia aquaculture industry in Thailand has developed to more intensive practices to increase productivity that caused in the susceptibility of disease outbreak. Recently, the Nile tilapia aquaculture industry still has been facing many problems related to disease and massive mortality. *Aeromonas hydrophila* is considered as a significant pathogen causing the motile aeromonad septicemia (MAS) in several fish species, including Nile tilapia (Abu-Elala *et al*., 2015). *A. hydrophila* has been causing outbreaks in fish farms with high mortality rates, resulting in severe economic losses to the aquaculture industry worldwide (Nielsen *et al*., 2001). The use of antibiotics and chemotherapeutics to control the diseases have been widely reviewed for their negative impacts such as drug resistance pathogen, aquatic animal immune system suppression and residue of aquatic animals and environment which directly impact to human and environment. Thus, an alternative approach to enhance disease resistance and other health benefits by herbal have been interested which are easy to find, economically and eco-friendly.

Garlic (*Allium sativum*) is an herb which can be stimulate feed intake, growth performance and immune system. It also has ability to resistance of bacteria, fungi, parasite and virus (Williams and Lloyd, 2012). There are many chemical compositions found in garlic; allistatin, allicin, alliin, garlicin and ajoene which are notable for inhibiting the growth of bacteria, fungi and viruses. In medical applications, herbs with high levels of allicin are good and interesting (Huchette *et al*., 2005). Allicin content can activate non-specific immunity by increasing cytokine gene expression (Fall and Tanekhy 2015). Additionally, there was also reported that garlic can stimulate immune responses such as: synthesis of lymphocyte, release of cytokine, phagocytosis and natural killer cell activities (Kyo *et al*., 1998). In aquaculture, garlic is used in many ways, including growth stimulation, antimicrobial ability, stimulate the immune system, stimulate the appetite and prevent stress. These benefits are very important in aquaculture (Guo *et al*., 2012). There are studies in aquatic animals reporting properties of garlic that can stimulate growth and the immune system, such as common carp, *Cyprinus carpio* (Karimi Pashaki *et al*.,

2020) Nile tilapia, *O. niloticus* (Seden *et al*., 2014), rainbow trout, *Oncorhynchus mykiss* (Farahi *et al*., 2010) African catfish, *Clarias gariepinus* (Thanikachalam *et al*., 2010), Asian sea bass, *Lates calcarifer* (Abdelwahab *et al*., 2020) and swordtail, *Xiphophorus Helleri* (Kalyankar *et al*., 2013). Incorporating immunostimulants with feed is an effective way to improve non-specific immune systems. The use of garlic as a feed additive is beneficial for use in aquaculture to enhance the immune response, acting as an immunomodulator in tilapia. Therefore, the current study was designed to investigate the effect of garlic supplemented diet on disease resistance and responses of blood cell count at pre-and post -challenged with *Aeromonas hydrophila* in Nile tilapia.

MATERIALS AND METHODS

Ethics statement

All study methods and experimental procedures were conducted following the guidelines and regulations reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (Reference No. 660201.2.11/40).

Experimental design

The research was conducted by using completely randomized design, CRD with 5 treatments and 3 replications. Each treatment was used diet containing 30% of crude protein by adding different levels of garlic (Diab *et al*., 2008) as following:

Treatment 1: Diet + garlic at 0 g/kg of feed (control)

Treatment 2: Diet + garlic at 1 g/kg of feed

Treatment 3: Diet + garlic at 3 g/kg of feed

Treatment 4: Diet + garlic at 5 g/kg of feed

Treatment 5: Diet + garlic at 7 g/kg of feed

Experimental diet

Garlic (*Allium sativum*) was procured from the local market, cleaned in water, aired and then dried in the hot air oven at 60 °C after that chopped into small piece and ground to become powder with motar. The basal experimental diets were formulated with the commonly available ingredients (Table 1). The ingredients were grinded, milled, weighed, mixed and pelleted with meat mincer at size 3 mm. After cold pelleting, the feeds were dried in oven at 60 °C and then stored in the refrigerator at 4 °C for further uses.

Table 1 Composition of experimental diets

Ingredients	Quantity of ingredients (g)				
	0	1	3	5	7
Fish meal	300	300	300	300	300
Broken rice	140	139	137	135	133
Garlic	0	1	3	5	7
Corn meal	70	70	70	70	70
Cassava starch	130	130	130	130	130
Soy bean meal	260	260	260	260	260
Rice bran	90	90	90	90	90
Premix	1	1	1	1	1
Total	1000	1000	1000	1000	1000

Experimental fish

Nile tilapia with initial weight of 10.00±0.05 g were obtained from the hatchery of aquatic animal production technology program, Khon Kaen University, Nong Khai Campus, Nong Khai Province. The fish were acclimatized in laboratory conditions for one week before starting the feeding trials. At the beginning of the experiment, 15 aquaria containers (0.45×0.90×0.45 m, water volume 150 liters) were each stocked with 15 fish. Each experimental diet was fed to fish in three aquaria. The fish were fed a diet of garlic at rate of 5% of body weight twice daily at 08:00 and 16:00 for 45 days.

Bacteria preparation

Aeromonas hydrophila was isolated from Nile tilapia by streak plate technique on Tryptic Soy Agar (TSA; Himedia). The species of bacteria was identified by morphology using Gram's stain and biochemical properties using API 20E strip (Biomerieux). The bacteria strain was cultured on TSA at 37°C overnight. The isolated colonies were cultivated in Tryptic Soy Broth (TSB; Himedia) and incubated with shaker at 34°C for 18 h. The turbidity of bacteria was adjusted to equivalent to 0.5 McFarland turbidity standards No.0.5 (1.5×10^8 CFU/ml).

Challenge test

After feeding on the test diet for 45 days, 30 fish from each treatment and control group (10 fish from each replication) were intraperitoneal injection with 0.1 ml of culture suspension of pathogenic *A. hydrophila* containing 1.5×10^8 CFU/ml. Mortality of fish was recorded every day for 10 days. The data were calculated mortality rate percentage and relative percent survival; RPS (Ellis, 1988) as following;

$$\text{Mortality rate (\%)} = \frac{\text{number of deaths in specific period}}{\text{total population during that period}} \times 100$$

$$\text{RPS} = 1 - \frac{\text{(percent mortality in treated group)}}{\text{(percent mortality in control group)}} \times 100$$

Hematological test

At the end of experiment, 3 fish from each replication were randomly to collect the blood sample before challenge with *A. hydrophila* (pre-challenge). Blood samples were collected from the caudal vein of fish by a sterile syringe containing EDTA as an anticoagulant. After 24 hours, these fish were intraperitoneal injection with 0.1 ml of culture suspension of pathogenic *A. hydrophila* containing 1.5×10^8 CFU/ml. After 24 hours, blood samples were collected from these fish again (post-challenge). Blood was used for calculation of red blood cell (RBC) and white blood cell (WBC). RBC and WBC were counted with a hemocytometer, RBC (10^6 cells/ml) in 5 squares, each having 16 smallest squares (4 corners and 1 central) and WBC (10^3 cells/ml) in the four 1 squares mm corner areas and was counted. All data was calculated follow the formula;

$$\text{red blood cell (cells/ml)} = \frac{\text{red blood cell x dilution factor}}{\text{volume (ml)}}$$

$$\text{white blood cell (cells/ml)} = \frac{\text{white blood cell x dilution factor}}{\text{volume (ml)}}$$

Statistical analysis

Data from each treatment were subjected to one-way analyses of variance (ANOVA) using SPSS for Windows version 17.0 (SPSS Inc., Chicago, USA). Means were compared after analysis of variance by Duncan's New Multiple Range Test ($p = 0.05$). The level of significance was chosen at $p \leq 0.05$, with results presented as mean ± standard deviation.

RESULTS AND DISSCUSION

After feed trial for 45 days, fish were challenged with *A. hydrophila*. The result showed that the control group progressed to the clinical sign of disease and died 24 hours after the challenge, while fish fed garlic supplemented in diet at 1 g/kg feed and 3 g/kg feed began to die after 48 hours and fed garlic supplemented in diet at 5 g/kg feed and 7 g/kg feed began to die after 72 hours (Figure 1). The lowest mortality rate was found in fish fed garlic supplemented in diet at 7 g/kg feed, with an average of 16.67±5.77% and showed statistically significant differences between the 1 g/kg feed and 3 g/kg feed garlic-fed experimental group and the control group (p<0.05). Fish fed garlic supplemented in diet at 5 g/kg feed was found the mortality rate at 23.33±5.77% and no significance different (p>0.05) with 7 g/kg feed group but significance difference with control group (p<0.05). Fish fed garlic supplemented in diet at 7 g/kg feed also showed the highest relative percent of survival (RPS) at 80.77 follow by 73.08, 46.15 and 34.62 in fish fed garlic supplemented in diet at 5 g/kg feed, 3 g/kg feed and 1 g/kg feed, respectively (Table 2).

Table 2 Mortality and relative percentage survival of Nile tilapia fed diets with different concentrations of garlic for 45 days after challenged with *Aeromonas hydrophila*

Gram of garlic per 1 kg of feed	Mortality (%)	Relative percentage survival (%)
0	86.67 ± 5.77^a	-
1	56.67 ± 5.77^b	34.62
3	46.67 ± 5.77^b	46.15
5	23.33 ± 5.77^c	73.08
7	16.67 ± 5.77^c	80.77
***p*-Value**	0.01	-

Different superscript letters within a column indicate significant differences at p<0.05

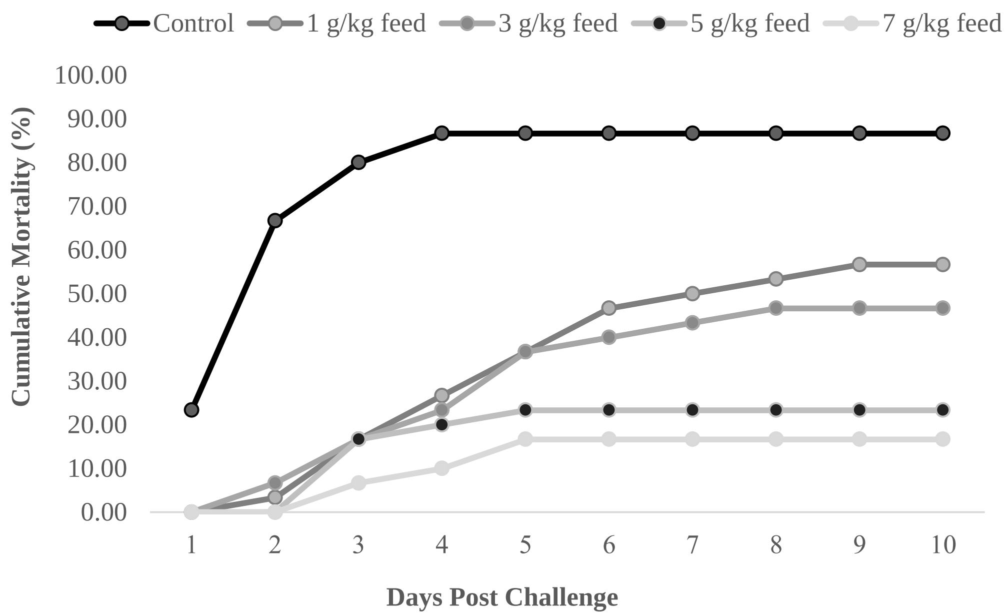

Figure 1 Cumulative percentage mortality (CPM) of Nile tilapia following intraperitoneal injection with *Aeromonas hydrophila* after feeding garlic-supplemented diet for 45 day

Hematological test

At the end of the experiment (45 days), fish were drawn blood before intraperitoneal injection with *A. hydrophila* (pre-challenge) for red blood cell (RBC) and white blood cell (WBC) counting. The results revealed that fish groups fed with diet containing 5 g of garlic/ kg of feed has had the highest RBC count of $10.63 \times 10^6 \pm 0.39 \times 10^6$ cell/ml, with no statistically significant difference with 7 g of garlic / kg of feed group (p>0.05) but statistically significant difference with control group (Figure 2). Similarly, there was no statistically significant difference between fish fed garlic with diet at 7 g/kg of feed and 5 g/kg of feed at WBC values of $19.92 \times 10^3 \pm 1.04 \times 10^3$ cell/ml and $18.33 \times 10^3 \pm 1.84 \times 10^3$ cell/ml, respectively). All treated groups also showed statistically significant differences with control group (Figure 3).

After intraperitoneal injection with *A. hydrophila* (post-challenge), fish were also drawn blood for red blood cell (RBC) and white blood cell (WBC) counting. The results showed that both RBC and WBC values of all groups were statistically significant different among groups (p<0.05). The highest RBC values was observed in fish fed garlic supplemented in diet at 7 g/kg of feed ($15.79\times10^6\pm0.75\times10^6$ cell/ml) follow by fish fed garlic supplemented in diet at 5 g/kg of feed, 3 g/kg of feed, 1 g/kg of feed and control group at $11.68\times10^6\pm0.48\times10^6$ cell/ml, $8.93\times10^6\pm0.78\times10^6$ cell/ml, $6.30\times10^6\pm0.55\times10^6$ cell/ml and $3.75\times10^6\pm0.20\times10^6$ cell/ml, respectively (Figure 2). Fish fed garlic supplemented in diet at 7 g/kg of feed was also found the highest significance WBC values at $29.67\times10^3\pm0.95\times10^3$ cell/ml followed by 5 g/kg of feed group, 3 g/kg of feed group, 1 g/kg of feed group and control group at $22.92\times10^3\pm0.63\times10^3$ cell/ml, $11.67\times10^3\pm0.88\times10^3$ cell/ml, $15.58\times10^3\pm1.37\times10^3$ cell/ml and $3.42\times10^6\pm0.38\times10^6$ cell/ml, respectively (Figure 3). After a challenge test, there was also a significant increase in WBC count of garlic treated groups compared to the control.

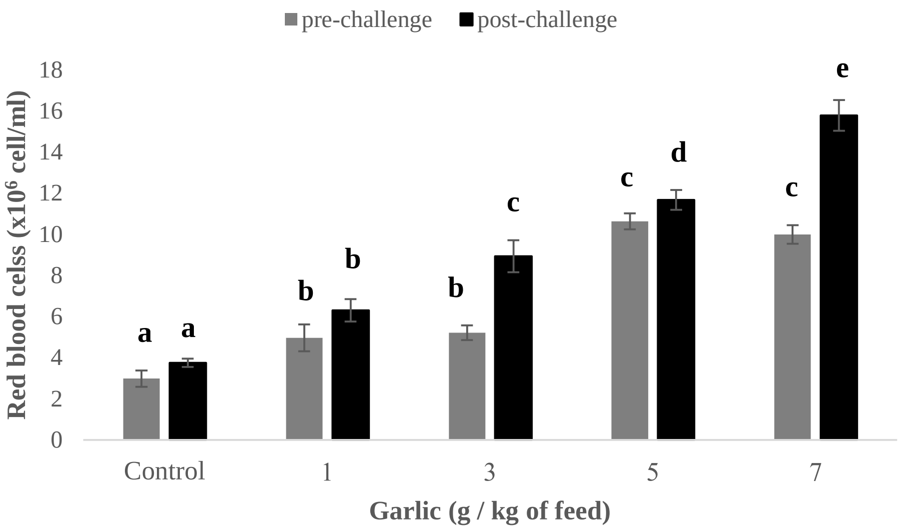

Figure 2 The number of red blood cells of Nile tilapia fed diets with different levels of garlic for 45 days at pre-challenge and post-challenge with *A. hydrophila*. Data (mean ± SD) with different letters significantly differ (p<0.05) among treatment

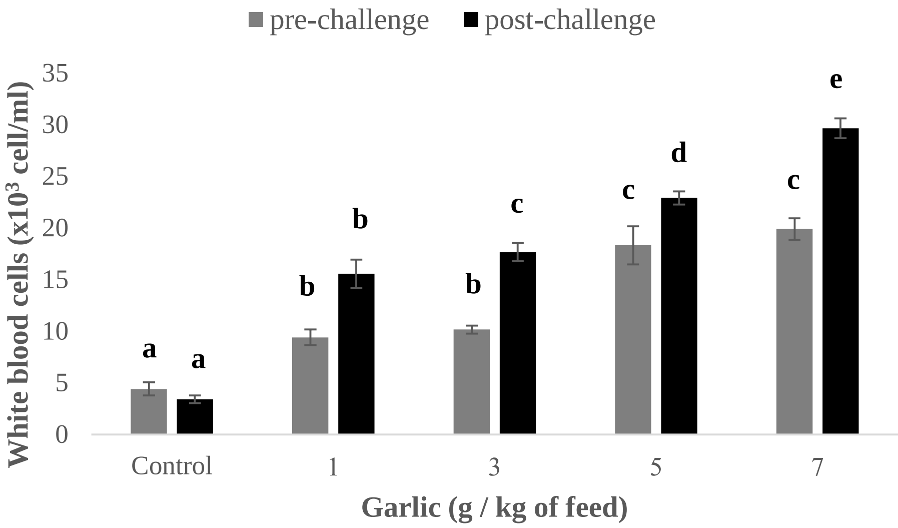

Figure 3 The number of white blood cells of Nile tilapia fed diets with different levels of garlic for 45 days at pre-challenge and post-challenge with *A. hydrophila.* Data (mean $\pm$ SD) with different letters significantly differ (p<0.05) among treatments

The use of natural products in aquaculture has risen dramatically for prophylaxis and to avoid the indiscriminate use of harmful antibiotics. Herbal plants are biocompatible, biodegradable and safe for the environment and human health. Some recent studies have confirmed that feeding fish with herbal plants resulted in enhance disease resistance and survival improvement which may be attributed to an immune function improvement such as *Phyllanthus niruri* and *Aloe vera* (Ahilan *et al*., 2010), *Ocimum sanctum* (Das *et al*., 2015), *Morus alba* (Mapanao *et al*., 2019), *Achyranthes aspera* (Kumar *et al*., 2019) and *Moringa oleifera* (Abd El-Gawad *et al*., 2020). Hence, the present study investigated the effect of garlic cloves supplemented in diet on red blood cell (RBC) and white blood cell (WBC) values and mortality rate after challenge with *A. hydrophila* in Nile tilapia.

Results of the challenge test with *A. hydrophila* showed that increased mortality rate was lowest (16.67$\pm$5.77%) in the treatment containing 7 g of garlic/ kg of feed, followed by 5 g of garlic/ kg of feed (23.33$\pm$5.77% mortality), 3 g of garlic/ kg of feed (46.67$\pm$5.77% mortality) and 56.67$\pm$5.77% mortality in the diet containing 1 g of garlic/ kg of feed. The fish mortality was much lower compared to the control group. Furthermore, the relative percent of survival (RPS), as one of the most visual parameters of immune evaluation in the challenge test, was higher in all fish groups fed with diet containing garlic than in the control group. This indicates that garlic could be produced more resistance to challenge infection. This result was also explained by the increase in WBC in this study that indicated an increase in the immune status in experimental groups which correlates with fish survival increasing. Garlic has traditional applications as an anti-infective agent against many bacteria (Ress *et al*., 1993), fungi (Adetumbi *et al*., 1986) and viruses (Weber *et al*., 1992). Many defense mechanisms activated by garlic counteract the challenge infection including the production of superoxide anions against the *A. hydrophila* (Aly and Mohamed, 2010). The antibacterial properties of garlic clove homogenates are attributed to allicin. Several studies have provided strong evidence that most of the biological function of garlic cloves are caused by active substance known as allicin (Li *et al*., 2007), which is the most important organosulphide in garlic. Similar results have been reported in previous studies. Rose *et al*. (2005) reported that the positive effects of garlic supplementation may be due to its medical effects based on organic sulfur compounds, particularly allicin which is an active but volatile compound of garlic and has been implicated in antibacterial activity against a wide range of gram-positive and gram-negative bacteria, and has antiviral, antifungal and antiprotozoal activity (Nya *et al*., 2010). Thus, it also could be suggested that high disease resistance in the experimental groups as compared to control group could be due to the presence of allicin in garlic, which further helps to inhibit pathogens.

Additionally, the result in this study also agrees with the results of several studies. For examples; Sahu *et al*. (2007) noted the effectiveness of adding 0.5 or 1% garlic powder in fish diets for controlling *A. hydrophila* infection in Indian major carp (*Labeo rohita*). Nya and Austin (2009) reported that feeding rainbow trout with 0.5 g and 1.0 g garlic per 100 g feed for 14 days showed fish mortalities after challenged with *A. hydrophila* only 4%, RPS 95%. Moreover, in rainbow trout, the administration of garlic dosed at 0.5 g/100 g of feed for 14 days, the RPS was 86%, decreasing to 75% and 68% after 21 and 28 days of dietary garlic treatments, respectively and 14 days after stopping feeding with 1.0 g garlic /100 g of feed, the RPS was 80%, reducing to 55% after 21 days, and 46% at 28 days (Nya and Austin, 2011). Sasmal *et al*. (2005) and Diab *et al*. (2008) found that feed containing 1% garlic powder was able to improve the disease resistance of Nile tilapia, particularly against *Pseudomonas fluorescens*. Furthermore, Shalaby *et al*. (2006) and Aly and Mohamed (2010) revealed that adding 3% garlic powder at Nile tilapia diet could reduce mortality after a challenged with *A. hydrophila*. Eirna-liza *et al*. (2016) reported that after African catfish juveniles were challenged with *A. hydrophila*, the highest survival (64%) was observed for fish fed with garlic cloves at 20g/kg. Also, Thanikachalam *et al*. (2010) showed that after challenge with *A. hydrophila* in African catfish, all the garlic peel fed groups showed a significantly (p<0.05) reduced mortality compared to the control group with the highest survival rate (96%) was recorded in fish fed with 1.5% of garlic peel. After challenged with *A. hydrophila*, the survival rate of swordtail, *Xiphophorus helleri*, increased and reached up to 95.8% in fish fed with the garlic diet (Kalyankar *et al*., 2013)

Fish species, size, age, physiological status, environmental conditions, dietary regime, quality and quantity of diet were reported to affect fish hematological parameters. Normal values of these parameters relate to dietary ingredients, protein sources, vitamins and probiotics (Osuigwe *et al*., 2005). The study of hematological values is also useful for physiological examination of changes in fish health or to determine the state of health. Complete blood cell count (CBC) is an important and effective diagnostic tool that can be used to monitor fish health status in response to changes in nutrition, water quality and disease therapy (Fazio, 2019). In this study, pre- and post-challenge with *A. hydrophila* test showed that there was significantly difference higher RBC and WBC counts in fish fed garlic cloves than control group (p<0.05). Especially, in post-challenge RBC and WBC counts in fish fed garlic cloves increased as the amount of garlic cloves in the diet increased. This indicated that the health of Nile tilapia improved with dietary addition of garlic cloves.

Leucocytes play an important role in nonspecific immunity and their count can be considered as an indicator of the fish health status (Harikrishnan *et al*., 2003). In current study, WBC counts significantly increased (p<0.05) following post feeding garlic cloves trial for 45 days and post challenge with *A. hydrophila* which supports the anti-infection properties of garlic. As Iranloye (2002) mentioned that it is well known that when infection occurs WBC increase rapidly which as first line of defense of body. This result is also supported by other studies by Thanikachalam *et al.* (2010) reported that significantly highest WBC count was found in African catfish fingerling fed with 1.5% garlic peel incorporated diet. Nwabueze (2012) found that WBC counts of African catfish fed 0.5%, 1.0% and 3.0% garlic supplemented diet were increased significantly by weeks. Ndong and Fall (2011) reported that WBC counts increased significantly in juvenile hybrid tilapia (*O. niloticus* x *O.*

aureus) fish fed 1% and 0.5% garlic supplemented diet. Heo *et al*. (2000) reported that the only changes in fish blood profiles, observed when l-carnitine was included in diet, were increased concentrations of WBC and lymphocytes, although there is no direct evidence from the literature on the effect of l-carnitine supplementation on immune-related blood cell counts. However, many previous reports have suggested that l-carnitine supplement may influence lipid metabolism. Dietary lipid may affect a great number of immune parameters, such as lymphocyte proliferation, cytokine synthesis, natural killer cell activity and phagocytosis (De Pablo and De Cienfuegos, 2000).

RBC count was also increase significantly in garlic fed groups when compared to control groups. RBC count increased with the administration of garlic, which might indicate an immunostimulant effect. The high red blood cell counts in garlic fed fish groups are presumed to be due to garlic's antioxidant properties that can ward off free radicals. Free radicals are compounds that can damage red blood cell membranes and other cells. Garlic also contains compounds that can act as antioxidants in blood cells. This antioxidant can act as a reservoir of free radicals to protect red blood cell membranes (Setijaningsih *et al*., 2021). The findings confirm to those by Farahi *et al*., (2010) observed erythrocyte count and hemoglobin content increased in rainbow trout fed on diets containing 20 and 30 g garlic which were significantly different from those of control. The red blood cells are significantly higher in garlic peel fed groups than control group (Thanikachalam *et al*., 2010). RBC counts in African catfish were observed to be significantly higher than fish fed 0.5% garlic group as compared to the control (Nwabueze, 2012). Setijaningsih *et al*. (2021) also found that the addition of 1% garlic extract to the feed had the highest value in erythrocyte and hemoglobin of Nile tilapia. Seeley *et al*. (1992) mentioned that the primary function of RBC in fish is to transport oxygen from the gills to the various tissues of the body, an action carried out by the haemoglobin found in RBC. Garlic increases superoxide dismutase (SOD) activity in blood serum and that this phenomenon has been used in fish farming to enhance the activity of non-specific defense system in *O. niloticus* (Diab *et al*., 2002). Also garlic has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen and bone marrow (Jeorg and Lee, 1998).

CONCLUSION

This study has highlighted the potential value of garlic to aquaculture in terms of protection against specific bacterial disease (*A. hydrophila*). The results shown that 0.7% (7 g/kg) garlic supplement in fish feeds elicited more increase in volume of RBC, WBC and survival rate of *O. niloticus*. Garlic inclusion in fish diet at 0.7% (7 g/kg) concentration is therefore beneficial for use in aquaculture to enhance the disease resistant status *O. niloticus*. This is a basic study provides a new perspective for the use of garlic cloves as a dietary supplementation to enhance the disease resistance and disease prevention of fish. Further purification of the active compounds and their evaluation may substantially improve the quality as well as their usage in aquaculture as immunomodulators.

ACKNOWLEDGEMENT

Author is grateful to Aquatic Animals Production Technology Program, Faculty of Interdisciplinary Studies, Khon Kaen University, Nong Khai Campus for financial support.

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