UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF MALACHITE GREEN TREATMENT ON THE HEMATOLOGICAL PARAMATER OF JADE PERCH

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It is hereby certified that I have read this project paper entitled "The Effect Of Malachite Green Treatment On The Hematological Paramater Of Jade Perch", by Hasni Nabilah Binti Yaacob and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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DEDICATION

To:

My family especially to my beloved mother, Shamsiah Mohd Zain who taught me about life

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ABSTRACT

The Effect of Malachite Green Treatment on the Hematological Parameter of Jade Perch (*Scortum barcoo*)

By

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2017

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Malachite green (MG) is extensively used as an antiprotozoal and antifungal agent in the aquaculture industry. Since MG is toxic, the present study was conducted to investigate the effect of MG on the hematological parameter of jade perch (*Scortum barcoo*). Sixty jade perch were divided into control and two treatment groups; one immersed in MG for bath at 0.5mg.l^{-1} daily changed for 5 days maintaining the same concentration and another treated for a single dip at 66.7 mg.l^{-1} for 30 seconds. For all groups, blood was collected on day 0, 1, 5, 7, 11, 13, 15, 19, and 21 for hematological parameters (RBC count, hemoglobin, PCV, MCV, MCHC, and WBC). Analysis of hematological parameter using T-test for dip treatment showed significant difference on PCV, MCHC and WBC while for bath treatment significant difference seen on PCV, RBC, MCHC, and WBC. However, there was no general trend seen in these changes. Fish given bath treatment showed reddening of pectoral and caudal fin,

petechial hemorrhage on the body, erratic swimming and anorexia during exposure to MG. In conclusion, although there were some significant differences of hematological parameter there was no obvious general trend. However, the clinical signs seen in bath treatment indicate that the fish were under stress.

Keywords: Jade Perch, malachite green, dip, bath, hematological parameter.

ABSTRAK

Kesan Malachite Green pada Hematologikal Parameter dalam Jade Perch (*Scortum barcoo*)

Disediakan oleh

Hasni Nabilah Binti Yaacob

2017

Penyelia: Dato' Dr Mohamed Shariff Bin Mohamed Din

Malachite green (MG) telah menjadi satu bahan kimia yang biasa digunakan dalam akuakultur sebagai agen anti-protozoa dan anti-fungal. Memandangkan MG merupakan bahan toksik, kajian dijalankan untuk mengkaji kesan MG pada hematologikal parameter dalam jade perch (*Scortum barcoo*). Enam puluh ekor jade perch dibahagi kepada kumpulan kawalan, kumpulan rawatan dengan MG mandian pada kepekatan 0.5mg.l^{-1} selama 5 days and satu rawatan rendaman pada kepekatan 66.7 mg.l^{-1} selama 30 saat. Kesemua sampel daripada setiap kumpulan diambil pada hari 0, 1, 5, 7, 11, 15, 19, and 21 untuk mengkaji hematologikal parameter (RBC count, hemoglobin, PCV, MCV, MCHC, and WBC). T-Test digunakan dan terdapat keputusan ketaradalam kumpulan rawatan rendaman PCV, MCHC dan WBC. Manakala kumpulan rawatan mandian menunjukkan keputusan ketara dalam parameter PCV, RBC, MCHC dan WBC. Walaubagaimanapun, tiada trend umun yang diperolehi daripada perubahan tersebut. Kumpulan ikan rawatan mandian menunjukkan tanda klinikal kemerahan pada sirip pektoral dan caudal, bintik-bintik kemerahan pada badan, berenang secara tidak menentu dan anoreksia semasa

didedahkan dengan MG. Konklusi, walaupun terdapat beberapa berubahan ketara dalam hematologikal parameter, tiada perubahan umun yang jelas. Walaubagaimanapun, tanda klinikal yang terdapat dalam kumpulan rawatan mandian menunjukkan ikan berada dalam keadaan stress.

Kata Kunci: Jade Perch, Malachite Green, rawatan rendaman, rawatan mandian, hematological parameter.

1. INTRODUCTION

1.1 Study background

Jade perch (*Scortum barcoo*) is a new favored freshwater fish used in aquaculture industry especially in Asian countries such as China, Hong Kong, Singapore and Malaysia (Real, 2011; Gao, 2014; FAO, 2014; Lim, 2015). As the industry continues to bloom, the farmers have been using malachite green (MG) to control and treat diseases that are commonly found in aquaculture. This chemical has been used in the aquaculture industry as an antifungal and antiprotozoal agent (Culp & Beland, 1996; Srivastava et al., 2004; Srivastava et al., 2016). However, the usage of MG has been of great concerned due to its toxicity. A study on the use of MG to treat fish showed a detrimental effect on the hematological parameter in freshwater catfish *Heteropneustes fossilis* when compared to the treatment with other alternative drug such as Pyceze (Srivastav & Roy, 2015). In addition, El-Neweshy et al.,(2011) also showed that the long term exposure to MG was able to induced anaemia and leukopenia in *Oreochromis niloticus.*

1.2 Justification

Malachite green has been commonly used in jade perch culture (Sambell, 2000) and as yet no study has been done on the effect of MG on the hematological parameter of jade perch. Therefore, this study was conducted using MG on jade perch to provide valuable data on the hematological parameter after treatment with MG.

1.3 Objective

Objective of this study:

I. To study the effect of MG treatment on the hematological parameter of jade perch.

II. To establish a preliminary hematological database reference for jade perch.

1.4 Hypothesis

I. Null hypothesis: There will be no significant changes of the hematological parameter after jade perch is treated with MG.

II. Alternative hypothesis: There will be significant changes of the hematological parameter after jade perch is treated with MG.

2. Literature reviews

2.1 Aquaculture Industry

As the human population grows, there is an increase in the demand for protein sources. This demand had led to the blooming of the aquaculture industry around the world. Based on the data from FAO (2016), the aquaculture production has grown from 145.9 million tonnes in 2009 to 167.2 million tonnes in 2014. In addition, total production of aquaculture for Asian country in 2014 has reach up to 65.6 million tonnes. In Malaysia, the total production for aquaculture in 2014 was 532 thousand matric tonnes (DOF, 2014). Moreover, the food consumption has increased along with the increase in human population which makes aquaculture a promising industry in providing a source of sustainable food supply (Jye et al., 2012)

2.2 Jade perch

Jade perch (*Scortum barcoo*) belong to order *Perciformes*, family *Terapontidae* and genus *Scortum* (McCulloch and White, 1917). The family *Terapontidae* has an oblong body to oblong-ovate, somewhat compressed, has two spines atopercle, longer lower spine and also dorsal fin notch .This family can be divided into 16 genera with *Scortum* as one of them (Nelson, 2006). The *S.barcoo* sp. can be recognized by its distinctive characteristics of having silvery-grey colour on the sides, darker dorsally and whitish on the ventral part of body. It also has translucent fin or whitish with bluish tinge and white stripe extending below the red eye to its snout. The habitat for *S.barcoo* is in turbid water of large rivers and also water holes. It has the capability to tolerate high water temperature of up to 40℃. It originated from central Australia

(Allen et al., 2002). It is also known as one of the greatest freshwater fishes in Australia, which is a native to Eyre Lake. This fish has a great future in aquaculture industry due to its rapid growth, disease tolerance and tolerance to hypoxia, and delicious meat (Chen et al., 2011).

2.3 Malachite green

According to Culp and Beland (1996), MG is actually not a well-defined chemical and the term MG itself may refer to a dye as either the chloride or oxalate salt (chromatic form), the neutral carbinol, or the reduce leuco form with the physical form of a green crystalline powder.

Besides that, Chemical Abstract Service (CAS) name and also the number for the MG oxalate compound is given as*N*-[4-[[4-(dimethylamino)-phenyl]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminiumoxalate and 2437-29-8. The MG also has the capability to decompose in air and light due to the oxidation to diarylketone. As for the biochemical disposition of MG, the carbinol forms of the MG dye are more lipophilic than in cations from which they are derived, and in carbinol forms, it most likely to enter cells. Once the MG enters the body, it would be reduced into leucomalachite green (Culp & Beland, 1996).

Back in 1968, study done by Werth and Boituex (1968) managed to detect leucomalachite green in the liver, kidney, heart, lungs, and muscle of rats after 2 hours MG injection via intravenous injection and also in Ehrlich's ascitic tumor cells after 3 hours injection via intraperitoneal. In a study by Alderman, (1985), the MG has been

used extensively in the aquaculture industry world-wide as a highly effective chemical against important protozoal and fungal infection. Malachite green is being used as a topical treatment by bath or flush method without paying any attention to the fact that topically applied therapy might also be absorbed systematically and produce the significant internal effect (Srivastava et al., 2004).

3.0 Materials and methods.

Jade Perch Transportation and Acclimatizing

Sixty Jade perch were collected from a local farm in Sepang. The total body length of the fish ranged from 16.0 to 19.5 cm and body weight was 67.4 to 144.0 gram. Before transporting the fish to laboratory, they were sedated with MS-222 and 10 fishes were placed into plastic bag filled with water and oxygen. The anesthesia agent used at dosage of 50 mg.l^{-1} was to reduce the transportation stress. On arrival at the Aquatic Animal Health Unit (AAHU), the fish were transferred into three glass aquarium, after the temperature in fish bag was equalized by floating it on the water in aquarium tank for 30 minutes. Each aquarium tank contained200 liter of fresh water and 20 fish were placed into each tank. The fish were conditioned for 1 week before conducting the experiment. Aeration was provided for each aquarium throughout the experiment and 100 liter of water from each aquarium was changed daily. The fish were feed ad libitium.

Malachite green treatment and control.

Three glass aquaria were used: one was used as a control tank and the other two were treatment with MG via bath and dip. The dosage of MG for bath treatment group was 0.5mg.l^{-1}. The water was changed daily for 5 days and a same dose of MG was introduced at the initial concentration. For the single dip treatment group, the dosage used was 66.7 mg.$l^{-1}$for 30 seconds .

Blood Sampling.

Two fish was sampled at 9 am from each tank on day 0, 1, 5, 7, 11, 13, 15, 19, and 21 for hematological parameter (RBC count, hemoglobin, PCV, MCV, MCHC, and WBC) using the facility at the Clinical Pathology Lab in the Faculty of Veterinary Medicine. The blood was collected from the caudal peduncle vein by approaching the lateral line. The amount of the blood collected per fish was 0.6 ml using sterile 23G needle with 1 ml tuberculin syringe. The blood collected was placed into the lithium heparin blood collecting tube. During the sampling, the fish was handled with minimal stress using the wet towel covering the head of the fish. The maximum duration for each sampling was 3 minutes.

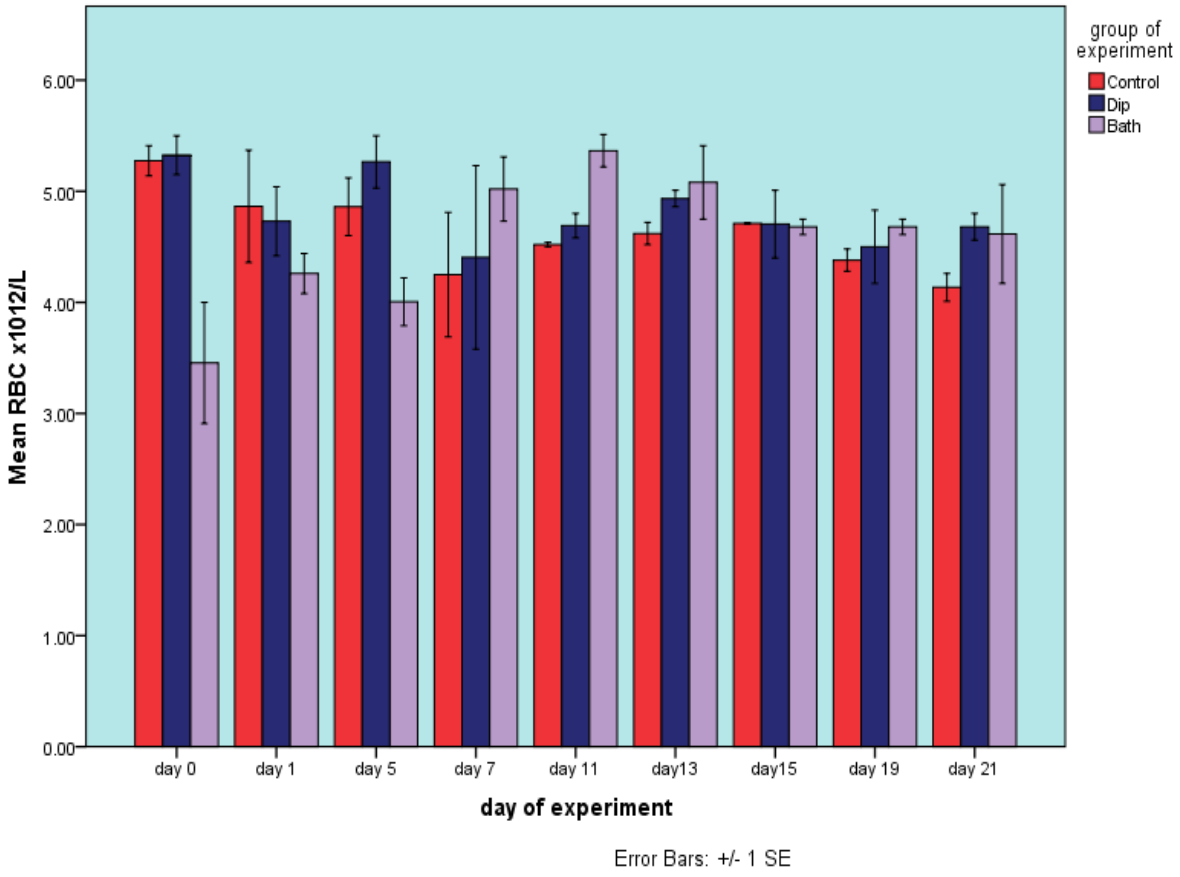
Statistical Analysis

All the data obtained was statistically analysed using the SPSS version 22.The well-distributed data was tested using the T-test.

4.0 Results

4.1 Red Blood Cells (RBC) counts

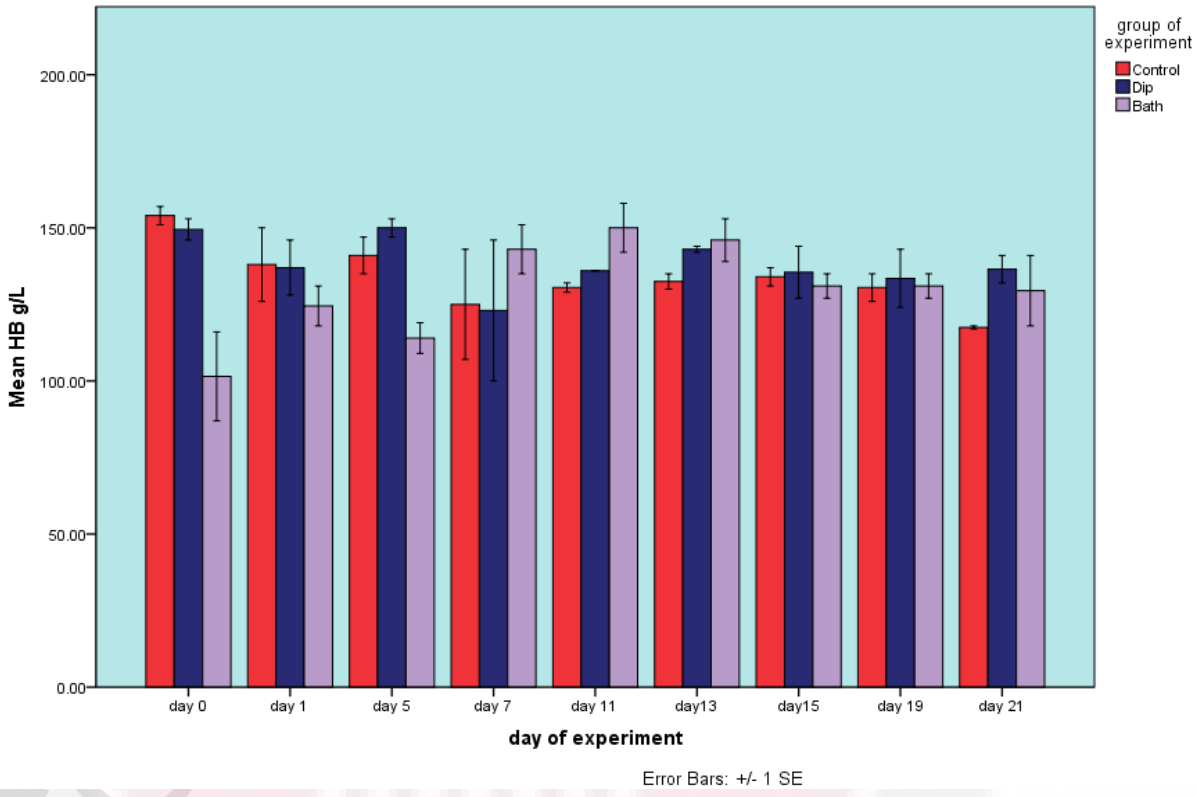
For the red blood cell (RBC) counts, there was a significant increase ($p \leq 0.05$) on day 11indication erythrocytosis in the bath treatment group when compared with the control (Graph 1).



Graph 1: Mean RBC readings for the treatment and control groups against day of experiment

4.2 Hemoglobin (HB)

Statistical analysis on the hemoglobin parameter, did not show significant difference in both the dip and bath treatment groups when compared with the control (Graph 2).

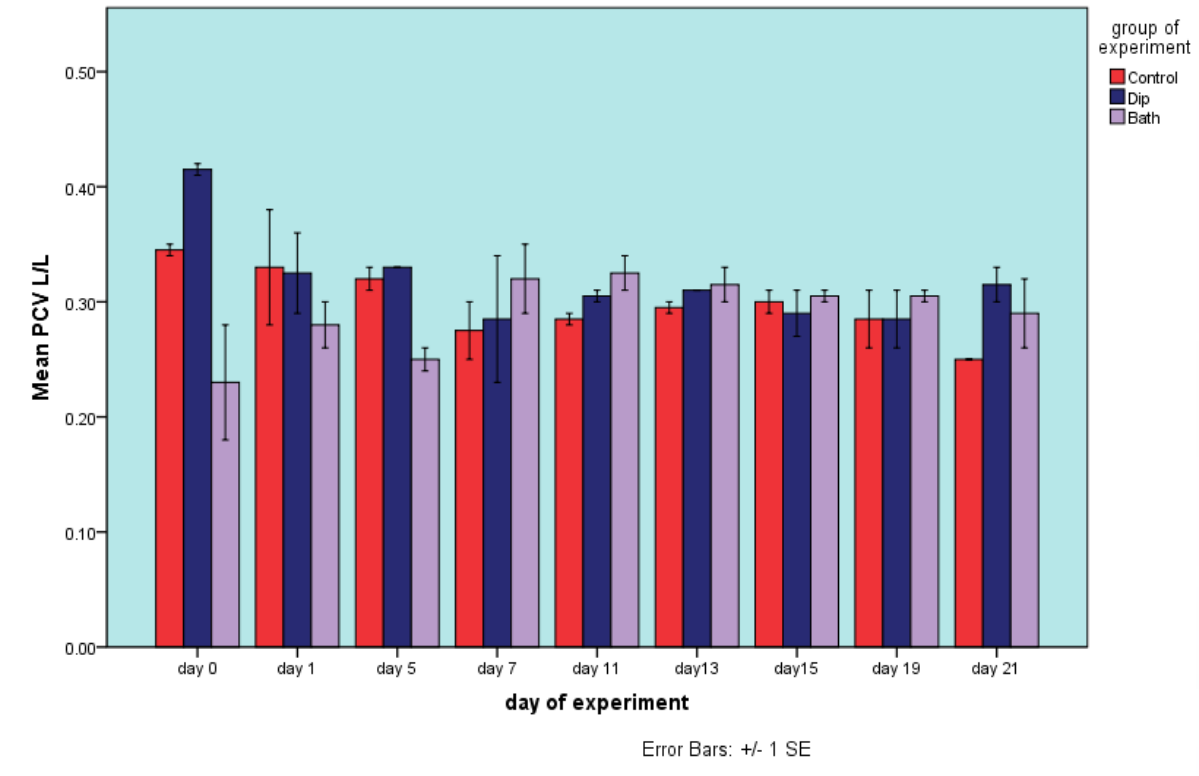


Graph 2: Mean HB readings for the treatment and control groups against day of experiment

4.3 Packed Cell Volume (PCV)

For the packed cell volume (PCV) parameter, the significant increase was found in the dip treatment group on day 0 ($p \leq 0.05$) and on day 21 when compared

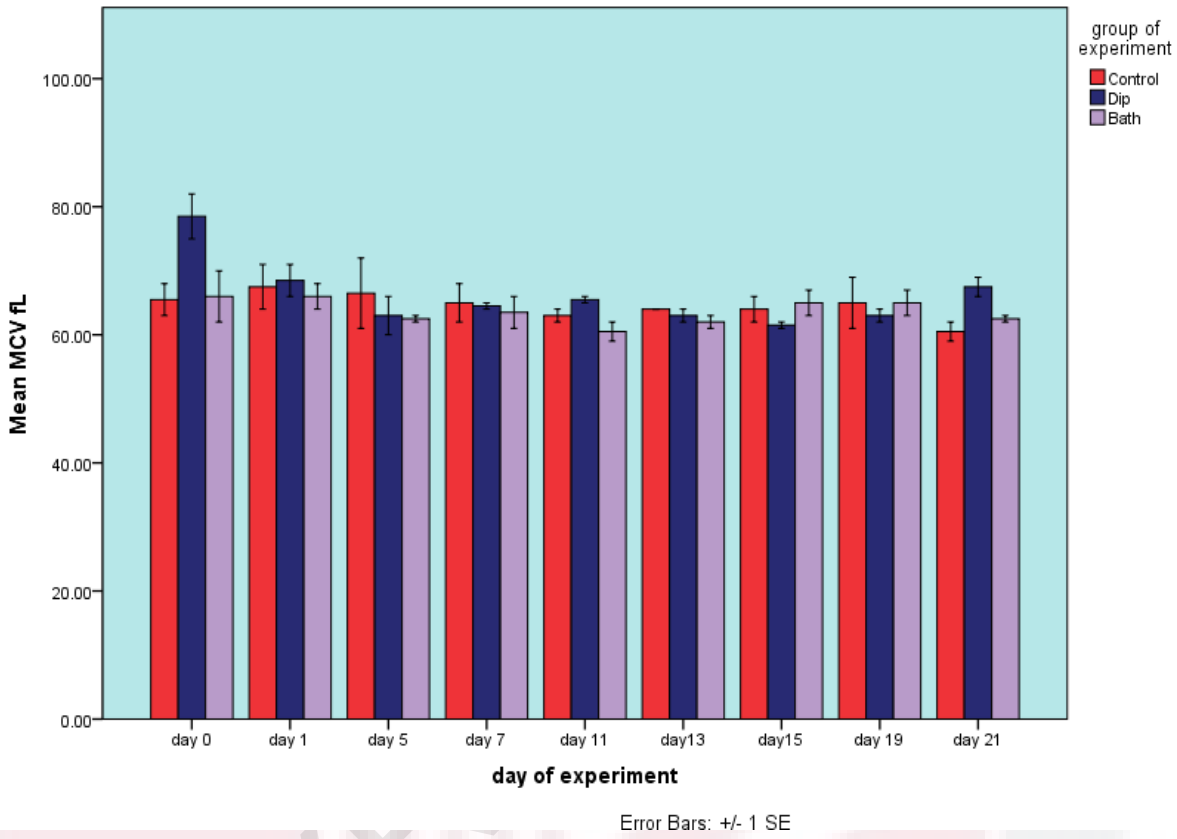
with the control (Graph 3). Whereas the bath treatment group, showed a significant decrease on day 5 when compared with the control.



Graph 3: Mean PCV readings for the treatment and control groups against day of experiment

4.4 Mean Corpuscular Volume (MCV)

There was no significant different in the dip treatment group and a bath treatment group (Graph 4) when compared with the control.

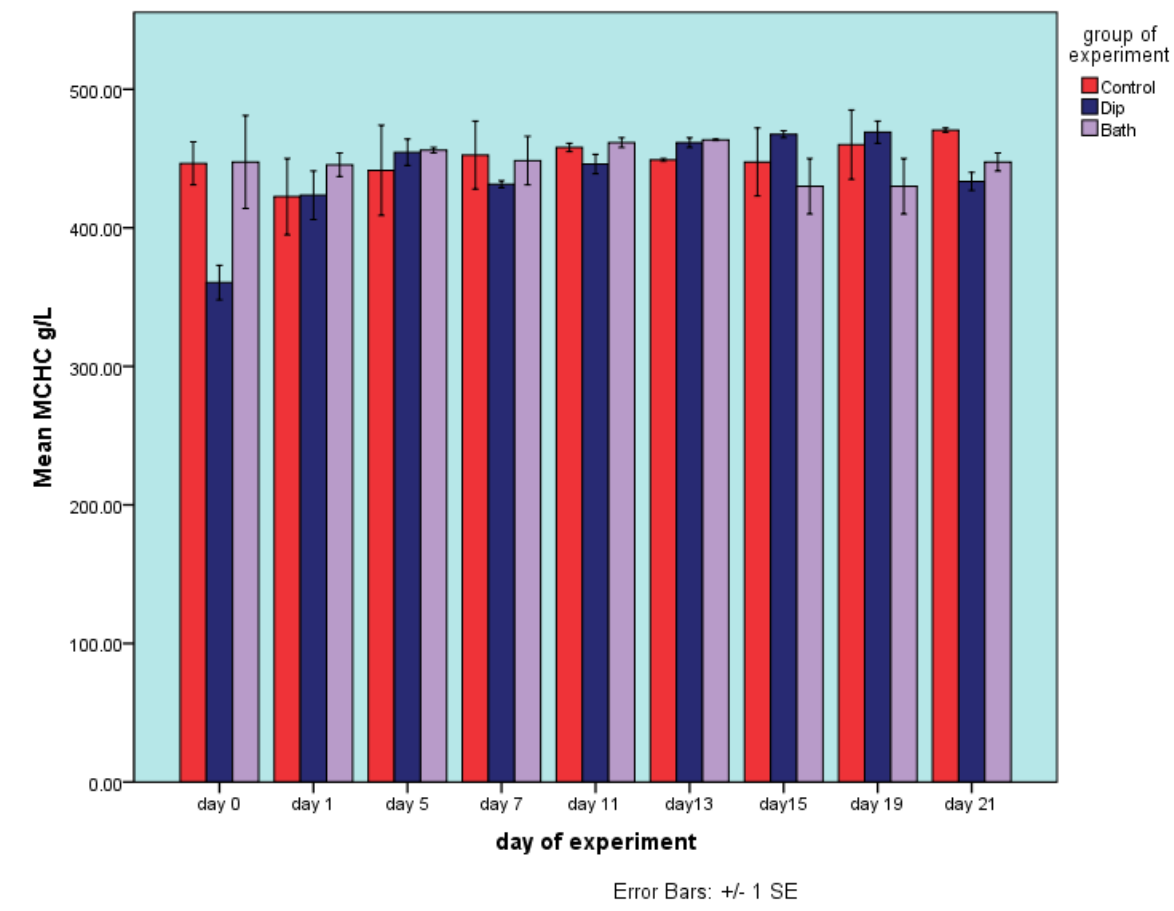


Graph 4: Mean MCV readings for the treatment and control groups against day of experiment

4.5 Mean Corpuscular Hemoglobin Concentration (MCHC)

There was a significant decrease found in the dip treatment group on day 0 ($p \leq 0.05$) and also day 21when compared with the control (Graph 5). For the bath

treatment group, there was a significant increase found on day 13.

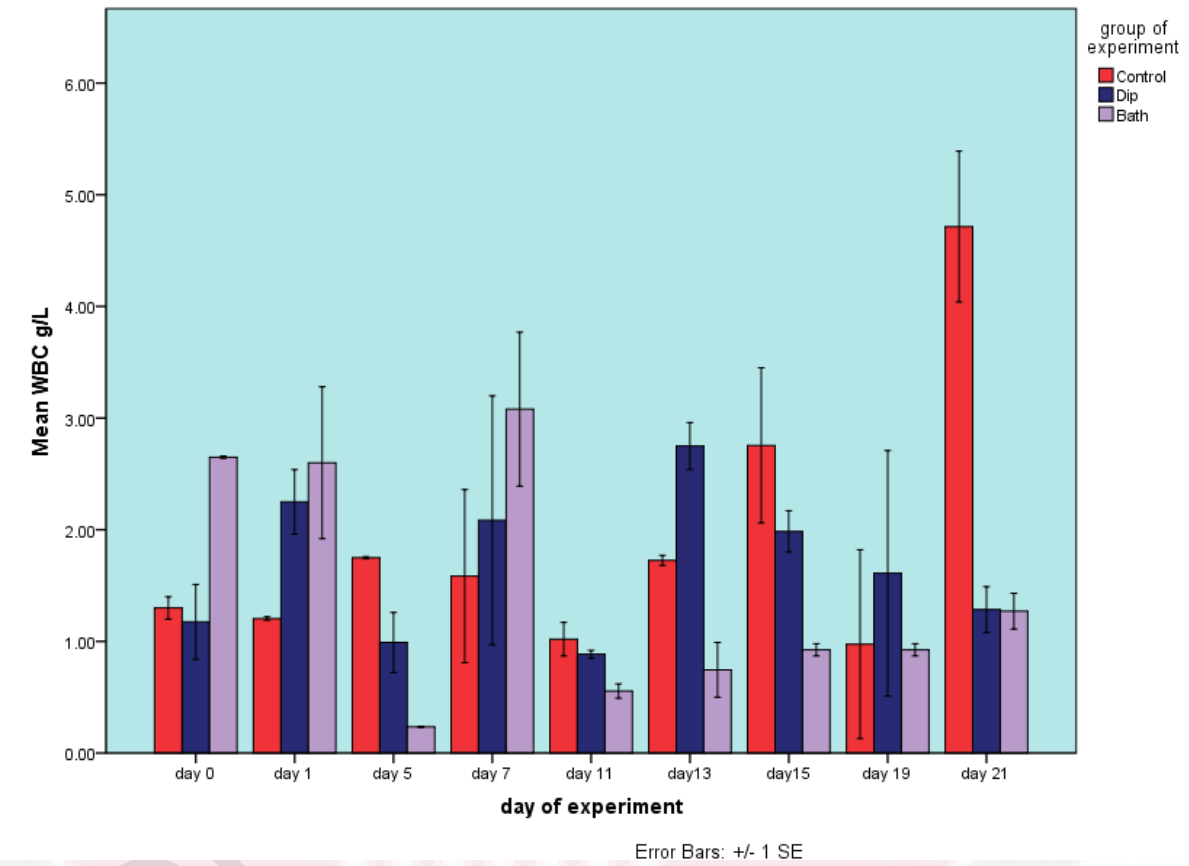


Graph 5: Mean MCHC readings for the treatment and control groups against day of experiment

4.6 White Blood Cells (WBC)

Results showed a significant fluctuation in both treatment groups when compared with the control. For the dip treatment group, a significant increase was seen on day 13 indicating leukocytosis and a significant decrease on day 21 indicating leucopenia ($p \leq 0.05$). In the bath treatment group, there was a significant increase on

day 0 and a significant decrease indicating leucopenia on day 5and also on day 21 (Graph 6).



Graph 6: Mean WBC readings for the treatment and control groups against day of experiment

4.7 Clinical Signs

During the experiment several clinical signs were seen in the bath treatment group and it started with reddening of the pectoral (Figure 1) and caudal fin on day 2 of experiment. Then on day 3, the fish started to show signs of erratic swimming.

On day 4, 5 and 6, in addition to the reddening on the pectoral and caudal fin, erratic swimming the fish also showed petechial hemorrhage (Figure 2) on the body. In addition, throughout the MG exposure in the bath treatment group, the fish were also anorexic and darkening of the body until day 8 of the experiment. The reddening of the pectoral and caudal finand petechial hemorrhage on the body were resolved on day 9 and for the erratic swimming on day 8.

Figure 1 . The reddening of the pectoral fins was seen on day 5 in the bath treatment group.

Figure 2. The darker body colour was seen on day 5 indicating that fish were under stressful condition. The petechial hemorrhage on the body are shown by the red circle.

5.0 Discussion

Based on the results obtained on the RBC parameter, which showed significant increase in RBC count on day 11 in the bath treatment group, was a similar finding with the study done by Grizzle (1997) where there was an increase of erythrocyte counts after 7 days of MG treatment in fingerling channel catfish. However, the results of the recent study differs with the study done by El-Neweshy and Abou Srag (2011). Their study showed that chronic exposure of MG in the Nile Tilapia shows a significant decrease in RBCs. In addition, the acute exposure of diazinon in the common carp caused a significant decrease of the RBC count (Svoboda et al., 2001).

The PCV results in the bath treatment group showed a significant decrease on day 5 which differed with the study done by Alderman and Clifton-Hadley (1993) who observed a significant increase of PCV in rainbow trout after a long exposure. In additions, study by Ojutiku et al., (2013) on hematological effect of acute concentration of cypermethrin on juveniles of African catfish which show significant increase.

The MCHC parameter showed a significant decrease in dip treatment group while the bath treatment group showed a significant increase. Other studies showed an increase on MCHC level with the increase in exposure time in the rainbow trout given MG therapeutic bath treatment (Saglam et al,. 2003). However the study by Srivastava and Roy (2015), stated that there was significant decrease of MCHC after 96 hours of MG exposure in the freshwater catfish.

The WBC parameter showed a fluctuation in both dip and bath treatment groups, however the on day 0 the bath treatment group showed a significant increase. This also indicated that the leucocytosis occur once the fish is exposed to the MG. The result obtained for WBC on day 0 is similar to the study by Srivastava and Roy (2015), where they also found a significant increase in WBC parameter in freshwater catfish when exposed to MG. They also stated that the leucocytosis can also be observed in other teleost fish after being exposed to other toxicant. However, the study of MG treatment in Rainbow trout showed a significant decrease in leucocytes (Saglam et al., 2003) and was supported by Musa and Omoregie (1999), where they also found a significant decrease of erythrocytes count in *Clarias gariepinus* when exposed to MG at dosage of 0.015 ppm, 0.02 ppm and also 0.025 ppm. The clinical sign of erratic swimming was also seen in the rainbow trout upon the exposure to MG (Saglam et al., 2003) which is similar to the findings of the present study in the bath treatment group of jade perch. Saglam et al., (2003) also stated that the rainbow trout also showed a rapid pectoral and opercula movement which was not seen in jade perch upon MG exposure.

6.0 Conclusion

In conclusion, although there were some significant differences of the hematological parameter, but there was no obvious general trend noted in any of the hematological parameter in the present study. However, based on the clinical signs that were seen in the bath treatment group which showed anorexia, swimming in the circle and the reddening of the fins along with darkening of the body and petechial hemorrhage were indication that the fish were in a stressed condition due to the exposure to MG.

7.0 Recommendation

In order to obtain a good data and for future studies, it is important that precautions are taken to minimise stress especially during the handling. In addition, stress must also be minimised due to the external factor such as temperature, pH and changes due to the weather.

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