



RESEARCH ARTICLE

REVISED Effectiveness of turmeric-enriched pellets to improve the immunity of *Clarias batrachus* toward motile *Aeromonas* septicemia disease [version 2; peer review: 2 approved]

Morina Riauwaty , Yusni I. Siregar , Isma Mulyani

Fisheries and Marine Science Faculty, Universitas Riau, Pekanbaru, Riau, Indonesia

V2 First published: 02 Mar 2021, 10:169
<https://doi.org/10.12688/f1000research.28260.1>

Latest published: 17 May 2021, 10:169
<https://doi.org/10.12688/f1000research.28260.2>

Abstract

Background: Turmeric is known as a natural remedy to improve the immunity of organisms. This study aims to understand the effectiveness of turmeric-enriched pellets to improve the immunity of *Clarias batrachus* to *Aeromonas hydrophila*.

Methods: The study was conducted from May to August 2020. *C. batrachus* fingerlings, 7-8 cm total length (TL) and 4-5 g (BW) at baseline, were kept in 30 L aquaria (10 fishes/aquarium; three replicated/treatment). Commercial pellets were mixed with turmeric powder. There were five treatment groups: P0 (control, no turmeric); P1 (0.5 g turmeric per Kg of pellets); P2 (0.7 g/Kg); P3 (0.9 g/Kg); Pp (positive control). Thirty days after being feed with turmeric-enriched pellets, all groups of fish were infected with 0.1 ml (10^8) of *A. hydrophila* suspension, intramuscularly. The P0 group did not receive injection, while Pp group were not fed with turmeric-enriched pellets but were infected with the bacteria. Fourteen days after infection, clinical signs and hematology of the fish were studied.

Results: Pp fish showed heavy clinical signs of *A. hydrophila*, such as loss of balance, pigmentation, hemorrhages and ulcers. P0 fish did not show any symptoms, while the treated fish revealed some clinical signs of *A. hydrophila* to a lesser extent than Pp, indicating that the fish is able to face the *A. hydrophila* attack. Hematology for Pp fish revealed high white blood cells, indicating that the fish were infected. The blood condition of the P0 fish, as well as those of the turmeric-treated fish were normal. In general, the P3 fish showed the least clinical signs of *A. hydrophila* and normal blood condition, indicating that P3 treatment is best.

Conclusion: The best turmeric dosage to improve the immunity of *C. batrachus* toward *A. hydrophila* infection is 0.9 g/Kg pellets.

Keywords

Aeromoniasis, catfish, clinical sign, MAS diseases, self-defense

Open Peer Review

Reviewer Status

Invited Reviewers

1

2

version 2

(revision)

17 May 2021



report



report



report



report

version 1

02 Mar 2021

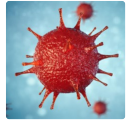
1. Christopher Marlowe A. Caipang ,

University of San Agustin, Iloilo City,
Philippines

2. Indra Junaidi Zakaria , Universitas

Andalas, Padang, Indonesia

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the [Disease Outbreaks](#) gateway.

Corresponding author: Morina Riauwaty (morina.riauwati@lecturer.unri.ac.id)

Author roles: **Riauwaty M:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Siregar YI:** Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Mulyani I:** Data Curation, Formal Analysis, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Riau University Research Centre, Riau University and the Ministry of Research, Technology and Higher Education (“Percepatan Guru Besar scheme research”).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2021 Riauwaty M *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Riauwaty M, Siregar YI and Mulyani I. **Effectiveness of turmeric-enriched pellets to improve the immunity of *Clarias batrachus* toward motile *Aeromonas* septicemia disease [version 2; peer review: 2 approved]** F1000Research 2021, **10**:169 <https://doi.org/10.12688/f1000research.28260.2>

First published: 02 Mar 2021, **10**:169 <https://doi.org/10.12688/f1000research.28260.1>

REVISED Amendments from Version 1

As I received comments from reviewer, I have revised the manuscript based on his comment. In the background, I have added information about the literature on the use of turmeric in fish farming. In the method, I have explained the method for mixing the turmeric powder with feed pellets. Also I have added information on the number of fish used in each blood sampling. I have add the statistical tool that was used. In the Result, Table 1 can not be removed as there is information on infected and non-infected fishes, while Table 2 has been removed and the information on growth pattern of the fish can be seen in [Figure 1](#). Table 3 has been removed and information on fish total length can be seen in [Figure 2](#). I have explained that the growth rate of the turmeric feeding fish is higher and the graph is valid as long as the fish is fed with turmeric enriched pellets. I have removed [Figure 4](#) and replace it with [Table 3](#). In discussion I focused more on the differences of leucocyte cell types proportion among the treatments, and I have add the photo of the WBC of catfish. In the Discussion, I have discussed white blood cells condition based on statistical analyses.

Based on comments from Mr Indra Junaidi Zakaria, I have revised the manuscript as follows: in the Result, I have explained that the growth rate of the turmeric feeding fish is higher and the graph is valid as long as the fish is fed with turmeric enriched pellets. In the Discussion, the water quality data have been provided and the relationship between water quality and the fish health has been explained.

Any further responses from the reviewers can be found at the end of the article

Introduction

Clarias batrachus or the catfish is a favored fish in Riau Province, Indonesia, due to its high economic value and high protein content. The demand of this fish is high, leading to the community culturing the fish, at large and household scale.

C. batrachus is relatively easy to be cultured. It is able to live in fair quality water and consumes a wide range of feed, including commercial pellets, food remains, and fish or chicken remains. This fish grows quickly and achieves marketable size, around 125 grams, within two months. However, this fish is vulnerable toward *Aeromonas hydrophila* attack, which causes motile *Aeromonas* septicemia (MAS) disease. This disease may cause mass death in fish or cause ulcers and hemorrhage in fish skin. Fish that suffer from the disease may die or be unmarketable, and this problem causes great loss in fish culture¹.

So far, MAS disease is commonly prevented or cured using antibiotics. The use of antibiotics, however, has negative impacts as its residues may stay in fish flesh and endanger the health of consumers². Another alternative in preventing MAS disease in fish is by improving the immunity of the fish using natural remedies, such as turmeric. The root of turmeric contains natural materials, namely curcumin that is antibacterial and has immune-modulatory agents¹. The chemical components of turmeric are curcumin (diferuloylmethane), desmethoxycurcumin, and bisdemethoxycurcumin³.

Turmeric is well known as a traditional remedy for humans and it has been widely used for its antimicrobial, anti-inflammatory, antioxidant properties, as a detoxification of toxins and is able to increase the immune system against disease². The use of curcumin in the diets of Nile tilapia improve the immune response towards the emerging diseases¹. While the addition of curcumin in *Oreochromis niloticus* diets also improve the growth performance and immunity, as indicated by significant increase of total protein, globulin, phagocytic activity and index of phagocytes as well as enhanced the resistance of fish toward *Pseudomonas fluorescens* infection².

Therefore, turmeric has been used to improve fish health through immersing method⁴. Unfortunately, that method is not very effective as the treated fish became stressed and it is not practical for large scale fish culture. Turmeric is not poisonous, and it can be consumed, but information on feeding fish with turmeric-enriched pellets is limited. To understand the effectiveness of turmeric-enriched pellets to improve the immunity of fish to *A. hydrophila*, this study aimed to assess the effectiveness of turmeric-enriched pellets in improving the immunity of *C. batrachus* towards *A. hydrophila*.

Methods

Study design and fish

This research was conducted from May to August 2020 at the Parasite and Fish Diseases Laboratory, Aquaculture, Fisheries and Marine Science Faculty, Riau University. The experiments were carried out within the ethical guidelines provided by the research institution and national or international regulations.

C. batrachus fingerlings were obtained from the hatchery of the Riau Province's Marine Fisheries and Department in Tibun, Pekanbaru. Fish chosen were actively swimming with no wounds or parasites. They were approximately 7–8 cm total length (TL) and 4–5 g body weight (BW). The fish were reared in aquaria (30×40×40cm³; 10 fish/aquarium) with aerators and filters. Prior to the treatment, the fish were acclimated to the laboratory environment for four days. A total of 150 fish (30 fish per treatment for five treatments). Blood samples were taken from 3 fish/treatment (total 15 fish).

Experimental design

A completely randomized design with five treatment groups (three replications per treatment) was used in this research. The aquaria were grouped based on the turmeric treatments and in each group the aquaria were placed randomly based on lottery method. The treatments applied are as follows:

- P0 = negative control, no turmeric feed, no infection
- Pp = positive control, no turmeric feed, infected with *A. hydrophila*
- P1 = 0.5g turmeric in 1 Kg feed, infected with *A. hydrophila*
- P2 = 0.7g turmeric in 1 Kg feed, infected with *A. hydrophila*
- P3 = 0.9g turmeric in 1 Kg feed, infected with *A. hydrophila*

Turmeric-enriched pellet preparation

Turmeric powder was made by slicing the turmeric, drying and grinding it using a blender. During the research, the fish were fed with commercial fish feed pellet (F999 with 35% protein content from the PT Central Proteina Prima Tbk). The powder was then mixed with fish feed pellets before the feed was given. The turmeric used in this study was obtained from the local market in Delima Street Pekanbaru and the turmeric was planted by local farmer. During the study the fish were fed *ad libitum*. The turmeric was mixed with a spoon of water and then mixed well with 1 Kg of pellets. As the turmeric powder was wet, it stick in the pellet granules and it was swallowed as the fish eating the pellets. During the research the fish was fed with turmeric enriched pellet three times per day (morning, noon and afternoon), *ad libitum*.

On the 30th day, the fish were infected with *A. hydrophila* bacteria (intramuscularly 0.1 ml with a bacterial density of 1.0×10^8 CFU/mL). Prior to injection, the fish was sedated using clove oil, approximately 0.25 ml or 5 drops/L fresh water. The fish was put in the clove mixture for around 3 minutes until it shown inactive movement. After the injection the fish was returned to the rearing tank.

After being infected, clinical sign of MAS disease (namely rotting of the tail, increased respiration rate and swollen abdomen, exophthalmia and lethargy) was monitored every day. By the 45th day of the experiment (14th days after infection) the blood condition of fish was studied.

Blood sampling were conducted three times, at baseline (prior to the treatment), in the 30th day (after being treated with turmeric for 30 days) and in the end of the research period (14 days after the infection). In this research there were 5 treatments and in each treatment there were 3 replications (in 3 aquaria). For blood sampling, 3 fishes were taken from each aquarium, it means that there were 9 fishes/ treatment or total 45 fishes were used for blood study per sampling.

Blood sampling were conducted two times, at baseline and in the 8th week (end of the research period). Three fish from each aquaria were taken and their blood were obtained; fish were anesthetized using clove oil (5 drops/L) and blood was taken from the caudal vein, by inserting an EDTA (Merck) 10% wet syringe. Blood samples was kept in EDTA moistened vials, in a cool box filled with crushed ice. Total erythrocytes and leukocytes were counted using a Neubauer hemocytometer and then were calculated³ and analyzed⁴. Hematocrite and leucocrite levels were determined using heparinized micro-hematocrit capillaries that was centrifuged at 12,000 rpm for 3 minutes. Hemoglobin content in blood was measured using Sahli method⁵.

Data analysis

The parameters studied are as follows:

1. **Survival rate:** survival of the fish was monitored every day and data obtained were analyzed using ANOVA.

2. **Growth:** growth of fish was monitored one per week and data obtained were analyzed using ANOVA.
3. **Clinical signs:** after being treated with turmeric enriched pellet for 30 days, the fish was injected with *A. hydrophila* and the clinical sign of the MAS diseases in fish was monitored everyday (started at the injection day) for 7 days. Data obtained were described.
4. **Hematological condition:** Blood samples were taken 2 times. The first samples were taken prior to turmeric enriched pellet treatment. The second blood sampling was conducted in the 45th day (14 days after the fish being infected with *A. hydrophila*). Hematological parameters measured were total erythrocyte, hematocrit levels, hemoglobin, total leucocyte and leucocyte differentiation. Data obtained were then described.

The data were subjected to one-way analysis of variance followed by the post hoc Newman–Keuls test using SPSS 18.0 software. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Survival

The survival of the fish various among treatment groups. After being fed with turmeric-enriched pellets for 30 days, the survival of the fish was 100% in all treatments. After infection, however it is clear that the survival rate of the infected fish decreased. In P0 group (no turmeric feed nor *A. hydrophila* infection), the survival rate was 100%. In contrast, in the Pp group (no turmeric with *A. hydrophila* infection), the survival of the fish by the end of the experiment was low (43.33%). The survival rate of *C. batrachus* for all groups is presented in Table 1.

All infected fish showed various clinical signs of MAS disease, namely ulcers, hemorrhage, pigmentation, swollen abdomen and eroded fins. MAS signs worsened as the turmeric

Table 1. Survival rate of *Clarias batrachus* after being fed a turmeric-enriched feed and infected with *Aeromonas hydrophila*.

Treatments	Survival rate (%)	
	Day 0	Day 45
P0	100	100.00 ± 0.00 ^d
Pp	100	43.33 ± 5.77 ^a
P1	100	50.00 ± 10.00 ^a
P2	100	66.66 ± 5.77 ^b
P3	100	80.00 ± 10.00 ^d

P0, control (no turmeric/not infected); Pp, positive control (no turmeric/infected); P1, 0.5g turmeric in 1 Kg feed and infected; P2, 0.7g/Kg and infected; P3, 0.9g/Kg and infected.

Mean with standard error followed by different letters are significantly different ($P < 0.05$)

dose was reduced: P1, rotting of the tail, increased respiration rate and swollen abdomen; P2, exophthalmia and lethargy; P3, clinical symptoms were unclear.

Growth

The growth pattern of *C. batrachus* is presented in Figure 1. In general, the growth of fish in all treatment groups showed a similar pattern: they gained length throughout the experiment. However, by the end of the experiment, TL varied between groups. Fish that were treated with turmeric shown better growth than that of fish that do not receive any turmeric. The growth of fish reduced as the turmeric dosages decreased (Figure 1).

As well as body length, BW of the treated fish increased throughout the experiment. The daily growth rate of fish in each treatment, varied. Fish that were fed with turmeric-enriched pellets showed a higher daily growth rate and as a consequence had a heavier BW than those with non-turmeric enriched pellets (Figure 2).

As shown in Figure 2, the highest BW is in fish that are fed with feed pellets with 0.9 turmeric/Kg pellets. The lowest BW is in the fish that were not fed with turmeric-enriched pellets and infected with *A. hydrophila*. From the beginning (D0) to the end (D45) of the research, fish in all treatments grew well. Even

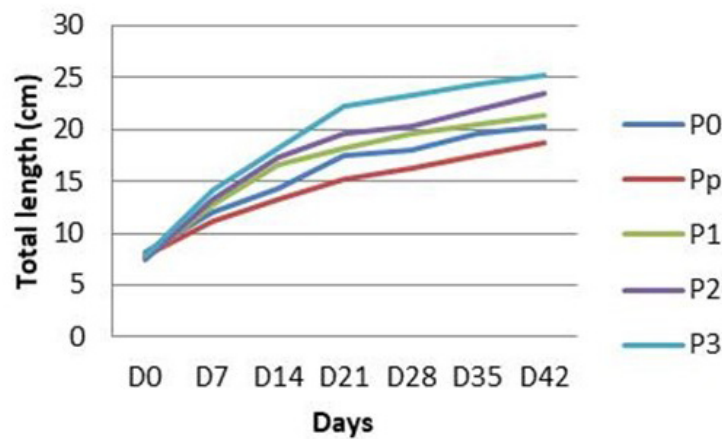


Figure 1. Total length of *Clarias batrachus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila* (on day 30). P0, control (no turmeric/not infected); Pp, positive control (no turmeric/infected); P1, 0.5g turmeric in 1 Kg feed and infected; P2, 0.7g/Kg and infected; P3, 0.9g/Kg and infected.

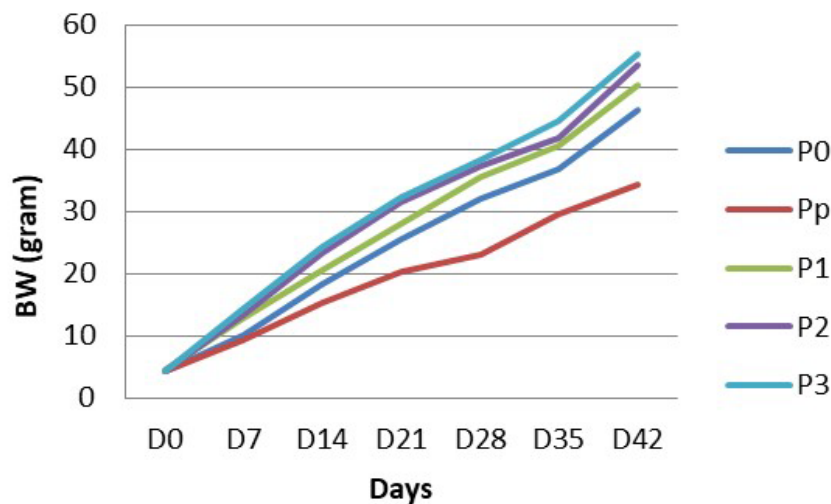


Figure 2. Body weight of *Clarias batrachus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila* (on day 30). P0, control (no turmeric/not infected); Pp, positive control (no turmeric/infected); P1, 0.5g turmeric in 1 Kg feed and infected; P2, 0.7g/Kg and infected; P3, 0.9g/Kg and infected.

after *A. hydrophila* infection, the growth of fish increased steadily. The growth of fish that are belonged to the Pp group had the lowest growth compared to growth of fish in other treatment groups. As the growth rate of the turmeric feeding fish is higher, it is predicted that the fish is fed with turmeric enriched pellets will perform better growth.

Hematological condition

In fish, the function of leucocytes is mainly related to the immune system. Feeding the fish with turmeric enriched pellets in this study aimed to improve the immunity of the fish. The immunity status of the fish was expected based on the leucocyte condition in general. The leucocyte condition of the fish before and after being fed with turmeric-enriched pellets is presented in Table 2.

Data obtained indicate that the number of leucocytes in the turmeric-fed fish and in the fish with no turmeric were different.

Table 2. Leucocyte number in *Clarias batrachus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila* (on day 30).

Treatments	Leucocyte number (x10 ⁴ cells/mm ³)		
	D0	D30	D14inf
P0	8.18	8.37 ± 0.12 ^a	8.59 ± 0.31 ^a
Pp	8.26	8.47 ± 0.15 ^a	9.71 ± 0.35 ^b
P1	8.35	8.87 ± 0.2 ^b	10.28 ± 0.31 ^b
P2	8.43	9.34 ± 0.25 ^c	11.08 ± 0.15 ^c
P3	8.42	9.60 ± 0.15 ^c	11.29 ± 0.45 ^c

Po, control (no turmeric/not infected); Pp, positive control (no turmeric/infected); P1, 0.5g turmeric in 1 Kg feed and infected; P2, 0.7g/Kg and infected; P3, 0.9g/Kg and infected. Day14inf = 14 days after infection with *A. hydrophila*. Mean with standard error followed by different letters are significantly different (P<0.05)

The turmeric-fed fish showed a higher number of leucocytes (2–15×10⁴cells/mm³). Before being feed with turmeric (day 0), the average number of leucocyte was 8.18–8.43×10⁴ cells/mm³ and after being fed with turmeric enriched pellets for 30 days, the leucocytes slightly increased. P3 group fish has the highest number of leucocytes at day 30 (9.60×10⁴ cells/mm³).

After being infected, the number of leucocytes in the fish of each treatment increased due to infectious agents. The leucocyte of the P0 group is steady as the fish were not infected. The highest number of leucocytes was again seen in group P3 (11.29×10⁴cells/mm³), while the lowest was in group Pp (9.71×10⁴cells/mm³) (Figure 3). These data suggest that the provision of turmeric increased the leucocyte number, before as well as after being infected with *A. hydrophila*.

The population of each leucocyte cell type in the treated fish are presented in Table 3. The composition of leucocyte types in all treated fish showed similar patterns. Lymphocyte were around 70% of the total population. From day 0 to the 30th day, the fish were fed on turmeric and it is clear that in turmeric fed fish the lymphocyte proportion is significantly higher than that of fish that was not fed with turmeric enriched pellets. After being infected with *A. hydrophila*, the lymphocyte of the turmeric fed fish decreased slightly (around 80%), but it was significantly higher than that of fish that was not fed with turmeric, which was around 72%. On the other hand, the proportion of monocyte and neutrophil in all fishes treated were increased. The proportion of white blood cells of *C. batrachus* treated with turmeric enriched pellets is presented in Table 3, while the white cell types of *C. batrachus* is presented in Figure 4.

Discussion

In general, the survival of the fish treated with turmeric-enriched pellets in this study varied. Survival of the control positive fish (infected with *A. hydrophila* and no turmeric fed) was low (43.33%). The infected fish showed various clinical symptoms of MAS disease, namely ulcers, hemorrhage, pigmentation,

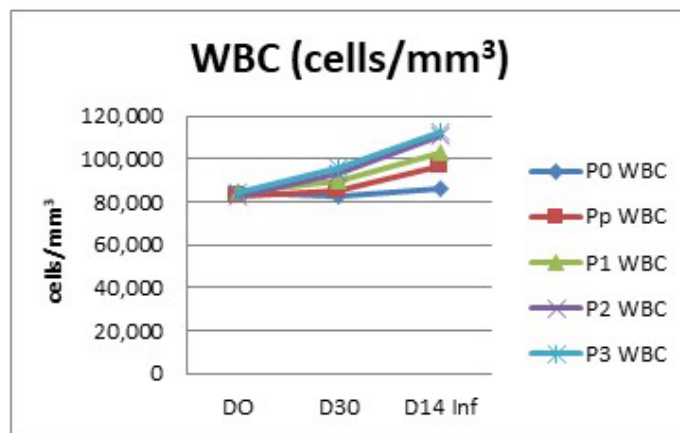


Figure 3. White blood cell count of *Clarias batrachus* fed on turmeric-enriched pellets and infected with *Aeromonas hydrophila* (on day 30). Po, control (no turmeric/not infected); Pp, positive control (no turmeric/infected); P1, 0.5g turmeric in 1 Kg feed and infected; P2, 0.7g/Kg and infected; P3, 0.9g/Kg and infected. Day14inf = 14 days after infection with *A. hydrophila*.

Table 3. The proportion leucocyte cell types in *C. batrachus* treated with turmeric enriched pellets.

Day	Treatments	Leucocyte types		
		Lymphocyte (%)	Monocyte (%)	Neutrophil (%)
D0	P0	72.66	15.66	11.66
	Pp	71.00	14.66	11.66
	P1	71.66	15.66	14.33
	P2	72.33	15.00	12.66
	P3	73.33	15.33	11.33
D30	P0	76.00±2.08 ^a	11.66±1.52 ^b	12.66±0.57 ^c
	Pp	76.00±1.00 ^a	13.66±1.15 ^b	11.66±0.57 ^c
	P1	83.66±1.152 ^b	6.66±1.15 ^a	10.00±1.00 ^b
	P2	86.33±1.52 ^{bc}	7.336±1.52 ^a	7.00±1.00 ^a
	P3	89.00±2.00 ^c	5.33±1.15 ^a	6.33±0.57 ^a
D14 Inf	P0	74.00±1.15 ^b	13.00±1.00 ^c	12.00±1.00 ^c
	Pp	72.00±1.15 ^a	9.66 ± 0.57 ^a	17.66±0,57 ^d
	P1	78.66±1.52 ^c	10.33±0.57 ^{ab}	10.33±0.57 ^b
	P2	82.33±1.52 ^d	11.00 ± 1.00 ^{ab}	7.66±0.57 ^a
	P3	79,66±1.52 ^c	12.00±1.00 ^{bc}	7.00±1.00 ^a

Po, control (no turmeric/not infected); Pp, positive control (no turmeric/infected); P1, 0.5g turmeric in 1 Kg feed and infected; P2, 0.7g/Kg and infected; P3, 0.9g/Kg and infected. Day14inf = 14 days after infection with *A. hydrophila* or 45 day after being treated with turmeric. Mean with standard error followed by different letters are significantly different (P<0.05).

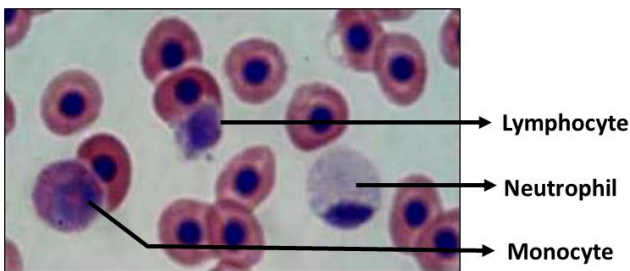


Figure 4. Leucocyte types in *Clarias batrachus* fed on turmeric-enriched pellets.

swollen abdomen and eroded fins. Similar clinical signs have been found in common carp that suffer from MAS disease⁶⁻⁸. In addition, fish infected with 1.8×10^8 CPU/ml of *A. hydrophila* die between 8 and 24 h and show alterations in behavior, which are not observed in control fish^{9,10}.

In this study, the growth of fish that were fed with turmeric-enriched pellets was higher than the growth of the control

positive fish. This suggests that turmeric improves the growth of fish, as shown by the increase in TL as well as BW. As the fish in the control positive group did not receive turmeric, their feeding appetite may be lower than the turmeric-fed fishes and this is reflected in their growth rate. The infection of *A. hydrophila* may worsen the health of the fish in general as their immunity is not being boosted by the turmeric and as a consequence, by the end of experiment, the BW of fish in Pp group is the lowest.

The fish that were treated with turmeric revealed better growth than that of the fish that do not receive any turmeric. This fact suggests that turmeric improves the growth of the fish. Curcumin supplementation has been shown to improve growth and feed appetite in *Nile tilapia*¹¹. Fish fed with feed enriched with curcumin exhibited enhanced antioxidant status and immune responses, and tilapia fed with curcumin supplemented diets had highest post-challenge survival rate¹². The higher curcumin content in fish feed resulted in a higher growth rate, as turmeric acts as a antibacterial, anti-inflammatory and antiviral agent¹³. Curcumin has been shown to improve the immunity of fish and acts as a defense agent to combat the *A. hydrophila* infection¹⁴.

Curcumin is a strong antioxidant and acts as anti-free radical that negatively affects the physiological process of the fish². Turmeric contains curcumin, an active compound that is able to improve immunity as well as increases the appetite of the fish toward feed provided¹. As the turmeric-fed fish had a strong immunity and good appetite, they grew better than the fish with non-enriched pellets. In our study, after being infected on the 30th day, the turmeric-fed fish was able to cope with the *A. hydrophila* attack and continue to grow.

In our study, after being infected, the number of leucocytes in the fish of each treatment group increased due to presence of an infectious agent. Among the infected fish, the increment of leucocyte number in the control positive group was the lowest, as most leucocytes are transferred to the infected area and leucocytes in blood vessels are reduced¹⁵. On the other hand, the fish provided with turmeric had higher leucocyte numbers, which means that they are better at facing the infection, as leucocytes act as non-specific defense agents that are able to localize and eliminate pathogens. The immunogenic agents in natural remedies may trigger the increment of leucocytes in general¹⁵. Increasing leucocyte numbers indicate that cellular immunity (non-specific immunity) of the fish is good¹⁶.

Blood condition of fish before being treated with turmeric (D0) was not different. All of them show normal condition, with around 72% lymphocyte, 15% monocyte and 13% neutrophil. Utami *et al.*, (2013) stated that the blood condition in normal fish contain of lymphocyte 68–86%, monocyte 3.9–15.9% and neutrophil 10–18.1%. After being treated with turmeric enriched pellets, however, there was significant difference in the blood condition. The lymphocyte of the turmeric fed fishes was significantly higher than those of fish that was not fed with turmeric. In the P1, P2 and P3 the lymphocyte increased, ranged from 78.66% to 82.33% by the 30th day, while that of the non-turmeric fed fish, the lymphocyte was around 74%. In contrast the monocyte of the turmeric fed fishes was lower than that of the non-turmeric fed fishes. The highness proportion of lymphocyte in the turmeric fed fishes indicate that the antibody of those fish was increased. Hardi (2015) stated that the increment of lymphocyte will result in increasing the antibody. Even though the blood condition of all fishes used in this study was various, the leucocyte cells proportion can be categorized as normal.

After being infected with *A. hydrophila*, all fishes shown infection symptoms. By the 14th day after the infection, the blood condition of the fish was tested. There was significant different among the non-turmeric and the turmeric treated fishes. The lymphocyte of fish that was fed with no turmeric fed shown lower lymphocyte proportion, but they had higher monocyte and neutrophil proportion. While in the turmeric fed fishes, the P2 show the highest lymphocyte proportion, which was around 82%. Data obtained in this study proved that the immune system of the turmeric fed fishes was better than that of the fish with no turmeric fed. Lymphocyte plays as important role in the immunity system of the fish in general¹⁵. The improvement in leucocyte cells reflects the immunity system ability in developing the non-specific defense to face pathogen¹⁶.

Improvement of the immune system can be studied based on leucocyte cell type composition. In this study, the leucocytes of the control positive group was relatively low. A decrease of lymphocytes on the 14th day after infection indicated that antibodies were formed to fight *A. hydrophila*. The fight may reduce lymphocyte cell numbers, as the lymphocyte in the peripheries are allocated to the infected area¹⁷. Even though the lymphocytes in P1, P2 and P3 groups decreased after infection, their amount remained in the normal range. The ability of the fish to maintain the amount of lymphocytes may be caused by the presence of curcumin, which has been shown to trigger the formation of those cells¹⁸.

The presence of pathogens in the fish may trigger monocyte cells to regenerate. If the immunity of the fish is good, phagocytosis succeeds and the pathogen is defeated. The fish becomes healthy and the monocyte number increases to a normal range. The production of antibodies is crucial for the immune response¹². The monocyte percentage of *N. tilapia* fish ranges between 17 and 25% when present in freshwater¹². In less healthy fish, immunity is low, and monocyte action may not succeed. As result many monocyte cells die, the monocyte number decreases and the pathogen thrives. Monocytes or macrophages are able to phagocyte any pathogens, and if there is infection monocytes will move to the infected area¹⁹. A decrease in monocytes may be caused by an increase in lymphocyte amount that produce antibodies; therefore leading to an obstruction of monocyte production. The pathogen present may disrupt the fish physiology and clinical signs of diseases occur. In this research, the clinical sign of MAS disease were present in the control positive group. This fact indicated that the fish with no turmeric fed had low immunity. In fish that were fed with turmeric (P1, P2 and P3) the number of monocytes was in the normal range. The condition of the fish in general showed few clinical symptoms of MAS disease. This fact indicates that the provision of turmeric is able to improve the immune system in fish, and fish in all treatment are able to defeat *A. hydrophila* infection.

Among the turmeric-fed fish, the P3 group showed the fewest clinical symptoms of MAS disease. This may be due to the best immune performance in P3 fish, as they had the highest number of lymphocytes (up to 80%). Based on data obtained, it can be concluded that the fish feed with 0.9g/Kg pellets provide the best result to improve fish immunity to fight *A. hydrophila* infection.

The lymphocyte level in this study ranged between 60–70%. The lymphocyte level of the freshwater fish ranged between 42 and 51%. The amount of lymphocytes could increase during stress. Stress in fish may interfere with non-specific immune responses, such as lymphocyte proliferation (increase in cell amount and alterations in T and B cells). Leukocyte increase is related to the decrease in cortisol levels in the body. When cortisol level decreased, DNA synthesis of the lymphocyte cells occurred and led to the high amount of lymphocytes²⁰.

In this research, the water quality is maintained. The water quality in general is slightly fluctuated. The temperature

ranged from 26.5 to 27.6°C, DO was 6.4 to 7.6 mg/L, pH was 6 to 7 and NH₃ was 0.05 to 0.07 mg/L. These data shown that the water quality during the research was normal and may support the life of the fish.

Conclusions

Feeding *C. batrachus* with turmeric-enriched pellets is effective in improving the immune system of the fish. The number of lymphocytes maintained a normal range even though the fish were infected with *A. hydrophila*. The clinical signs of MAS disease were fewer lighter in fish that were fed with turmeric, and the most effective dose of turmeric for improving the immune system of the fish was shown to be 0.9g/Kg of pellets.

Data availability

Underlying data

Figshare: Survival Rate, TL and Growth, <https://doi.org/10.6084/m9.figshare.13606265.v1>²¹.

This project contains the following underlying data:

- Survival rates for all aquaria (n=15),
- Clinical signs of MAS for all aquaria (n=15),
- Body weight of all fish (n=15),
- Total length of all fish (n=15),
- Number of leucocytes in all fish (n=15),
- Number of lymphocytes, monocytes, neutrophils, thrombocytes in all fish (n=15).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

Acknowledgements

The authors thank the students who supported the research.

References

1. Sahu S, Das BK, Mishra BK, *et al.*: **Effect of dietary *Curcuma longa* on enzymatic and immunological profiles of rohu, *Labeo rohita* (Ham.), infected with *Aeromonas hydrophila*.** *Aquac Res.* 2008; **39**(16): 1720–1730. [Publisher Full Text](#)
2. Nagpal M, Sood S: **Role of curcumin in systemic and oral health: An overview.** *J Nat Sci Biol Med.* 2013; **4**(1): 3–7. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Blaxhall PC, Daisley KW: **Routine haematological methods for use with fish blood.** *J Fish Biol.* 1973; **5**(6): 771–781. [Publisher Full Text](#)
4. Anderson DP, Siwicki AK: **Simplified assays for measuring nonspecific defense mechanisms in fish.** Seattle, WA: Fish Health Section/American Fisheries Society Meeting. 1994; 26–35.
5. Patil PJ, Thakare GV, Patil SP: **Variability and accuracy of Sahli's method in estimation of haemoglobin concentration.** *Natl J Integr Res Med.* 2013; **4**(1): 38–44. [Reference Source](#)
6. Saharia P, Pokhrel H, Kalita B, *et al.*: **Histopathological changes in Indian Major Carp, *Labeo rohita* (Hamilton), experimentally infected with *Aeromonas hydrophila* associated with hemorrhagic septicemia of Central Brahmaputra valley of Assam, India.** *J Entomol Zool Stud.* 2018; **6**(5): 6–11. [Reference Source](#)
7. Song X, Zhao J, Bo Y, *et al.*: ***Aeromonas hydrophila* induces intestinal inflammation in grass carp (*Ctenopharyngodon idella*): an experimental model.** *Aquaculture.* Elsevier, 2014; **434**: 171–178. [Publisher Full Text](#)
8. Sarker J, Faruk M: **Experimental infection of *Aeromonas hydrophila* in pangasius.** *Progress Agric.* 2016; **27**(3): 392–399. [Publisher Full Text](#)
9. Dias MKR, Sampaio LS, Proietti-Junior AA, *et al.*: **Lethal dose and clinical signs of *Aeromonas hydrophila* in *Arapaima gigas* (Arapaimidae), the giant fish from Amazon.** *Vet Microbiol.* 2016; **188**: 12–15. [PubMed Abstract](#) | [Publisher Full Text](#)
10. Van Doan H, Doolgindachabaporn S, Pensee AS, *et al.*: **The LD50 of Asian Catfish (*Pangasius bocourti*, Sauvage 1870) challenge to pathogen *Aeromonas hydrophila* FW52 strain.** Accessed: Nov. 26, 2020. [Reference Source](#)
11. Hassan S, Abdel-Rahman M, Mansour ES, *et al.*: **Prevalence and Antibiotic Susceptibility of Bacterial Pathogens Implicating the Mortality of Cultured Nile Tilapia, *Oreochromis niloticus*.** *Egypt J Aquac.* 2020; **10**(1): 23–43. [Publisher Full Text](#)
12. Rahman AA, El-Bouhy Z, Wahbah M, *et al.*: **Effects of dietary turmeric and clove powder on growth and immune response of the Nile tilapia.** *Egypt J Aquat Biol Fish.* 2020; **24**(5): 589–608. [Publisher Full Text](#)
13. Khalil F, Emeash H: **Behavior and Stereotypies of Nile Tilapia (*Oreochromis niloticus*) in Response to Experimental Infection with *Aeromonas hydrophila*.** *Aquat Sci Eng.* 2018; **33**(4): 124–130. [Publisher Full Text](#)
14. Riauwati M, Sari TEY, Adelina A: **Potency of Turmeric in Reducing Motile *Aeromonas Septicaemia* (Mas) in *Pangasius hypophthalmus*.** *IOP Conf Ser Earth Environ Sci.* 2020; **430**(1): 012025. [Publisher Full Text](#)
15. Parrino V, Cappello T, Costa G, *et al.*: **Comparative study of haematology of two teleost fish (*Mugil cephalus* and *Carassius auratus*) from different environments and feeding habits.** *Eur Zool J.* 2018; **85**(1): 193–199. [Publisher Full Text](#)
16. Hoseinifard SH, Zoheiri F, Dadar M, *et al.*: **Dietary galactooligosaccharide elicits positive effects on non-specific immune parameters and growth performance in Caspian white fish (*Rutilus frisii kutum*) fry.** *Fish Shellfish Immunol.* 2016; **56**: 467–472. [PubMed Abstract](#) | [Publisher Full Text](#)
17. Dawood MAO, Abdel-Kader MF, Moustafa EM, *et al.*: **Growth performance and hemato-immunological responses of Nile tilapia (*Oreochromis niloticus*) exposed to deltamethrin and fed immunobiotics.** *Environ Sci Pollut Res Int.* 2020; **27**(11): 11608–11617. [PubMed Abstract](#) | [Publisher Full Text](#)
18. Riauwati M, Windarti W, Sari TEY, *et al.*: **Blood condition of *Pangasius hypophthalmus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila*.** *IOP Conf Ser Earth Environ Sci.* 2019; **348**(1): 012036. [Publisher Full Text](#)
19. Riauwati M, Windarti W, Sari TEY, *et al.*: **Blood condition of *Pangasius hypophthalmus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila*.** *IOP Conf Ser Earth Environ Sci.* 2019; **348**(1): 012036. [Publisher Full Text](#)
20. Fazio F: **Fish hematology analysis as an important tool of aquaculture: a review.** *Aquaculture.* 2019; **500**: 237–242. [Publisher Full Text](#)
21. Riauwati M, Siregar YI, Mulyani I: **Survival Rate, TL and Growth.** *figshare.* Dataset. 2021. <http://www.doi.org/10.6084/m9.figshare.13606265.v1>

Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 09 June 2021

<https://doi.org/10.5256/f1000research.56750.r85485>

© 2021 Zakaria I. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Indra Junaidi Zakaria 

Department of Biology, Universitas Andalas, Padang, Indonesia

Okay.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquaculture (marine and freshwater), Ichthyology, Fish Biology, Marine Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 09 June 2021

<https://doi.org/10.5256/f1000research.56750.r85484>

© 2021 Caipang C. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Christopher Marlowe A. Caipang 

College of Liberal Arts, Sciences, and Education, University of San Agustin, Iloilo City, Philippines

The authors have addressed the comments and suggestions that were made in the original submission.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: fish health management, aquatic biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 22 March 2021

<https://doi.org/10.5256/f1000research.31256.r80815>

© 2021 Zakaria I. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Indra Junaidi Zakaria 

Department of Biology, Universitas Andalas, Padang, Indonesia

- What about the influence of the water qualities in which the fish were kept during the experiment? Such as temperature, pH, DO, BOD, ammonia and phosphate and otherwise; is there no data? The health of the fish during cultivation also depends on the water quality. Or you should make the statement that the aquatic environment has no effect.
- On the tree treatment of adding turmeric to the pellets, it was seen that up to the day of observation, the movement of the graph was almost the same, which was the same as upward. Will this be valid for how many days after that?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquaculture (marine and freshwater), Ichthyology, Fish Biology, Marine Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Apr 2021

Morina Riauwaty, Universitas Riau, Pekanbaru, Indonesia

- What about the influence of the water qualities in which the fish were kept during the experiment? Such as temperature, pH, DO, BOD, ammonia and phosphate and otherwise; is there no data? The health of the fish during cultivation also depends on the water quality. Or you should make the statement that the aquatic environment has no effect.

OK, the water quality data has been provided and the relation between water quality and the fish health has been explained.

- On the tree treatment of adding turmeric to the pellets, it was seen that up to the day of observation, the movement of the graph was almost the same, which was the same as upward. Will this be valid for how many days after that?

I have explained that the growth rate of the turmeric feeding fish is higher and the graph is valid as long as the fish is fed with turmeric enriched pellets.

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 March 2021

<https://doi.org/10.5256/f1000research.31256.r80812>

© 2021 Caipang C. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Christopher Marlowe A. Caipang 

College of Liberal Arts, Sciences, and Education, University of San Agustin, Iloilo City, Philippines

The present study describes the use of turmeric in improving immunity of *Clarias batrachus* against infection with MAS. Results showed incorporation of 9g turmeric powder per kilogram of feeds improved immunity of the fish.

The results presented are straightforward. However, the manuscript needs improvement on the following aspects, which I hope the authors would consider:

1. The authors mentioned that turmeric causes stress when fish are immersed in a solution containing that rhizome. Please cite studies that turmeric is beneficial to fish when incorporated in the diets.

2. The authors claimed that feeding fish with turmeric-enriched diets has not been studied. I suggest that the authors make a thorough literature search to indeed claim that no studies have done so.
3. In the preparation of the enriched diets, there was no mention how turmeric powder was incorporated in the feed pellets. Based from the manuscript, the turmeric powder was just mixed with the feed pellets. If this the case, upon feeding the turmeric powder would immediately leach out from the feeds and thus may not be consumed by the fish.
4. For the blood sampling, only 3 fish per treatment was used and there were 3 replicate tanks per treatment with 10 fish in each replicate. So this means that only 1 fish was taken per replicate tank to make up for 3 fish/treatment? Please explain.
5. Were the same fish used for blood sampling at initial, day 30 of feeding and at 14th day of infection?
6. Please indicate the statistical tool that was used.
7. As all fish survived the challenge, then there is no need for Table 1. The authors can mention in the manuscript that all fish survived the experimental infection.
8. I suggest to combine Table 2 and Fig 1, as well as Table 3 and Fig 2 as these provide same information.
9. If the authors can show photos of the WBC for catfish that would add more strength to the manuscript, as the morphology of leukocytes in fish varies. This would be helpful to other researchers who would be working similar studies in the future as they can refer to the photos of the different leukocytes from this manuscript.
10. I suggest to revise Fig 4 to make the data presentation clear. The trend of each leukocyte type in the different treatments must be compared statistically rather than presenting the leukocyte populations of every treatment as what the authors presented in the present manuscript.

I hope that the authors will incorporate these comments/suggestions when they revise their manuscript.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: fish health management, aquatic biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Apr 2021

Morina Riauaty, Universitas Riau, Pekanbaru, Indonesia

The present study describes the use of turmeric in improving immunity of *Clarias batrachus* against infection with MAS. Results showed incorporation of 9 g turmeric powder per kilogram of feeds improved immunity of the fish.

The results presented are straightforward. However, the manuscript needs improvement on the following aspects, which I hope the authors would consider:

1. The authors mentioned that turmeric causes stress when fish are immersed in a solution containing that rhizome. Please cite studies that turmeric is beneficial to fish when incorporated in the diets.

I have added the literature on the turmeric beneficial in culturing the fish.

2. The authors claimed that feeding fish with turmeric-enriched diets has not been studied. I suggest that the authors make a thorough literature search to indeed claim that no studies have done so.

I have added the literature on the use of turmeric for feeding fish.

3. In the preparation of the enriched diets, there was no mention how turmeric powder was incorporated in the feed pellets. Based from the manuscript, the turmeric powder was just mixed with the feed pellets. If this the case, upon feeding the turmeric powder would immediately leach out from the feeds and thus may not be consumed by the fish.

I have explained that the turmeric powder was mixed with water and the powder was stuck in the pellet granule. The powder was swallowed when the fish was eating the pellets.

4. For the blood sampling, only 3 fish per treatment was used and there were 3 replicate tanks per treatment with 10 fish in each replicate. So this means that only 1 fish was taken per replicate tank to make up for 3 fish/treatment? Please explain.

I have added information on the blood sampling. In this research there were 5 treatments and in each treatment there were 3 replications (in 3 aquaria). For blood sampling, 3 fishes were taken from each aquarium, it means that there were 9 fishes/ treatment or total 45 fishes were used for blood study.

5. Were the same fish used for blood sampling at initial, day 30 of feeding and at 14th day of infection?

The fish for blood sampling were returned to each aquarium and for the next sampling the fish was taken randomly. It means that the sampled fish might be accidentally captured for the next samplings.

6. Please indicate the statistical tool that was used.

I have added the statistical tool that was used.

7. As all fish survived the challenge, then there is no need for Table 1. The authors can mention in the manuscript that all fish survived the experimental infection.

I have removed Table 1 and explained the survival information in manuscript.

8. Suggest to combine Table 2 and Fig 1, as well as Table 3 and Fig 2 as these provide same information.

OK, I have combined Table 2 and Fig 1, as well as Table 3 and Fig 2.

9. If the authors can show photos of the WBC for catfish that would add more strength to the manuscript, as the morphology of leukocytes in fish varies. This would be helpful to other researchers who would be working similar studies in the future as they can refer to the photos of the different leukocytes from this manuscript.

I have added the photo of the WBC of catfish.

10. I suggest to revise Fig 4 to make the data presentation clear. The trend of each leukocyte type in the different treatments must be compared statistically rather than presenting the leukocyte populations of every treatment as what the authors presented in the present manuscript.

OK, I have revised Fig 4.

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research