journal homepage: www.pktm.my

International Journal of Technical Vocational and Engineering Technology

e-SSN2710-7094, Vol. 5, No. 1 (2024)

Survival Response of African Catfish (Clarias Gariepinus) On Bitter Bytes Dietary Against Aeromonas Hydrophila

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ARTICLE INFO

Article History:
Received 31 January 2024
Revised 10 May 2024
Accepted 10 June 2024

Keywords:
Aeromnas Hydrophila;
African Catfish;
Bitter Bytes.

ABSTRACT

Aeromonas hydrophila is widely known as one of the common bacteria species in freshwater habitats and occasionally been recognized as a fish pathogen which lead to disease and cause economic losses for African catfish (*Clarias gariepinus*) industry. Antibiotic, vaccine, and several chemical drug therapies used in aquaculture resulted in resistant bacterial strains as well as created unsafe condition to fish and environment. This study was investigated the efficiency of Bitter Bytes as an alternative formulated pellet in treating bacterial disease of African catfish. African catfish juvenile (n=300) was fed twice daily for 8 weeks with different inclusion level of bitter leaf in Bitter Bytes diet (T1=0gkg^{-1} (control), T2=10gkg^{-1}, T3=20gkg^{-1}, T4=30 gkg^{-1}). After 8 weeks of post feeding, 20 fish in each treatment group (T1, T2, T3, T4) were randomly selected for *A. hydrophila* bacterial challenge (10^{8} cell/ml). The survival response of African catfish was observed until 12 days of infection. The result demonstrated, African catfish were fed with 10g of bitter leaf inclusion level (T2) showed the higher survival rate as compared to the other treatment group (T1, T3 and T4). Moreover, African catfish were fed with bitter leaf diet (T2, T3, T4) show poor clinical sign after infected with *A. hydrophila* as compared to control group (T1, 0g). The result indicated the application of bitter leaf in Bitter Bytes diet capable to enhance disease resistant of fish and can act as immunostimulants.

1.0 Introduction

Aquaculture has recently been recognized as a potential fisheries sector that can contribute significantly to the country's future fish requirement. According to Dauda et al., (2018) aquaculture contributes a total of 44.14% which is 73.8 million tonnes from the total of fish global production which is 167.2 million tonnes. African catfish (*Clarias gariepinus*) is one of the common species that have a commercial value in aquaculture because it has ability to growth fast, can fed in large variety of agriculture by product and high disease resistant (Eirna-Liza et al., 2018). Ibrahim et al., (2020) reported that African catfish became the highest produced finfish cultured and contribute about 10% of freshwater aquaculture production compared to red tilapia and other species. However, in recent time, African catfish was reported to be infected by bacteria disease.

According to Adah et al., (2024) stated that African catfish in Malaysia commonly infected by bacteria *Aeromonas hydrophila.*

In this respect, vaccines, chemical agents, and antibiotic are being used to treat infectious diseases of fish which can affect the huge economic loss in industry. However, vaccines are expensive, not available everywhere and not efficient against various type of disease (Ibrahim et al., 2020). Aquaculturist hence, has started to find other approaches to prevent and treat disease. Since chemical agents are reported as unsafe to use due its side effects, researcher had shifted their focus to use alternative source such as natural herbs to treat and control fish diseases which is cheap, available in large quantity and safe to fish and environment. Therefore, this study has been done to investigate the efficiency of natural feed additives (bitter leaf) that include in different inclusion in formulate feed Bitter Bytes diet for African catfish against *A. hydrophila* infection.

2.0 Literature review

2.1 Chemical and drug therapies usage in Aquaculture

Oxytetracycline and sulfamethoxine were reported to be effective in treating of bacteria disease in fish which is *A. hydrophila* (Smith, 2008). In other hand, formalin, Dipterex, chroloramphenicol and terramycin having a great potential in prevention of parasites diseases by reducing the number of parasite and promote to minimize of fish mortality (Bondad-Reantaso et al., 2005). There are only limited chemical agents that allowed to be used in aquaculture practise. Some of chemical agent needed of approval by government caused of negative effect to fish and environment. According to Williams and Lloyd (2012), Canada, Norway and the United State are needed permit of oxytetracycline and flofenicol usage in aquaculture. Moreover, Rodgers and Furones (2009) stated that Norway also required permitting of quinolones application in farming practise. In contrast, certain country not only allows usage of chemical drugs in aquaculture sector but also, not required to permits. Therefore, several chemical drugs such as oxytetracycline, flofenicol and amoxicillin are allowed to use in countries where is less stringent or lacking in controlling of chemical and drug application in aquaculture.

2.1 Natural herbs as alternative antimicrobial in aquaculture

Antimicrobial is defined as agents that inhibiting the growth or kills microorganism (Cabello, 2013). In recent time, several researchers were found the alternative method to treat diseases outbreak in aquatic animal using natural herbs application. They trusted that natural herbs have their own active substance that could be used to inhibit bacterial and parasite infection as well as promoted fish growth then increase the production. Referring to Cristae et al., (2012), herbs plant has natural compound whenever embedded into feed which enhanced animal productivity. Many plant products such as Aloe vera, onion, ginger, garlic, thyme, neem, peppermint, and rosemary were reported to be used as immunity stimulation and growth promoter in aquaculture. According to Olusola and Nwokike, (2018) proved that leaves extracts of bitter (*Vernonia amygdalina*) and pawpaw (*Carica papaya*) enhance the growth of juvile African catfish as well as immunity resistance against bacterial infection. Moreover, Harikrishnan et al., (2010) suggested that lymphocystis disease virus (LDV) infection inhibited by intraperitoneal administration of *P. granatim* leaf extracts at 50 or 100mg/kg. Therefore, it has been shown that herbal plants lead to reduced risk of fish disease as well as environmental pollution.

3.0 Methodology

3.1 Experimental Fish

African Catfish fingerlings with fingerlings size average length of 5 ± 2cm and weight of 1.8 ± 0.2 g were selected for this study. The fish were acclimated for a week under aerated conditions at 28 ± 1.5 ^{o} C. Fish were feed with a commercial catfish with 3% of body weight percentage twice daily during acclimatized period which they were randomly assigned to four treatments.

3.2 Experimental diet

The dried leaves leaf was bought from local by online shop (Shoppe) and were grinded into fine powder using heavy duty blender and stored in an air-tight plastic container. The fine powder bitter leaf was incorporated into basal diet at different inclusion level; $10gkg^{-1}$, $20gkg^{-1}$, and $30gkg^{-1}$. No inclusion of bitter leaf treated as control (0g). A total of 300 ml water added to the dry ingredients and mixed thoroughly using mixer (Artisan – Kitchen mixer D300T) for 20 min at low speed until it became dough. The dough was further made into pellet using pellet machine (TJS22 Dual- Purpose machine) and dried using oven at 60°C for 24 hr. The modified feeds (Bitter Bytes) were stored in screw cap bottle at room temperature for further use.

3.3 Experimental design and feeding trial

African catfish fingerlings (n=300) randomly divided into four groups (refer Table 1) in the aquarium after acclimatized period. Each group consisted of 25 fingerlings with three replicates. Each aquarium supplied with continuous aeration. Group T1 feed with basal diet without inclusion of bitter leaf as the control (0 g). The remaining groups feed with experimental diet with inclusion of bitter leaf at $10gkg^{-1}$ (T2), 20 gkg^{-1} (T3) and $30gkg^{-1}$ (T4). The fish will feed twice daily at 09:00 and 18:00 hr 3% of body weight for 8 weeks (60 days).

Table 1. Experimental groups of African catfish feed with different inclusion level of bitter leaf in formulated diet Bitter Bytes

Serial No.	Treatments
T1 (Control)	No inclusion of bitter leaf
T2 (BL 10g)	Inclusion of bitter leaf at 10gkg-1
T3 (BL 20g)	Inclusion of bitter leaf at 20gkg-1
T4 (BL 30g)	Inclusion of bitter leaf at 30gkg-1

3.4 Experimental challenge

After post feeding trial (8weeks), 10 fish from each treatment group (refer Table 1) with average length of 15 ± 2cm and weight of 12 ± 2g were randomly selected in preparation for the bacterial challenge. The fish was placed in a new glass aquarium and each treatment group was done in triplicated. The fish were injected with 0.1 mL of *A. hydrophila* by intramuscular injection (IM) at concentration of 10^8 $cellmL^{-1}$. Clinical sign of African catfish after challenge with *A. hydrophila* was observed and recorded until 12 days.

3.5 Cumulative mortality and relative survival (RPS)

The cumulative mortality of each treatment was recorded daily until at the end of the experiment (12 days). The relative percent survival was calculated based on the following equation (refer with equation labelling [1]):

$$RPS = (1 - \text{treatment mortality} / \text{control mortality}) \times 100 \quad [1]$$

3.6 Statistical analysis

The data obtained from this study which is cumulative mortality and relative survival (RPS) were subjected to statistical analyses of mean by using Ms Excel and presents in table. While observation data of clinical and physical sign was summarised and presented in table.

4.0 Discussion of analysis and findings

4.1 Relatively percentage survival (RPS)

The results of fish resistance to the bacteria disease *A. hydrophila* showed that the bitter leaf extracts significantly affected the survival rate of the African catfish fingerling. The first mortality was occurred on day 1 after infected with *A. hydrophila* for control group (T1, 0%). By the first post injection, fish with no inclusion of bitter leaf (T1, control) were observed to be stagnant and reluctant to eat. Fish were observed to swim closer to the surface and aquarium wall. On the second day, a mild hemorrhage of the skin was identified. The ulceration of the skin and inflammation observed were obvious after a few days. The highest total of mortality was observed in control group (T1) with 8 fish followed by treatment T4 (BL 30g) and T3 (BL 20g) with 6 and 4 fishes respectively. The lowest number of total mortalities was recorded in treatment T2 (BL 10g) where only two fish were dead. Meanwhile, the results of RPS (Table 2) showed that the African catfish fed on diet with 10g of bitter leaf (T2) inclusion level had the highest percentage (75%) followed by 20g of bitter leaf (T3) with 50% and 30g of bitter leaf inclusion level (T4) was 25%.

Table 2: Relative percentage survival of African catfish fed with different inclusion level of bitter leaf in Bitter Bytes diet after challenged with Aeromonas hydrophila

	TREATMENT			
RPS %	T1 (Control)	T2 (10g)	T3 (20g)	T4 (30g)
	0	75	50	25

**Control (T1): no inclusion level of bitter leaf; T2, T3 and T4: inclusion of bitter leaf at 10, 20, 30 gkg^{-1} in Bitter Bytes diet, respectively.

4.2 Clinical and physical signs of African catfish after challenges with *Aeromonas hydrophila*

The summary of clinical physical signs of African catfish after challenges with *A. hydrophila* was completely defined in Table 3. The control group (T1, 0g) showed signs of swimming abnormalities, skin discoloration also has hemorrhage and ulcer with inflammation at the fish skin. These all signs were observed at all duration times which were day 3, 6, 9 and 12 after challenge. Interestingly, the treatment group with inclusion of 10g bitter leaf (T2) showed no ulcer with inflammation, hemorrhage skin as well as skin discoloration and swimming abnormalities at all duration times. While treatment T3 (20g) and T4 (30g) showed the sign of mild ulceration at day 9 and 12 after challenge.

Table 3: Summary of clinical and physical sign of the African catfish fed with different inclusion level of bitter leaf in Bitter Bytes diet after challenge of 10^8 of *Aeromonas hydrophila*.

Days	Treatment	Ulcer with inflammation	Hemorrhage skin	Skin discoloration	Swimming abnormalities
3rd	T1 (Control)	+	+	+	+
	T2 (10g)	-	-	-	-
	T3 (20g)	-	-	-	-
	T4 (30g)	-	-	-	-
6th	T1 (Control)	++	++	++	++
	T2 (10g)	-	-	-	-
	T3 (20g)	-	-	-	-
	T4 (30g)	-	-	-	-
9th	T1 (Control)	++	++	+++	+++
	T2 (10g)	-	-	-	-
	T3 (20g)	+	+	-	-
	T4 (30g)	+	+	-	-
12th	T1 (Control)	+++	++	+++	+++
	T2 (10g)	-	-	-	-
	T3 (20g)	+	+	-	-
	T4 (30g)	+	+	+	+

**Control: no inclusion of bitter leaf; T2: inclusion of bitter leaf at 10 gkg-1 Bitter Bytes diet, T3: inclusion of bitter leaf at 20 gkg-1 Bitter Bytes diet and T4: inclusion of bitter leaf at 30 gkg-1 Bitter Bytes diet respectively. Data were shown (-) none, (+) Mild, (++) Moderate, (+++) Severe.

According to the result, treatment T2 (10g) showed the highest percent of relatively percentage survival (RPS) and showed no clinical and physical signs after challenges with *A. hydrophila*. During the feeding trial for 8 weeks, the formulate Bitter Bytes diet with inclusion of 10g of bitter leaf (T2) was well consumed by the fishes as compared to the T3 (20g) and T4 (30g). The feed intake reducing might be the bitter leaf has bitter taste when it was used in higher inclusion level. In this study, it could be confirmed that higher disease resistance in treatment group (T2, 10g) as compared to the control group could be due to the presence of bioactive compound which is flavonoid, saponins, tannins and alkaloid in bitter leaf (*V. amygdalina)* that helps in inhibiting the pathogen activity (Kafri et al., 2021). This result was supported by previous study that has been done by Abarike et al., (2022) that mentioned a blend of guava, bitter leaf and neem leaf extract helps in inhibiting *Streptococcus* and *Aeromonas* bacteria growth toward Nile tilapia as compared to control group.

5.0 Conclusion and future research

Aquaculture sector not only being the fish protein sources contributor, but it also provided a great job opportunity to Malaysian. Unfortunately, intensification of fish culture has led to disease problems, where bacterial disease was detected as the main problems that happened in local fish farmers. Diseases have resulted in economic losses to aquaculture farmers. The improvement of the immune system of fish is considered to be the most significant method of preventing fish diseases in aquaculture. The used of traditional herbs as alternative antimicrobial might be eco – friendly as biodegradable. In addition, herbs plants also an inexpensive source of antimicrobial and safe to use better than other chemical and drug agents (Punitha et al., 2008). Shankar and Kiran (2013) stated that plant herbs generally produce active substances such as alkanoid, allicin, tannins and flavonoids which provide and important source of hampering of pathogens. Besides, another further *in vitro* as well *in vivo* studies in needed to determine the efficiency of Bitter Bytes formulated diet in fish growth performance and other disease pathogens infection as well as bacterial infection.

Acknowledgements

Deepest gratitude to Politeknik Sandakan Sabah and University College Agroscience Malysia (UCAM) for cooperation in completing this research. And special acknowledgement to those who are involved directly and indirectly in producing this research.

Author Contributions

Eirna-Liza: Conceptualization, Methodology, Writing- Original Draft Preparation; **Mimi Fadzlin**: Data Curation, Writing-Reviewing and Editing.

Conflicts of Interest

The manuscript has not been published elsewhere and is not under consideration by other journals. All authors have approved the review, agree with its Submission and declare no conflict of interest in the manuscript.

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