Effect of Dietary *Persea americana* on the Organosomatic Indices, Diseases Resistance and Liver Histopathlology of *Klebsiella pneumonia* Infected *Clarias gariepinu*s

Abstract

The experiment was carried out to evaluate the prophylactic efficiency of dietary powdered *Persea americana* leaves on the organosomatic indices, disease resistance and liver histopathology of *Klebsiella pneumonia* infected *Clarias gariepinus*. Five (5) different isonitrogeneous diets with varying percentages of *P. americana* powered leaves inclusion were formulated as follows: Do(0%); D1(3%); D3 (6%); D3 (9%) and D4 (12%). One hundred and fifty (150) *C. gariepinus* were distributed in five groups in triplicates and fed diets Do-D4 accordingly. After eight (8) weeks of feeding, they were injected intraperitioneally at days 1, 7 and 14. After twenty one (21) days post infection period, three fish from each group were scarified for evaluation of the Hepatosomatic index (HSI); Cistosomatic index (CSI) and Spleenosomatic index (SSI), and the liver was taken to the laborary for histopathological analysis. The survival rate was calculated in each of the group and the disease resistance was determined. At the end of the experiment, serious ulcerations was observe on the fish fed Do and infected with *K. pneumonia*, while the fish fed D1 – D4 were ulcer free. The result reveals that the SSI and CSI were similar in all the groups (Do-D4), but the HSI was higher ($P>0.05$) in the group fed Do (control) compared to the group fed D1-D4. The survival rate and the diseases resistance were lover ($P<0.05$) in the group fed Do compared to the group fed D1-D4, though it was not does dependant. The result of the histopathology shows that the liver of the fish fed Do had blood stain in the portal vein and sinusoid, while the liver of the fish fed D1-D4 have no blood stain the experiment reveals that *Persea americana* powdered leaves as applied is an anti-liver inflanmatory herb, disease resistant and bacterial.

Keywords: Aquaculture, Histology, Organosomatic Indices, Disease and *Klensilla pneumonia*

1. Introduction

Aquaculture is the rearing of aquatic organism in a conducive and controlled environments or enclosures. It is one of the fasted growing food sector in the world [1]. The demand for fish and fish products have been on the high side due to population increase, and this cannot be met by the supply from natural water bodies. Environmental degradation and

population increase have affected aquaculture in developing countries [2], but agriculture and science have been developing ways to enhance productivity in aquaculture to meet up the high population demand and reduce the effects of environmental degradation [3]. Nutrients contain in fish and fish products could go a long way to solve the world malnutrition problems [4], and more than half billion people all over the world are relying on aquaculture and fisheries for a living [5].

One of the problems associated with aquaculture practice is the presence of micro-organisms. And this is as a result of so many factors, including maladministration of feed [6]. The presence of micro-organisms makes fish susceptible to diseases, which causes death and retard growth [7], and most of these diseases are caused by bacteria, viruses, fungi, parasites etc. [8]. There is serious need to improve disease resistance in aquaculture to enhance feed acceptability and growth [9; 10].

Synthetic drugs and chemicals have been used in aquaculture for the prevention and eradication of diseases, but there uses are associated with disadvantages such as polluting the environment, depositing on fish flesh, drug resistance, etc [10]. The use of herb and herbal products is fast replacing the use of chemical, because it dose not pollute the environment, deposit on fish flesh, and are not immunospecific [10].

The presence of disease or pathogen causing disease can be assess in fish in so many ways: Haematological analysis [11; 12]; Organosomatic indices [13; 14], Biochemcial parameters [15; 16] Histopathological analysis [17] among others.

Several herbs and herbal extracts have shown their potency as antibacterial in aquaculture, they includes: *Caricapapay* aqueous root extracts [18], *Persea amerciana* aqueous leaves extracts [19], *Azardirachta/curcuma lionga* aqueous extracts [20] etc.

The *Persea americana* leaves extracts have been reported to contain medicinal phytochemicals [21; 22]. This research work tents to assess the efficacy of *P. americanan* powered leave as in *Clarias gariepinus* infected with *Klebsiella pneumonia*.

2. Material and Methods

2.1 Experimental Fish

The experimental fish was purchased in Idi-Onyana farms along Abua Ahoada road in Abua/Odual Local Government Area of Rivers State.

2.2 Experimental Diets

Persea americana leaves were harvested within Port Harcourt, Rivers State, Nigeria. They were air dried to constant weight, grounded to powdered, sieved and stored. The sieved powered leaves were added to 38.35cp formulated diets Do, at 3%(D1), 6% (D2), 9% (D3) and 12% (D4) respectively following the method of [23].

2.3 Experimental Procedure

150 catfish (117.80 ± 0.11g and 25.88 ± 0.14cm) were stocked in the experimental tanks in triplicate at 10 fish per tank. Feeding commenced 24 (twenty four) hours after stocking, and

the fish were fed with the experimented diets (Do – D4) accordingly at 5% body weight per day, two (2) times daily. After 8 weeks of feeding, the fish were infected intraperitionearly at the day 1, 7 and 14 with 1.5ml of $1.9x10^5$cfu/ml of overnight grown *K. pneumonea.* After 28 days post infection, the liver, spleen, heart were harvested from the experimental fish for organosomatic indices, the liver was analysed for histopathology, and the disease resistance ability and survival rates were determined.

2.4 Determination of Organosomatic Indices

This was determined using the formula

$$\frac{Weight\ of\ Organ}{Weight\ of\ \ fish} x100 \qquad [15]$$

2.5 Histopathogical Analysis

The fish liver was taken to the laboratory in sample bottles containing 10% formaline solution. The samples (liver) were manually processed and trimmed using a rotary microtone (LEICA RM 2125 RTS), manufactured by LEKA Brosysteo, Buffalo Grove, U.S.A. Tissues were dewaxed, stained in hematoxylin and eosin for display of tissue architecture. Stained slides were examined under light micro scope at x 10 magnification.

2.6 Anti-pathogenic Ability (Disease Resistance)

This was determined as relative percentage survival, using:

$$RSP = 1 - \frac{\%\,Mortality\,in\,treated}{\%\,Mortality\,in\,control} x100 \qquad [24]$$

Where RSP = Relative Survival Percentage

2.7 Data Analysis

The collected data were analysed using SPSS statistics software 17.0 windows. A one way analysis of variance (ANOVA) was employed to reveal significant difference between control and treated groups. Tukey's multiple comparism test was applied to separate treatments with significant difference [25].

3. Results

The result of the Organosomatic indices is shown in table 1, there were no significant difference in the spleenosomatic and cardiosomatic indices in the experimental fish fed Do – D4. The hepatosomatic index was significantly higher in fish fed Do, but significantly the same in fish fed D1 – D4 diets. The result for the disease resistance fluctuated across the diets, but it was significantly lower in the fish fed Do (control) followed by the fish fed D1 and significantly the same in the fish fed D2 – D4 (Table 2).

Plate 1 (a-f) shows the liver histopathology of the experimental fish infected with *K. pneumonia* 4.1a is the liver of fish fed Do without infection, it has normal portal vein (P) and there are patches of liver vocuolations; 4.1b is the liver of fish fed Do and infected with *K. pneumonia*, it has blood stained portal vein with micro liver vacuoles and sinusoid; 4.1c is the liver of fish fed D1 and infected with *K. pneumonia*, it has central vein (CV) with no liver vacuoles and sinusoid; 4.1d is the liver of fish fed with D2 and infected with the *K. pneumonia*, it has micro liver vacuoles and sinusoid; 4.1e is the liver of fish fed D3 and infected with *K.*

pneumonia, it has portal vein, micro vacuoles and sinusoid; while 4.1f is the liver of fish fed D4, it has patches of liver vacuolations. Figure 1 shows picture of fish Do and infected with *K. pneumonia* and figure 2 shows picture of fish fed supplemented diets and injected with *K. pneumonia.*

Table 1: Organosomatic indices of some organs of *Clarias gariepinus* fed with Avocado pear leaf Supplemented diets and infected with *Klebsiella pneumonia* (Mean ±SD)

Parameters	**Diets**				
	D0	**D1**	**D2**	**D3**	**D4**
HSI	2.00±0.25 [a]	1.71±0.09 [b]	1.06±0.21 [b]	1.19±0.02 [b]	1.20±0.08 [b]
CIS	0.08±0.02 [a]	0.15±0.00 [a]	0.78±0.01 [a]	0.10±0.02 [a]	0.06±0.05 [a]
SSI	0.06±0.04 [a]	0.08±0.04 [a]	0.06±0.02 [a]	0.07±0.01 [a]	0.03±0.02 [a]

Means within the same roll with different superscript are significantly different (p<0.05)

HSI: Hepetasomatic Index; CSI: Cardiosomatic Index and SSI: Spleenosomatic Index

;

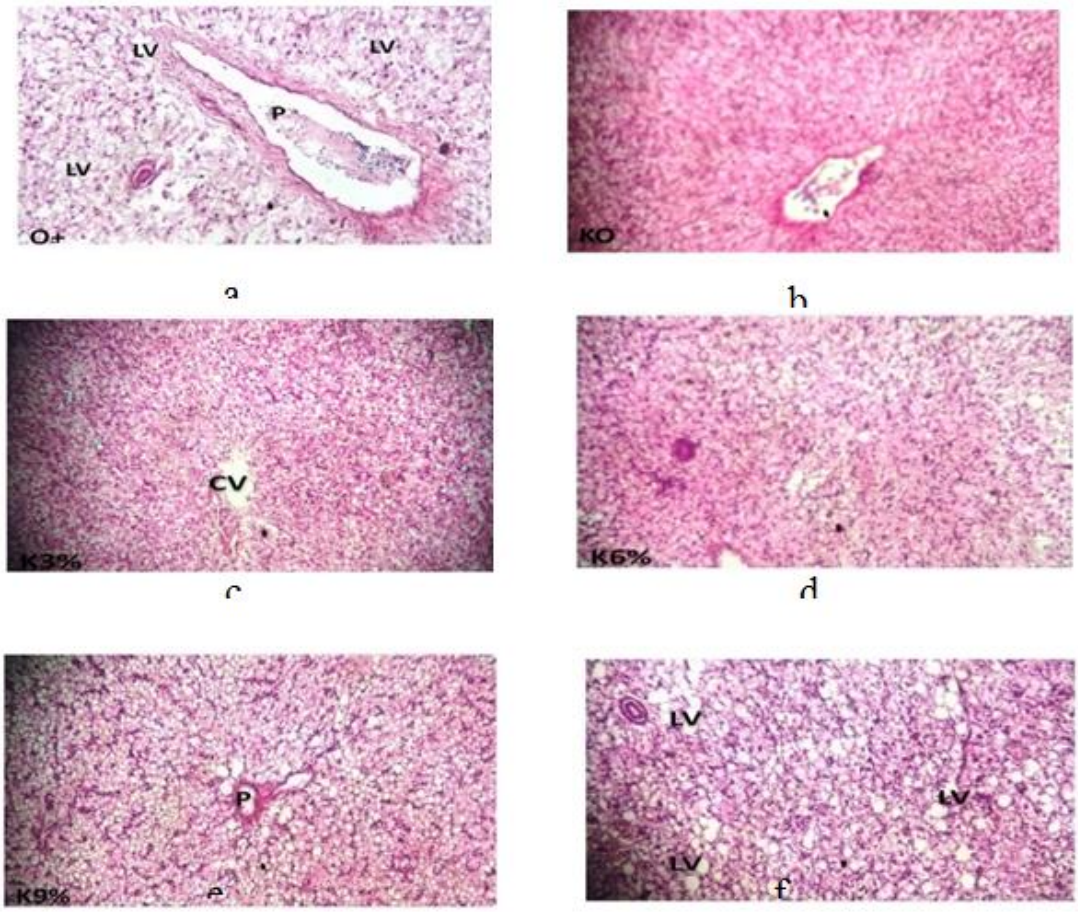
Table 2: Percentage Survival and Relative Survival Percentage (Disease Resistance) of *Clarias gariepinus* fed with Avocado pear leaf Supplemented diets and infected with *Klebsiella pneumonia* (Mean ±SD)

Parameters	Diets				
	D0	D1	D2	D3	D4
% Surv	66.66±5.77 [c]	90.00±10.00 [b]	96.66±5.77 [a]	96.66±5.77 [a]	93.33±11.54 [a]
RSP	0.00±0.00 [d]	69.33±33.85 [c]	91.66±14.43 [a]	91.66±14.43 [a]	77.66±38.68 [b]

Means within the same roll with different superscript are significantly different ($p<0.05$)

% Surv: Percentage Survival; RSP: Relative Survival Percentage

Plate 1: **Pathology of liver of *C.gariepinus* fed the experimented diets and exposed to *Klebsiella Pneumonae***



a: **liver of fish fed (Do) 0% leaf inclusion diet without infection**

b: **liver of fish fed (Do) 0% leaf inclusion diet exposed to *K.pneumonae***

c: **liver of fish fed (D1) 3% leaf inclusion diet exposed to *K.pneumonae***

d: **liver of fish fed (D2) 6% leaf inclusion diet exposed to *K.pneumonae***

e: **liver of fish fed (D3) 9% leaf inclusion diet exposed to *K.pneumonae***

f: **liver of fish fed (D4) 12% leaf inclusion diet exposed to *K.pneumonae***

Figure 1: Fish Fed Do and Infected with *K. pneumonia*

Figure 2: Fish Fed *P. americana* supplemented diet and infected with *K. pneumonia*

4. Discussion

4.1 Behaviour Observation

There were no ulcerations on the body of the fish fed D1 – D4 after the infection with *K. pneumonia*, but there were serious ulceration in the fish fed Do and exposed to *K. pneumonia.* Similar result was obtained when *C. gariepinus* was challenged with *P. aeruginosa* and exposed to *Carica Papaya* aqueous root extracts [11]. [26] also reported similar result when *punica* peel and oxytetrayclia were administered on *A. hydrophila* infected *C. gariepinus*. This could be as a result of the phytochemicals present in the *P. americana* powdered leaves that are antibacterial [27; [28]. [29] also reported that phenotic compounds (as contained in *P. americana* in this work) seriously inhibites microbial activities. The result obtained in this work could also be as a result of improved thrombocytes formation in fish fed D1 – D4, which is believed to prevent injuries infected fish [30; 13].

4.2 Organosomatic Indices

Organosomatic indices such as HSI, SSI and CSI are used to evaluate the health status of fish and other organisms [31; 14; 32]. Some of the factors that affects the organosomatic indices in fish includes water quality, feed type, presence of pathogen/disease etc. [14; 32; 13]. The result of the organosomatic indces after the period of infection in this work reveals that there were no significant difference in the CSI and SSI in the experimental fishes but the HSI value was higher ($P>0.05$) in the fish fed Do compared to the fish fed D1 – D4. This result is in agreement with the reports of [33] who reported increase in HSI when *rattus rattus* was infected with *cysticeness fasciolaris* and [13] when *C. gariepinus* fed dietary mango back were experimentally infected the *P. aeruginosa.* [15] also reported increase in the size of the liver,

spleen and intraperitoneal fat in the control, when *C. gariepinus* was challenged with *P. aeruginesa* and exposed to *P. americana* aqueous extracts.

The increased HSI in the infected fish fed Do (control) could be as a result of glycogen depletion [34] arising from the restlessness and rapid opercula movement observed in the fish fed Do when they were injected with the *K. pneumonia* and depletion of glycogen leads to liver inflammation [35]. Lack of glycogen also leads to metabolic formation of fat in the liver [36] and this could lead to liver weight increase. The lower HSI in the fish fed D1-D4 compared to fish fed Do could be as a result of the antibactical activities in the experimental herb [40; 18]. It could also be as a result of the anti-inflammatary effects of flavonoids and Saponins present in the *P. americana* powdered leaves [41; 15].

4.3 Liver Histopathology

There were high presence of liver vacuoles in the liver of fish fed 0% leaf inclusion diet without exposure to *K. pneumonia* (positive control), but the liver of the fish fed 0% leaf inclusion diet and exposed to *K. pneumonia* (negative control) had no vacuoles, but minor necrosis. This result is similar to the result of [41] when *Lactobacillus acidophilus* was used as a biocontro agent against *Clarias gariepinus* juveniles infected with pathogenic bacteria (*Aeromodas hydrophila*). The liver of the fish fed D1 – D4 and exposed to *K. pneumonia* had little presence of liver vacoulation, with more in the fish fed D4. The high presence of liver vacoulation in the positive control could be as a result of the presence of high glycogen due to energy intake and use, as a result of artificial feeding and rearing conditions [43], which is believe to be nutritionally induced [44]. The presence of necrosis in the infected fish fed Do could be as a result of poison released by the pathogenic bacteria (*K. pneumonia)* during

infection [42], and the absence of liver vacuoles in the same fish could be the fish way of responding to infections diseases [45], as loss of glycogen has been associated with concurrent diseases and stress [43]. The presence of liver vacoulation was noticed in the fish fed D1-D4 after the infection, but was prominent on fish fed D4. This could be as a result of the antimicrobial activities of the avocado pear powdered leaves [11], [19], due to the presence of medicinal phytochemicals [46, [47], [48].

4.4 Disease Resistance

The disease resistance ability which was calculated as relative percentage survival (RSP), and the percentage survival were lowest ($P<0.05$) in fish fed Do compared to the fish fed D1-D4, and it increased as the percentage inclusion of *P. americana* leaves increased in the diets. The result is similar to the reports of [13] who reported the effect of mango bark extract on *C. gariepinus* infected with *P. aeruginosa*; [49] who reported the effect of neem leaf powder on common carp infected with *A. hydrophila*. [18] also reported high disease resistance when *P. aeruginosa* infected *C. gariepinus* was expose to *Carica papya* aqueous extracts, and attributed the results to the presence of phytochemicals such as phenols. The high disease resistance in the fish fed D1-D4 could be as a result of the phytochemicals such as flavonoids and other alkaloids present in the *P. americana* leaves that are bactericidal or bacteriostatic [18; 21].

5. Conclusion

The result of this research work shows that powered *P. americana* is a good prophylactic agent against ulceration and other skin diseases in *Clarias gariepinus* that may arise from the attack of *K. pneumonia*. Despite been a good antibacterial as seen in the result of the disease resistance in this work, *P. americana* powdered leaves has also proven to be a good anti-

inflammatory and anti-necrosis agent in *C. gariepinus* liver in the presence of pathogens such as *K. pneumonia. P. americana* powdered leaves should also be tested for their efficacy against other fish pathogens, to enhance its usage in aquaculture. To this end, we adviced that *P. americana* trees should be planted in our farms for availability of the leaves when needed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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