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Effect of Probiotic (*Klebsiella pneumonia*) on Growth Performance and Immune Response of *Clarias gariepinus* Fingerlings Challenged with *Clostridium botulinum*

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ABSTRACT
This study was conducted to evaluate the effect of Klebsiella pneumonia on the growth performances and immune response of Clarias gariepinus against Clostridium botulinum. Two hundred and twenty five (225) C. gariepinus fingerlings, average body weight of 5.32g + 0.02 were randomly selected and distributed into 15 glass tanks at the rate of 15 fish per tank srepresenting five treatments and three replicates. Treatment 1 was the control (basal diet). Treatment 2, 3, 4 and 5 were the basal diet with 10^3cfu/g, 10^5cfu/g, 10^7cfu/g and 10^9cfu/g of K. pneumonia respectively. The fish were fed to visual satiation twice daily for 70 days. Results revealed that the first group ($10^{3)}$ of fish fed K. pneumonia supplemented diets showed significant increase (P>0.05) in body weight gain, specific growth rate and feed efficiency ratio. Survival percentage was higher in fish fed probiotic supplementation diets when compared with the control groups. Varying inclusion levels of K. pneumonia in the diets improved the blood profile and carcass composition as there were significant differences (P<0.05) in the parameters tested. Challenge test revealed that fish fed control diet had the highest mortality percentage (100%) during the 10 days observation period. It may be concluded that K. pneumonia can be used as a growth promoter in C. gariepinus fingerlings.

Key words: *Clarias gariepinus, Klebsiella pneumonia, growth performances, Clostridium botulinum*

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I. INTRODUCTION

Probiotics are live microorganisms that act beneficially in the host, promoting the balance of the intestinal microbiota, favoring the health of the animals (Fuller, 1989). Nayak (2010) stated that an ideal probiotic, irrespective of its source must be able to colonize and multiply in the intestine of the host. Several studies have exhibited promising results of the use of probiotics in fish, mollusk, crustacean and amphibian farming Kesarcodi-Watson *et al*., 2008; El- Rhman *et al*., 2009; Zhou *et al*., 2009, which enables the probiotics to substitute the antibiotics as growth promoters. Nowadays, probiotics are becoming an integral part of the aquaculture practices to obtain high production (Saini *et al.,* 2014). Feed supplemented with probiotics improve appetite and growth performance of the farmed fish (Geovanny *et al.,* 2007). Generally probiotics are included in the genera of *Lactobacillus, Bacillus, Bifidobacterium, Vibrio, Saccharomyces, Enterococcus, Bacillus subtilis* (Pannu *et al.,* 2014). Disease outbreaks are one of the important problems that affect aquaculture production, suppressing both economic and social development in many countries (Qi *et al.,* 2009). The need for increased disease resistance, growth of aquatic organisms and feed efficiency has brought about the use of probiotics in aquaculture practices (Cruz *et al.,* 2012). The most widely used disease prevention method was using antibiotics and chemotherapy ingredients. The intensively or repeatedly use of antibiotics or chemicals had caused various negative effects such as bioaccumulation, pollution, antibiotic resistant pathogens, damaging the microbial population of the environment and immunosuppression (Biswas *et al*., 2012; Karthik *et al.,* 2014). Antibiotic residues can be accumulated in the fish's body, and are harmful to people health (Payung *et al*., 2017).

Klebsiella pneumoniae is a non-motile, lactose-fermenting, capsulated bacillus belonging to the Enterobacteriaceae family. It can be found in the soil, water, plants and in the gastrointestinal tracts of animals and humans ((Bagley, 1985; Kim *et al.,* 2005; Barati *et al.,* 2016). *K. pneumoniae* has both clinical and non-clinical habitats (Abbott, 2015). In fact, due to its widespread nature, even in the environments apparently free from obvious fecal contamination, *K. pneumoniae* is usually considered as a member of total coliforms with insignificant public health risk (WHO, 2004). The United State Food and Drug Administration has identified and published *Clostridium botulinum* as a major bacterium on their Import Alert 16-74 of June 23, 2014. This is tagged Type DWPE, that is, detention without physical examination of uneviscerated fish or partially eviscerated fish that are either salted, smoked, picked or brined. There is need to conduct a research on the use of some microorganisms as probiotic to eliminate the possibility of infection from pathogenic *C. botulinum* bacteria, thereby reducing economic loss through foreign exchange earnings. Therefore, the aim of this study was to evaluate the effects of dietary supplementation of *K. pneumonia* on growth performances and immune response of *C. gariepinus* fingerlings challenged with *Clostridium botulinum*.

II. MATERIALS AND METHODS

Study Area

The present work was conducted at the research laboratory of the Department of Biology, Adeyemi College of Education Ondo State, Nigeria for a period of 70 days.

Preparation of Probiotic Microorganism Used For The Study

Klebsiella pneumonia and *Clostridium botulinum* samples used for this experiment were isolated from adult *Clarias gariepinus* (500 – 1000 g) and pond sediment using standard method of bacteria isolation (Cowan and Steel, 1993). Morphological and biochemical tests that were performed on the bacterial isolates included, Gram reaction, catalase, coagulase, nitrate reduction, starch hydrolysis, indole (Lactose, sucrose, glucose, maltose, galactose and dextrose) using standard microbiological techniques (Cowan and Steel, 1993).

Pathogenicity

Test was conducted on the *C. botulinum* to confirm its pathogenicity potential using the method of Sharada *et al*, (2010). Appearance of red colonies was recorded as congo red (CR+) positive and colonies that remained white or grey were considered as congo red (CR-) negative. The result of the test (congo red (CR+) confirms *C. botulinum as a pathogen.*

Safety Test

Safety test was conducted on *K. pneumonia* isolates to determine whether it is pathogenic or non-pathogenic before using it as probiotics for the fish. This was done by acclimatized 40 apparently healthy *C. gariepinus* fingerlings for two weeks in indoor tanks and divided into two equal groups comprising twenty fish per treatment. Group one served as control and was injected 0.3ml of sterile normal saline solution intraperitoneally while group two was injected with 0.3 ml of *K. pneumonia* of 10^7cfu/ml. The two groups were kept under observation for two weeks to monitor their mortality and morbidity rate.

Experimental Fish

Four hundred (400) healthy *C. gariepinus* fingerlings with mean weight of 5.32±0.02g were obtained from the research farm of Federal University of Technology, Akure. The fish were allowed to acclimatize for 7 days, during this period, fish were hand-fed with 40% crude protein of a commercial diet (control diet) to visual satiation twice daily.

Experimental Diets

Diets were formulated to contain 40% crude protein using fish meal, soya bean meal, yellow corn, ground nut cake, vitamin and mineral premix, ascorbic acid and cassava starch was added as a binder. The experimental diets were prepared at different probiotics levels comprising of five treatments of *K. pneumonia* including the control (T1). Probiotic treated diets were prepared at T2 (10^3), T3 (10^5), T4 (10^7) and T5 (10^9) representing 0.00, 0.27, 0.19, 0.10 and 0.05 concentration respectively at wavelength of 460 nm as shown in Table1. The experimental diets were thoroughly mixed in a plastic bowls and pelleted using Hobbart A-2007 mixing and pelleting machine (Hobart Ltd London,) to obtain a homogeneous mass. The pellets produced were dried at room temperature (26 ^oC) for three days and packed in polyethylene bags and marked according to treatment and stored in a freezer at -2^oC until needed.

Table 1: Gross composition of the experimental diets *(K. pneumonia* inclusion*)* used to feed *Clarias gariepinus* for 70 days (g/100 g)

Ingredients	T1	T2	T3	T4	T5
Fish meal (72% CP)	25.4	25.4	25.4	25.4	25.4
Soybean meal (45% CP)	35.4	35.4	35.4	35.4	35.4
Groundnut cake (48% CP)	12.1	12.1	12.1	12.1	12.1
Yellow maize	15.0	15.0	15.0	15.0	15.0
Oyster shell	2.00	2.00	2.00	2.00	2.00
Vegetable oil	5.60	5.60	5.60	5.60	5.60
Vitamin-premix	2.50	2.50	2.50	2.50	2.50
Binder(starch)	2.00	2.00	2.00	2.00	2.00
K. pneumonia dose ($cfug^{-1}$)	0.00	10^3	10^5	10^7	10^9

Composition of vitamin-mineral mix (Aquamix) (quantity/kg), Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium; Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450. L- Lysine, 10 g; Selenium, 50 ppm

Key: T1- basal diet, T2 – basal diet + 10^3 *K. pneumonia***,** T3 – basal diet + 10^5 *K. pneumonia***,** T4 -basal diet + 10^7 *K. pneumonia,* T5 - basal diet + 10^9 *K. pneumonia.*

Experimental Design and Management

The experimental design was a complete randomized design. Out of the four hundred (400) *C. gariepinus* fingerlings obtained, a total of two hundred and twenty five (225) fish with mean weight of 5.32±0.02g were randomly selected and distributed into 15 glass Tanks (70 litre) each measuring (70 x 45 x 45 cm^3) at the rate of 15 fish per tank representing five treatments and three replicates**.** Fish were fed with their respective diets to visual satiation twice daily between (08:00 and 09:00 and 16:00 and 17:00 hours) for 70 days. Fish mortality was monitored daily, dead fish were removed, counted and recorded. Left over feed and faeces in each tank were siphoned out every morning before feeding. The water in the tank was partially flushed and replenished every morning while total changing was done every three days to avoid fouling resulting from faeces and uneaten food. Water quality parameters were monitored throughout the period of the experiment. Calculation of the growth performance was according to Cho and Kaushik (1985). Haematological parameters of the fish at the end of the feeding trials was determined according to adapted method of Svobodova *et al.* (2006). Proximate analysis of the experimental diets and carcass composition were carried out according to AOAC (1990).

Challenge Test

After feeding for 70 days, three fish from each treatment and replicate were challenged by inoculating Intraperitoneally (IP) with different concentration of pathogenic *C. botulinum* (0.1ml of 10^7cells/ml) which was isolated from Catfish purchased from above stock. The second subgroup was injected with 0.2 ml of saline solution only as control. Both subgroup were fed with controlled diet for 10 days and kept under observation for daily monitoring of the clinical signs and survival percentage.

Statistical Analysis

Data collected from the experiments were subjected to one-way analysis of variance (ANOVA) using the SPSS (Statistical Package for Social Science Version 21) as described by Steel and Torrie (1980) and Duncan new multiple range tests (Duncan, 1955) was used to separate the significantly different among the means.

III. RESULTS

Water Quality Parameters

The results of water quality measurements in glass tanks for the maintenance of catfish fingerlings are presented in Table 2. There were no significant variations (p>0.05) in temperature, dissolved oxygen and hydrogen ion concentration (pH) values in all treatments.

Table 2: Water qualities parameter measured during the experimental period

Parameters (g/100g)	T1	T2	T3	T4	T5
Temperature (^{O}c)	28.05±0.15^a	28.01±0.15^a	28.01±0.15^a	28.01±0.15^a	28.01±0.15^a
pH	7.2±0.19^a	7.1±0.15^a	7.3±0.10^a	7.2 ±0.11^a	7.1±0.21^a
Dissolved Oxygen (mg/l)	7.20±0.50^a	7.30±0.51^a	7.11±0.32^a	7.25±0.45^a	7.31±0.24^a

Means in same row with same superscripts are not significantly different (P>0.05)

Proximate Composition of Experimental Diets Fed to *C. gariepinus* Fingerlings

Proximate composition of experimental diets fed to *C. gariepinus* fingerlings is shown in Table 3

The control diet with 0% inclusion level had the highest value of moisture and ash content and lowest was in T2. Moisture content ranged between 11.37% in T1 to 9.48% in T4. Diet with 10^3 inclusion level of *K. pneumonia* recoded the highest value of crude protein and diet with 10^9 inclusion level had the lowest, Diet with 10^9 inclusion level recorded the highest percentage crude fibre and lowest was in control group.

Table 3: Proximate composition of experimental diets

Parameters (%)	**T1**	**T2**	**T3**	**T4**	**T5**
Moisture	11.37	9.21	9.39	9.48	9.27
Crude fat	9.85	10.22	11.07	10.08	10.11
Crude protein	40.02	40.07	40.02	40.05	40.01
Crude fibre	3.22	4.71	4.15	4.17	4.64
Ash	7.96	7.18	7.33	7.34	7.55
Nitrogen free extract	27.58	28.61	28.04	28.88	28.42

Key: T1- basal diet, T2 – basal diet + 10^3 *K. pneumonia***,** T3 – basal diet + 10^5 *K. pneumonia***,** T4 -basal diet + 10^7 *K. pneumonia,* T5 - basal diet + 10^9 *K. pneumonia.*

Growth Performance and Nutrient Utilization

The growth performance and nutrients utilization of *C. gariepinus* fed the experimental diets were presented in Table 4. There was variation in the growth indices and nutrient utilization parameters among the fish fed different inclusion levels of *K. pneumonia* and the control group. Highest growth performance was obtained for the group fed with T2 which indicate that (10^3) inclusion level of *K. pneumonia* can be considered as the most optimal level of inclusion in the diet of the fish. The highest feed intake (53.79) was recorded in fish fed diet T3 while the lowest feed intake was recorded in fish fed T1. Best FCR was recorded in fish fed T2 while the poorest FCR was recorded in fish fed T1. There were significant difference (p<0.05) in specific growth rate and feed efficiency ratio between the treatment groups and the control group. Survival percentage was higher in fish fed probiotic supplementation diets when compared with the control groups.

Table 4: Growth and nutrient utilization in *Clarias gariepinus* fed diets supplemented with *K. pneumonia*

Parameters	T1	T2	T3	T4	T5
MIW	5.32±0.01^{a}	5.33±0.02^{a}	5.32±0.01^{a}	5.33±0.02^{a}	5.32±0.03^{a}
MFW	29.27±0.06^{a}	40.62±0.06^{c}	37.47±0.01^{bc}	35.00±1.00^{b}	33.50±0.48^{ab}
MWG	23.98±0.01^{a}	35.29±0.01^{c}	32.15±0.01^{bc}	29.68±1.00^{b}	28.18±0.48^{ab}
SGR (%/day)	2.44±0.01^{a}	2.90±0.01^{c}	2.79±0.01^{bc}	2.69±0.01^{b}	2.71±0.02^{b}
FI	43.47±0.02^{ab}	48.79±0.01^{b}	53.79±0.01^{c}	52.43±0.06^{bc}	50.43±0.86^{a}
FCR	1.81±0.02^{c}	1.38±0.01^{a}	1.67±0.03^{b}	1.77±0.02^{bc}	1.79±0.14^{c}
FER	0.55±0.04^{a}	0.72±0.07^{b}	0.60±0.03^{c}	0.57±0.04^{c}	0.56±0.04^{bc}
Survival (%)	85.0±1.67^{a}	87.8±6.94^{a}	93.3±1.42^{b}	86.7±0.7.26^{a}	93.3±1.42^{b}

Means in the same row followed by different superscripts letters differed significantly ($P<0.05$)

Key: MIW= Mean initial weight, MFW= Mean final weight, MWG= Mean weight gain, SGR=Specific growth rate (%/day), FI=Feed intake, FCR=Feed conversion ratio, FER=Feed efficiency ratio.

T1- basal diet, T2 – basal diet + 10^3 *K. pneumonia*, T3 – basal diet + 10^5 *K. pneumonia*, T4 -basal diet + 10^7 *K. pneumonia,* T5 - basal diet + 10^9 *K. pneumonia.*

Haematological Parameters

The haematological parameters of the fish fed the experimental diets are presented in Table 5. The results showed there are significant differences among all the fish fed the experimental diets ($p<0.05$). Highest white blood cell was recorded in fish fed T4 while the lowest was recorded in fish fed T1. Fish fed T5 had the highest value of heamoglobin, packed cell volume and red blood cell. There was no significant difference ($P>0.05$) in mean cell haemoglobin concentration in probiotic treated groups and the control group. There was no significant difference ($P>0.05$) in MCHC which was highest in fish fed T5 and lowest in fish fed T4. The highest values of mean cell haemoglobin and mean corpuscular volume was recorded in fish fed T4 while the lowest values was recorded in T5 respectively.

Table 5: Haematological Parameter of *Clarias gariepinus* fed experimental diets

Parameters	T1	T2	T3	T4	T5
WBC (x10^3/mm^3)	5.24±0.05^{a}	5.63±0.56^{b}	5.86±0.05^{c}	6.18±0.54^{d}	5.83±0.54^{c}
RBC (x10^3/mm^3)	3.15±0.02^{b}	3.75 ±0.03^{d}	3.15±0.03^{b}	2.80±0.10^{a}	4.08 ±0.03^{d}
Hb (g/100ml)	9.39±0.05^{a}	11.0±0.50^{c}	9.30 ±0.05^{a}	9.27±0.07^{a}	11.9±0.01^{d}
PCV (%)	28.0±1.00^{a}	33.0±1.00^{c}	28.7 ±1.15^{a}	28.0±1.00^{a}	35.0 ±0.50^{c}
MCHC (%)	33.5±1.42^{a}	33.3±0.59^{a}	32.4 ±0.82^{a}	33.1±0.06^{a}	34.0±2.82^{a}
MCH (pg)	29.8±0.10^{a}	29.3±0.15^{a}	29.5 ±0.12^{a}	33.1±0.41^{b}	29.2 ±0.83^{a}
MCV (fl)	88.9±3.49^{a}	88.0±1.13^{a}	91.1 ±2.11^{ab}	100.0±1.16^{b}	85.8 ±4.59^{a}

Means in the same row followed by different superscripts letters differed significantly ($P<0.05$)

Key: WBC -White Blood Cell, RBC -Red Blood Cell, Hb-Haemoglobin, PCV-Pack Cell Volume, MCHC= Mean cell haemoglobin concentration, MCH= Mean cell haemoglobin, MCV= Mean corpuscular volume.

T1- basal diet, T2 – basal diet + 10^3 *K. pneumonia*, T3 – basal diet + 10^5 *K. pneumonia*, T4 -basal diet + 10^7 *K. pneumonia,* T5 - basal diet + 10^9 *K. pneumonia.*

Carcass composition

The whole body composition (% Dry Weight) of *Clarias gariepinus* fed experimental diets is shown in (Table 6). The results of chemical analysis of the fish showed significant different in their body composition. The moisture content was higher in fish fed T1 and lowest in fish fed T5. There was significant difference ($P<0.05$) in the crude protein content of all the fish with fish fed T1 having the highest of 75.45%. There was also significant difference ($P<0.05$) in the lipid values of some of the fish with those fed control group having the highest of 7.58%. These changes in protein and lipid contents in the fish body could be linked with changes in their synthesis, deposition rate in muscle and or different growth rate. There was no significant difference ($P<0.05$) in the ash content of fish fed the experimental diets.

Table 6: Carcass composition of *Clarias gariepinus* fed experimental diets.

Parameters (%)	T1	T2	T3	T4	T5
Moisture	75.45±0.07^{d}	73.36±0.27^{b}	73.32±0.06^{b}	74.03±0.05^{c}	72.33±0.72^{a}
Ash	6.52±0.26^{b}	5.62±0.05^{c}	6.57±0.07^{b}	5.17±0.21^{a}	5.71±0.02^{ab}
Crude protein	68.05±0.03^{b}	69.91±0.01^{d}	68.90±0.36^{c}	69.25±0.29^{cd}	65.67±0.23^{a}
Crude lipid	7.58±0.06^{b}	7.18±0.01^{c}	7.20±0.02^{c}	6.82±0.04^{a}	7.53±0.02^{b}

Means in the same row followed by different superscripts letters differed significantly ($p<0.05$)

Key: T1- basal diet, T2 – basal diet + 10^3 *K. pneumonia*, T3 – basal diet + 10^5 *K. pneumonia*, T4 -basal diet + 10^7 *K. pneumonia,* T5 - basal diet + 10^9 *K. pneumonia.*

Challenge Test

The challenge test (Table 7) showed that fish fed the control diet has the highest mortality rate 100% during the 10 days observation period. The survival rate was highest in fish fed diet T4. Though some parent clinical signs were observed in the fish fed with probiotic diets after the challenge test, such as darkening of pigmentation area, lesions all over the body, bleached skin and white caudal tail, the fish retired to the fish tank bottom and eventually death occurred. The mean time to death ranged from 10 to 72 hours and was significantly longer at the lower dose than at the higher doses.

Table 7: Challenge test on *Clarias gariepinus* fed experimental diets supplemented using *C. botulinum*

Parameters (%)	T1	T2	T3	T4	T5
Number of fish treatment	15	15	15	15	15
C. botulinum cfu/ml	10^9	10^9	10^9	10^9	10^9
Type of inoculate (0.2ml L^{-1} of water)	*C. botulinum*	*C. botulinum*	*C. botulinum*	*C. botulinum*	*C. botulinum*
Period of test	10 days	10 days	10 days	10 days	10 days
Mortality %	100	90	45	32	35
Survival (%)	0	10	55	68	65

IV. DISCUSSION

Water Quality Parameters

The mode of action of probiotic in aquaculture industry has not been fully explicated and there is incessant argue about its effect on the water quality parameters. The water quality parameters values recorded in this present study were within the acceptable range recommended for rearing and culture of most tropical fishes, including the *C. gariepinus* for optimum growth and survival as there was no obvious effect of the *K. pneumonia* added to the feeds on water quality. This finding agrees with that reported by (Putra *et al.*, 2017) in *C. gariepinus* fed a commercial diet and reared in the biofloc system enhanced with probiotic.

Proximate Composition of Experimental Diets

The proximate composition of the experimental diets obtained from this study gave a 40% crude protein for all the diets as desired implying that there was no significant difference (P>0.05) among the diets. Boonyaratpalin (2008) reported that protein requirement for most tropical catfish including *C. gariepinus* has been estimated to ranged (35 and 40%) which is within the range reported in this study.

Growth Performance and Nutrient Utilization

Dietary probiotic supplementation in aquaculture has been reported to improve, growth performance and nutrient utilization, disease resistance and enhance immune responses (Talpur *et al.*, 2014; Zhang *et al.*, 2014). The growth parameters, such as weight gain, specific growth rate and feed intake were significantly (P<0.05) higher in probiotics incorporated diet fed fish when compared with the control group which is in agreement with the findings of Ali *et al*. (2018) who reported similar results in *Mystus cavasius* fry fed with selected probiotics for 60 days. El-Haroun (2007) found that application of probiotics (Biogen) of *Bacillus* in the fish diet increased the growth rate and reduced the feed conversion ratio of *C. gariepinus.* Rahmawan *et al*. (2014) also revealed that the addition of probiotic in feed increased feed utilization, growth and survival of *Clarias gariepinus*. *C. gariepinus* showed maximum growth performances with the inclusion level of *K. pneumonia* at 10^3 cfu/g in their diet. This result compare favourably with the report of Nwanna *et al.* (2017) on *C. gariepinus* fed *Lactobacillus plantarum* at 10^3 cfu/g. Also, Dawood *et al.* (2016) concluded that incorporation of 10^3 to 10^6 cells/g *Lactobacillus rhamnosus* enhance growth of Red sea bream. In a study conducted by Gobinath and Ramanibai (2012) the application of probiotic *Lactobacillus sp*. isolated from *O. mossambicus* in feed significantly increased the size and weight of infected tilapia as compared with fish fed without addition of probiotic.

The better feed intake in *K. pneumonia* supplemented diets may have been due to the increased fish appetite, triggered by quick digestion, resulting in a higher feed intake and therefore increased growth of the fish. The lower feed conversion ratio (FCR) values resulted in probiotics treatment indicated that probiotics supplementation enhanced feed utilization of the experimental fish. The best FCR values observed with probiotic supplemented diet suggests that, addition of probotic improved feed utilization. Lara-Flores *et al*. (2003) reported that total feed intake by fish consequently had direct effect on feed conversion ratio and specific growth rate in *O. niloticus* fed different probiotics. Moreover, Dennis and Uchenna (2016) studied the influence of probiotics on the survival rate of *C. gariepinus* larvae and found better survival in probiotics treatment (mixture of *L. acidophilus*, *B. subtilis* and *L. bugaricus*) compared with the control group, which is consistence with the report from this study.

Haematological Parameters

The haematological data obtained in this study were within the recommended physiological requirement for C*larias gariepinus*. The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) are useful in the diagnosis of anaemia in most animals (Fagbemi *et al*., 2013). According to Nwanna *et al* (2014) Blood parameters are good bio-indicators or diagnostic tools to study the effects of diets on the organ function thus providing vital information for health assessment and management of cultured fish. The application of hematological techniques have proved valuable for fishery biologists in assessing the immunological responses of fish. The results of the present study showed that some of the values were fluctuating due to the condition under which the fishes were kept, the condition based on the fact that the fishes are not in their natural habitat. The heamatological results of the present study showed that HB, WBC, RBC, and PCV were affected by diet treatment with probiotics the highest values were recorded in fish fed probiotics diet. The results of White Blood Cell (WBC) on the present study indicated that all the microorganisms are good probiotics that are capable of providing immunity that involved in protecting the fish against both infectious disease and foreign invaders.

Carcass Composition

The results of proximate analysis of the fish showed significant variations ($P < 0.05$) in their body composition. Probotic supplementation significantly affected the whole fish body composition. The moisture content recorded in this study was higher than (70.03-73.71 %) reported by Nwanna *et al*. (2017) in *C. gariepinus* juveniles fed different probiotics level but compared favourably well with Ali *et al*. (2018) who reported (75.04-76.56 %) in *Mystus cavasius* fry fed with selected probiotics and control group for 60 days. The ash content reported in this present study was higher than (1.07 and 1.06 %) reported by Pal *et al.* (2017) in *Ompok bimaculatus* fry fed probiotics supplemented diets. Crude protein content of the fish is in line with the findings of Degani (1989) who reported that proteins are the major material in fish tissue and could make up to (65-75%) of the total organic materials on a dry matter basis, but higher than (20.12-23.98%) reported by Saini *et al.* (2014) in *L. rohita*. fed probiotic supplemented diets. Lipid content reported in this study was lower than that reported by Masuma *et al.* (2019) on *Ompok pabda* fed probiotics. (Abdel-Tawwab *et al*., 2006) added that changes in protein and lipid contents in the fish body could be linked with changes in their synthesis, deposition rate in muscle and or different growth performance.

Challenge Test

It was clear that immune response of fish was good and that appear from survivability. However, *C. botulinum* groups gave good survival percentage which agree with the findings of Hussein (2016) on *O. niloticus* with *A. hydrophila*. Similar result was also reported by Hala *et al.* (2019) on immune parameters of *O. niloticus* challenged with *Aeromonas hydrophila*.

V. CONCLUSIONS

This research found that the inclusion of probiotic bacteria isolated from *Clarias gariepinus* and Pond sediment into feed was able to improve growth performance, nutrient utilization, survival percentage, haematological parameters and carcass composition in *C. gariepinus*. The best growth performance was recorded in fish fed food supplemented with *K. pneumonia* containing 10^3 cfu/ml. The information generated from present investigation might contribute to the incorporation of the *K. pneumonia* in aquaculture industry as supplement in formulated fish feed.

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