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Growth Performance of *Clarias gariepinus* on Diets Fortified with *Lactobacillus plantarum* and *Psidium guajava* Leaf

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Abstract

This study was conducted to assess the effect of dietary inclusions of *Lactobacillus plantarum* (*L. plantarum*) and *Psidium guajava* (*P. guajava*) diets on the growth performance of *Clarias* gariepinus (*C. gariepinus*). Seven treatments were administered to 420 *C. gariepinus* juveniles. They were: Control (Basal diet of 42% crude protein), *L. plantarum* (LPc) (cell/1000 mL) at 40, 60, 80 and *P. guajava* leaf meal (PGLM) (g/1000g) at 40, 60 and 80 per kg of feed. Body Weight Gain (BWG) (g), Specific Growth Rate (SGR) (g/day), Feed Conversion Ratio (FCR), Survival Rate (SR) (%), Protein Efficiency Ratio (PER) and Nitrogen Metabolism (NM) were evaluated. Data was analyzed using descriptive statistics and ANOVA at $p<0.05$. From the results, 40LPc had the highest body weight gain (73.52±2.30) g and Specific Growth Rate (SGR) (1.19±0.02) g/day, while 80 PGLM had the least body weight gain (46.10±0.12) g and SGR (0.99±0.00) g/day. No significant difference ($p>0.05$) was observed in feed conversion ratio across all treatments. There was no significant difference ($p>0.05$) observed in the survival rate between control (93.80±4.98)% and 80 PGLM (96.77±4.09)%. No significance difference ($p>0.05$) was observed in protein efficiency ratio between control (1.01±0.36) and 60 PGLM (0.95±0.36). There was no significant difference ($p>0.05$) observed in nitrogen metabolism across all treatment groups. Hence, the inclusion level of 40 cells/1000 mL of *L. plantarum* fortified diet in cultured juvenile *C. gariepinus* was observed to have a better growth enhancing performance and nutrient utilization than *P. guajava* leaf meal at 40g/1000g.

Keywords: *Clarias gariepinus*, growth, phytobiotics, probiotics

Introduction

Aquaculture is recognized for its rapid growth in the food producing sector globally (Hassan and Ngaski, 2007). The growth of aqua-farming has led to high stock densities of fish, which cause stressful conditions for fish, thus resulting in increased disease incidence and decreased fish productivity (Bondad et al., 2005). Hence, functional feeds are vital in fish nutrition and management. Functional feeds help to reduce fish diseases, boost productivity and enhance profitability. High stocking densities have elicited antibiotic use as growth promoters and immunostimulants. However, restrictions have been imposed regarding the use of antibiotics in fish culturing as they [antibiotics] leave resistant and harmful residues in human and animal flesh (Sayed et al., 2011).

In relation to this, research has been on-going on environmentally-friendly additives that have no residual effects on man and animals. It is worth noting that such additives contain substances that enhance fish growth and stimulate their immune system against diseases. Among the additives used are probiotics such as *Lactobacilli* and phytobiotics such as *P. guajava*. *Lactobacilli* are non-pathogenic facultative organisms that are employed as probiotics in aqua-farming. Lactic acid bacteria (LAB) produce bacteriocins and organic acids that aid in inhibiting the growth/replication of pathogens detrimental

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microorganisms (NRC, 2011). These beneficial properties have made LAB more suitable than other microorganisms used as probiotics (Arimah and Ogunlowo, 2014). *Lactobacillus plantarum* are probiotic bacteria derived from fermented cow milk known as 'wara' in Nigeria (Adegbehingbe, 2013). Even though LAB are currently being used as probiotics, there has been limited information published on the use of LAB isolated from food sources in feed inclusion of African catfish.

To improve aquaculture, the use of natural compounds from plants is highly desired. According to Welker and Lim (2011) phytobiotics are substances derived from plants to enhance fish health and growth. They include *P. guajava*, *Echinacea purpurea* (Guz et et al., 2011) and *Allium sativum* (Shalaby et al., 2006). However, there is on-going research into the use of *P. guajava* leaves as immunomodulators and phytoconstituents in aquaculture industry.

In Nigeria, African catfish (*Clarias gariepinus*) are widely cultured due to their hardiness to stress, high resistance to diseases, spawning ability, fast growth rate and acceptability to consumers' taste (Adewolu et al., 2008).

This study focuses on the comparative evaluation of the efficacy of *L. plantarum* isolated from cow milk, and *P. guajava* leaves on the growth response and utilization of nutrients in *C. gariepinus* juveniles after an 84 day feeding trial.

Materials and Methods

Ethical statement
The experimental procedure followed the International ethical standard of animal use. The ethical report statement was issued by University of Ibadan in 2017 with an ethical statement number of 0028 (UI/ACUREC/App/2017/0028).

Experimental system and design
This study was carried out in the Department of Aquaculture and Fisheries Management wet Laboratory located in the Faculty of Agriculture and Forestry, University of Ibadan, Nigeria.

Experimental procedure
Four hundred and twenty *C. gariepinus* juveniles of the average live weight of 7.79±0.01g were purchased from a known breeding fish farm in Ibadan. They were individually transported inside a bag that was adequately filled with water and sufficiently aerated to the Department of Aquaculture and Fisheries Management Laboratory. Records of the average weights of the fish were taken prior to the experiment. Experimental fish were acclimatized for 14 days and fed with commercial feed. Afterwards, the feeding experiment was carried out for 84 days.

The fish were distributed equally into 7 experimental treatments, which were triplicated. Twenty African catfish juveniles were housed in rectangular fish holding facilities (Neoplastics, Lagos, Nigeria) with each individual facility having the dimensions 50x34x27cm and a 40 liter capacity. Twenty-one fish holding facilities were employed in the experiment with each treatment holding 60 juveniles of African catfish. The fish were weighed individually and collectively in each facility. Weighing was carried out fortnightly. The average weight of fish was determined by dividing the total weight of fish by the number of fish in each facility. Feed was administered to the fish twice daily at 8:00 AM and 6:00 PM (Greenwich Merridian Time-GMT) and was weighed at every feeding period. The fish were fed until satiation. Records of feed consumed and fish weights were kept to calculate feed conversion ratio and feed intake throughout the experiment.

This study followed a Completely Randomized Design (CRD) for 84 days. Water was obtained from the University of Ibadan water depot. Each holding facility was sufficiently oxygenated by using aerators (Cosmos aquarium air pump, double type 3500 50 Hz, 2.5-3 W) as described by Lashkar et al. (2011). Water quality indices were monitored with the use of dissolved oxygen meters (Jenway 3015DO meter, 0.01 accuracy, Genway, Staffordshire, UK), mercury-in-glass thermometers (producer Paragon Scientific Ltd, Birkenhead, Wirral, UK) and pH meters (Jenway 3015pH meter, 0.01 accuracy, Genway, Staffordshire, UK). This was carried out after the standardization of each meter.

Procurement of probiotic candidates
Samples from fermented cow milk (wara) were collected and stored in ice according to Iranmanesh et al. (2014). Homogenised samples of these products were diluted serially by using 10-fold serial dilution in peptone water (Rapids Lab, Essex, England). De Man, Rogosa and Sharpe (MRS) agar (Rapids Lab, Essex, England) and De Man, Rogosa and Sharpe (MRS) broth (Rapids Lab, Essex, England) were prepared and used for bacterial growth according to the manufacturer's recommendations. These were incubated under anaerobic conditions for 24-48 hours at 30°C. The pH of the media was regulated to 5.5 using 0.1 normal sodium hydroxide (N NaOH) and 0.1 normal hydrogen chloride (N HCl). Representative colonies on the MRS agar plates were selected and identification of LAB was presumptively carried out by physiological and biochemical tests. Isolates were re-streaked out on MRS agar medium to collect a pure culture. The culture was maintained on MRS agar plates and in MRS broth and stored at 4°C. These cultures were reactivated on MRS agar for 24 h before experimental use (Mourad and Eddine, 2006; Todorov et al., 2011).

Molecular characterisation
Extraction of Deoxyribonucleic acid (DNA) was carried out by employing the method outlined by Saraniya and Jeevaratnam (2012). Polymerase Chain Reaction (PCR) was carried out with 10 µL of 5x GoTaq colorless reaction buffer, 3 µL of $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'-AAGGAGGTGATCCAGCC-3' Primers. PCR was carried out in a GeneAmp 9700 PCR Sys-

tem Thermal cycler (Applied Biosystems Life Technologies Corporation, Carlsbad, USA). PCR profile had the first denaturation at 94°C for 5 min for 30 cycles, then at 94°C for 30 sec, 50°C for 60 sec and 72°C for 90 sec. The last extension was at 72°C for 10 min and the extracted DNA was iced at 4°C in gel (Agaliya and Jeevaratnam, 2013).

Imaging

A 1% Agarose gel (Thermo Fisher Scientific, Vigo, Spain) run was used to confirm the integrity of the amplified product of the roughly 1.5 Mb fragment. The process was done with a mixture of 8 µL of amplified product with 4 µL of loading dye. This was run for about an hour on solidified Agarose gel (Thermo Fisher Scientific, Vigo, Spain) at 110 V. Afterwards, quantification of the concentrated product was carried out using a nano drop of model 2000 (Applied Biosystems Life Technologies corporation, Carlsbad, USA) (Agaliya and Jeevaratnam, 2013).

Purification of amplified product

PCR reagents were removed from the DNA extraction with the use of ethanol (Presco-Beam Roanke,Virginia, USA) to purify the amplified fragments. Seven point six microliters of sodium acetate 3M, 240 µL of 95% ethanol and 40 µL PCR amplified product were added to a clean 1.5 µL tube eppendorf. It was vortexed and kept at -20°C for about 30 min. After this, it was centrifuged for 10 min at 13000xg and kept at 4°C. This was followed by the removal of supernatant, washing of the pellet with 150 µL of 70% ethanol (Presco-Beam Roanke, Virginia, and USA). The mixture was centrifuged at 7500 g for 15 min and at 4°C. The supernatant was removed and content was turned-over onto paper tissue and then dried in a fume hood at room temperature for 10-15 min. This was re-suspended in 20 µL of distilled water and kept at -20°C before sequencing. The purified fragment, which was examined on a 1.5% Agarose gel and run at 110 V for about 1 h, confirmed the presence of the purified product (Hata et al., 2010).

Sequencing

A Genetic Analyzer 3130xl sequencer (Applied Biosytems Life Technologies Corporation, Carlsbad, USA) was employed in sequencing the amplified fragments. The kit used was Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems Life Technologies Corporation, Carlsbad, USA). All genetic analyses were carried out using Bio- Edit software and MEGA 6 (Tom hall, North Carolina State University, USA) (Hata et al., 2010).

Plant collection and identification

Guava leaves were purchased from a reputable botanical garden in Akure, Ondo State, Nigeria. These leaves were identified by the Forestry Herbarium of Forestry Research Institute of Nigeria (FRIN) with the identification number 110937 (Sang et al., 2011).

Guava leaf preparation

Guava leaves of 1000 g were thoroughly washed with distilled water and air-dried for three weeks between 20-25°C. Thereafter, these were ground finely using an electric grinder and kept in an air-tight jute bag until used (Sang et al., 2011).

Diet preparation

Fish feed ingredients were obtained from a well-established fish farm in Ibadan, Nigeria. A basal diet of 42% crude protein was prepared by using Pearson Square Method. Guava leaves and single-cell protein of LAB were included in feed ingredients. These were mixed, ground and pelletized using water and a binding medium. A 2mm die was employed in a paste extrusion process, the paste formed was sun-dried and kept in a jute bag for later use. The feed ingredients used for the experiment included: soybean meal, groundnut cake, fishmeal, wheat meal, yellow maize, palm oil, common salt, and a vitamins and minerals premix. There were 7 experimental diets prepared including a control diet. *L. plantarum* and guava leaf meal were added as feed additives at various concentrations as stated below according to Ajani et al., (2011); Owodeinde and Ndimele (2011).

Control- Basal diet of 42% crude protein level

40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed

60 LPc-Basal diet+60 cells/1000 mL *L. plantarum* per kg of feed

80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed

40 PGLM-Basal diet+40 g/1000 g guava leaf meal per kg of feed

60 PGLM-Basal diet+60 g/ 1000 g guava leaf meal per kg of feed

80 PGLM-Basal diet+80 g/1000 g guava leaf meal per kg of feed

Feeding experiment

The feeding trial was conducted for 84 days in a semi-static environment. Fish were fed to satiation two times a day (8:00 am and 6:00 PM GMT). Left-over feed was removed from each facility and the polluted water was replaced with clean water. Water quality indices monitored included dissolved oxygen, pH, and temperature of each aquarium biweekly. Body lengths and weights of the fish were recorded fortnightly as the growth the fish increased.

Biological evaluation

Below are the growth indices taken during the experiment according to Hassan and Ngaski (2007).

Mean weight gain (MWG):

MWG= (W2-W1) g

Where W1= Initial mean weight (g)

W2= Final mean weight (g) (Brown, 1957)

Specific growth rate (SGR)

$$SGR\ (\%) = \frac{\text{Loge W2-logeW1 x100}}{\text{T2-T1}}$$

Where W2=Final weight (g) at time T2 (end of experiment)

W1=Initial weight (g) at time T1 (Beginning of the experiment)

Loge=Natural Logarithm (Brown, 1957)

Percentage weight gain (PWG)

$$PWG\ (\%) = \frac{\text{Mean weight gain} \times 100}{\text{Final mean weight}}$$ (Brown, 1957)

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Dry weight of fish fed (g)}}{\text{Fish weight gain (g)}}$$ (Halver, 1972)

Protein efficiency ratio (PER)

$$PER = \frac{\text{Wet weight Gain}}{\text{Protein fed}}$$

Where protein fed [percentage of protein in diet x total diet consumed/100. (Sandre et al., 2017)

Feed intake (g) = Total Feed consumed by juvenile *C. gariepinus* throughout the 84 day experimental period

Analytical methods

Proximate analyses of fish carcasses and experimental diets were carried out in the Department of Animal Science of University of Ibadan, Nigeria using the methods outlined by the AOAC (1990).

Statistical analysis

Biological indices were subjected to One-way ANOVA using SPSS version 20.0. (International Business Machines, New York, USA). Duncan multiple range tests were used to assess the differences among individual means.

Results and Discussion

Percentage body constituents of *C. gariepinus* after the 84 days feeding trial

Table 1 shows the total composition of the feed formulated. The results in Table 2 show the carcass composition of *C. gariepinus* fed experimental diets for 84 days. With the exception of the control, there was no significant difference ($p>0.05$) in the carcass crude protein content across all treatment groups. There was no significant difference ($p>0.05$) in carcass ash, carcass ether extract, carcass moisture content and carcass crude fiber content across all treatment groups. Control (17.78±0.12) and 80 PGLM (17.73±0.12) had the highest level of carcass nitrogen-free extract without any significant difference ($p>0.05$).

Growth performance and nutrients utilization of juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* leaf based diets for 84 days

Table 3 shows the growth performance and nutrients utilization of juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* leaf based diets for 84 days.

Table 1. Total ingredient constituents (g/1000g diet) of *L. plantarum* and *P. guajava* at various inclusion levels

Feed Ingredients (g)	T1&T2	40 LPc	60 LPc	80 LPc	40 PGLM	60 PGLM	80 PGLM
Fish meal	127.6	127.6	127.6	127.6	127.6	127.6	127.6
Groundnut cake	255.1	255.1	255.1	255.1	255.1	255.1	255.1
Soybean meal	392.7	392.7	392.7	392.7	392.7	392.7	392.7
Yellow maize	34.7	34.7	34.7	34.7	34.7	34.7	34.7
Wheat meal	99.9	99.9	99.9	99.9	99.9	99.9	99.9
Guinea corn	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Starch	5	5	5	5	5	5	5
Palm oil	10	10	10	10	10	10	10
Premix	10	10	10	10	10	10	10
Salt	5	5	5	5	5	5	5
Total	1000	1000	1000	1000	1000	1000	1000
L. plantarum (cells/1000 mL)	0	40	60	80	0	0	0
Guava leaves (g/1000 g)	0	0	0	0	40	60	80

Control- basal diet of 42% crude protein level, 40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed, 60 LPc-Basal Diet+60 cells/1000 mL *L. plantarum* per kg of feed, 80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed, 40 PGLM-Basal diet+40 g/1000 g guava meal per kg of feed, 60 PGLM-Basal diet+60 g/1000 g guava meal per kg of feed, 80 PGLM-Basal diet+80 g/1000 g guava meal per kg of feed.
Premix Composition: Vitamin A=20.500.00 IU, Vitamin B1=20,00.00 mg, Vitamin B2=15,000.00 mg, Vitamin B3=90.000.00 mg, Vitamin B4=4.000.00 mcg, Vitamin B5=40.00 mg, Vitamin B6=20.000.00 mg, Vitamin B=500.00 mcg, Vitamin B12=15.00 mcg, Vitamin C=350.000.00 mg, Vitamin D3=4.250.00.00 IU, Vitamin E=250.00000.00 IU, Vitamin K=8.000.00 mg, Copper Sulphate=4.000.00 mg, Inositol=50.000.00 mcg, Potassium Iodine=2.000.00 mg, Inositol=50.000.00 mg, Methionine=50.000.00 mg, Choline Chloride=600.000.00 mg, Ferrous Sulphate=40.000.00 mg, Manganese oxide=30.000.00 mg, Magnesium=60.000.00 mcg, Molybdenum=100.00 mg, Antioxidant=125.000.00 mg, Lysine=50.000.00 mg, Cobalt=750.00 mg, Sodium Selenite=200.00 mcg, Zinc oxide= 40.000.00 mg

Table 2. Carcass composition of *C. gariepinus* fed *L. plantarum* and guava (*P. guajava*) leaf diets at varying inclusion levels for 84 days

Parameters (%)	Initial	Control	40 LPc	60 LPc	80 LPc	40 PGLM	60 PGLM	80 PGLM	Sig.
Crude Protein	23.13±0.01	50.08±0.01^{b}	53.71±0.01^{a}	52.57±0.01^{a}	52.07±0.01^{a}	53.68±0.01^{a}	52.13±0.01^{a}	52.07±0.01^{a}	0.99
Ash	8.97±0.01	11.31±0.01	10.25±0.04	11.88±0.01	10.39±0.01	10.93±0.01	10.42±0.01	10.39±0.01	0.96
Ether Extract	7.46±0.02	9.55±0.01	9.36±0.01	9.11±0.01	10.59±0.01	9.80±0.01	10.07±0.01	9.80±0.01	0.95
Moisture	6.30±0.01	5.29±0.01	5.86±0.01	5.25±0.01	5.48±0.04	6.25±0.01	5.49±0.01	5.01±0.01	0.97
Crude Fibre	5.06±0.26	6.07±0.12	5.00±0.00	6.00±0.00	5.81±0.02	5.00±0.00	6.00±0.00	5.07±0.12	0.89
NFE	49.87±0.26	17.78±0.12^{a}	16.24±0.02^{b}	15.98±0.01^{c}	15.73±0.04^{c}	15.02±0.13^{c}	16.02±0.01^{b}	17.73±0.12^{a}	0.03

^{a,b,c} Mean values in each row lacking a common superscript differ ($p<0.05$)

NFE: Nitrogen-Free-Extract, Control-Basal diet of 42% crude protein level, 40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed, 60 LPc-Basal Diet+60 cells/1000 mL *L. plantarum* per kg of feed, 80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed, 40 PGLM-Basal diet+40g/1000g guava meal per kg of feed, 60 PGLM-Basal diet+60g/1000g guava meal per kg of feed, 80 PGLM-Basal diet+80g/1000g guava meal per kg of feed.

Table 3. Growth performance and nutrients utilization of *C. gariepinus* juveniles fed *L. plantarum* and *P. guajava* leaf based diets for 84 days

Parameters	CONTROL	40 LPc	60 LPc	80 LPc	40 PGLM	60 PGLM	80 PGLM	Sig.
IMW (g)	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	1.00
FMW (g)	67.10±2.06^{cd}	81.31±2.29^{a}	70.02±0.17^{bc}	69.96±2.35^{b}	70.89±0.11^{bc}	65.54±2.87^{d}	53.89±0.10^{e}	0.04
MWG (g)	59.31±2.05^{c}	73.52±2.30^{a}	62.22±0.18^{bc}	62.16±2.35^{bc}	63.10±0.10^{b}	57.74±2.86^{cd}	46.10±0.12^{d}	0.01
%MWG	88.39±2.97^{bc}	90.42±2.94^{a}	88.86±0.32^{b}	88.85±3.34^{b}	89.01±0.03^{b}	88.10±4.35^{bc}	85.54±0.30^{c}	0.03
F I (g)	138.80±0.52^{e}	166.42±2.30^{a}	147.61±0.18^{c}	146.44±0.40^{c}	153.12±0.23^{b}	143.18±2.18^{d}	109.64±0.14^{f}	0.02
F C R	2.34±0.53	2.26±0.81	2.37±0.43	2.36±0.68	2.43±0.53	2.48±0.10	2.38±1.83	0.87
SGR (g/day)	1.11±0.02^{cd}	1.19±0.02^{a}	1.14±0.00^{bc}	1.13±0.02^{bc}	1.14±0.00^{bc}	1.10±0.02^{d}	0.99±0.00^{e}	0.03
SR (%)	93.80±4.98^{c}	98.81±2.69^{a}	97.62±3.40^{a}	96.42±4.51^{ab}	98.33±2.89^{a}	97.62±3.01^{a}	96.77±4.09^{bc}	0.02
PER	1.01±0.36^{a}	1.00±0.38^{ab}	1.00±0.00^{ab}	1.01±0.36^{ab}	0.98±0.00^{ab}	0.95±0.36^{b}	1.00±0.00^{ab}	0.02
NM	1449.27±811.47	1633.81±953.08	1474.91±841.69	1485.24±835.00	1523.97±853.91	1444.18±789.04	1147.41±621.12	0.85

$^{a, b, c}$ Means within the same row lacking a common superscript differ ($p<0.05$)

IMW: Initial Mean Weight; FMW: Final Mean Weight; MWG: Mean Weight Gain; FI: Feed Intake; FCR: Feed Conversion Ratio; SGR: Specific Growth Rate, PER: Protein Efficiency Ratio; SR: Survival Rate; NM: Nitrogen metabolism. Control- Basal diet of 42% crude protein level, 40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed, 60 LPc-Basal Diet+60 cells/1000 mL *L. plantarum* per kg of feed, 80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed, 40 PGLM-Basal diet+40g/1000g guava meal per kg of feed, 60 PGLM-Basal diet+60g/1000g guava meal per kg of feed, 80 PGLM-Basal diet +80g/1000g guava meal per kg of feed, significance.

There was significant difference ($p<0.05$) in the mean weight gain (g) across all treatments in Table 3. The highest value of final weight gain was 40 LPc (81.31±2.29 g) and least final weight was 80 PGLM (53.89±0.10 g). A significant difference ($p<0.05$) was observed in the final weight among control (87.10±2.06 g), 40 PGLM (70.89±0.11 g) and 60 PGLM (65.54±2.87 g).

The highest percentage mean weight gain was 40 LPc (90.42±2.94%) with a significant difference ($p<0.05$) compared to 60 PGLM (88.10±4.35%) and 80 PGLM (85.54±0.34%). The treatments with least mean weight gain were 60 PGLM (88.10±4.35%) and 80 PGLM (85.54±0.34%) with no significant difference ($p>0.05$). Feed intake was highest in 40 LPc (166.42±2.30 g) and least in 80 PGLM (109.64±0.14 g) with a significant difference ($p<0.05$). No significant difference ($p>0.05$) was obtained in the feed conversion ratio across all treatments. The highest specific growth rate was 40 LPc (1.19±0.02 g/day) with a significant difference ($p<0.05$) from all other treatments. There was no significant difference ($p>0.05$) in survival rate among 40 LPc (98.81±2.69%), 60 LPc (97.62±3.40%), 80 LPc (96.42±4.51%), 40 PGLM (98.33±2.89%), and 60PGLM (97.62±3.01%). There was a significant difference ($p<0.05$) in the survival rate between control (93.80±4.98%) and 80 PGLM (96.77±4.09%). There was significant difference ($p<0.05$) in the protein efficiency ratio of control (1.01±0.36) and 60 PGLM (0.95±0.36). No significant difference ($p>0.05$) was observed in the nitrogen metabolism across all treatment groups.

This research evaluates the impact of *L. plantarum* and *P. guajava* leaf diets on growth response and nutrient utilisation of *C. gariepinus* juveniles. The results of this experiment revealed that *L. plantarum* and *P. guajava* leaf diets enhanced growth performance of fish appreciably. This conforms to the result of El-Haroun (2007) who observed that there was a faster growth

rate in *C. gariepinus* fingerlings fed diets supplemented with commercial feed additive than those fed control diets. This was confirmed further, in the result of Olmedo-Sanchez et al. (2009) who reported a faster growth rate of Shrimps (*Paneaus indicus*) fed feed supplemented with additives.

These results suggest that dietary additions of *L. plantarum* and *P. guajava* leaf diets at varying levels can enhance fish growth. Protein Efficiency Ratio (PER) and gross feed conversion efficiency (GFCE) measure the efficient conversion of protein sources in fish feed into body tissues in fish (DeSilva and Anderson, 1995). However, from this experiment, there was good growth performance across all treatments in relation to their weights, FCR, SGR, PER and FI. This may be due to the minerals present in guava leaves such as calcium, potassium, magnesium and phosphorus. Additionally, the presence of phytochemicals such as terpenoids, steroids, flavonoids and phenols in guava leaf diets may have contributed to the good growth performance of treatments fed guava leaf diets (NRC, 2011).

The low level of weight gain in 80 PGLM (80g/1000g) may have been due to the presence of anti-nutrient substances, which are present in plants such as saponins, tannins and anti-vitamins. They have been proven to disturb the gastro-intestinal-tract (GIT) of animals. These may negatively affect the feeding efficiency and the process of digestion in fish leading to low nutrient utilisation. This culminates to low feed intake which manifests in the slow growth response of fish (NRC, 2011). Probiotics such as *L. plantarum* aid in improvement of digestion in animals which is stimulated by digestive enzyme production. Improvement of digestion could also be through other routes such as the gut environment of the fish. This invariably is transformed into improved growth performance in fish. Hence, a good feed conversion ratio is influenced by feed absorption and utilisation (Welker and Lim, 2011).

A confirmatory report in the study conducted by Lashkar et al. (2011) and Waché et al. (2006) revealed that live yeast addition into fish diet as a probiotic improved the growth performance and food efficiency ratio. This is in agreement with the result of this experiment, which showed a better weight gain in *L. plantarum* diet (40LPc) compared to *P. guajava* and the control diets. This may have been caused by the increased appetite of fish, which led to a better body composition of fish fed dietary *L. plantarum* compared to other additives. Contrary to the results of this study, Albuquerque et al. (2014) and Moura (2011) reported that there were no significant differences in the addition of dietary probiotics on the growth performances of Nile Tilapia fingerlings of GIFT strain. Reports from Nwanna et al. (2012) and Nwanna et al. (2013) showed improved growth performance in fish fed probiotics fortified diet. Further results of Abdel-hamid et al. (2009), Diab et al. (2002) who administered commercial feed additives to fish and reported that the survival of *C. gariepinus* was enhanced by feed additives supplementation confirmed the results of this experiment.

Conclusion

From the findings of this study, it can be deduced that the inclusion of 40 cells/1000 mL of *L. plantarum* in the diet of *C. gariepinus* resulted in enhanced growth when compared to those of *P. guajava* leaf diets.

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References

Abdel-hamid, A.M., Mehrim, A.I., El-barbary M.I., Ibrahim, S.M., Abd El-wahab, A. I. 2009. Evaluation of a new Egyptian probiotic by African catfish fingerlings. Journal of Environment Science Technology 3, 133-145.

Adegbehingbe, K.T., 2013. Fermented sprouted and unsprouted maize for ogi production. International Journal of Advanced Research 1, 428-434.

Adewolu, M.A., Adeniji, C.A., Adejobi, A.B., 2008. Feed utilization, growth and survival of *Clarias gariepinus* (Burchell 1822) fingerlings cultured under different photoperiods. Aquaculture 283, 64-67. [CrossRef]

Agaliya, P., Jeevaratnam, K., 2013. Molecular characterization of *Lactobacilli* isolated from Idli batter. Brazilian Journal of Microbiology 44, 1199-1206. [CrossRef]

Ajani, F., Dawodu, M.O., Bello-Olusoji, O.A., 2011. Effects of feed forms and feeding frequency on growth performance and nutrient utilization of *Clarias gariepinus* fingerlings. African Journal of Agricultural Research 6, 318-322.

Albuquerque, D.M., Marengoni, N.G., Mahli, I. Riberro, R.P., 2014. *Bacillus* in diets for fingerlings of nile tilapia, Gift variety. Biosciences Journal 31, 532-540. [CrossRef]

Arimah, B.D., Ogunlowo, O.P., 2014. Identification of lactic acid bacteria isolated from Nigerian foods: medical importance and comparison of their bacteriocins activities. Journal of Natural Science Research 4, 76-86.

AOAC., 1990. AOAC method 973.18-Fiber (Acid Detergent) and Lignin in Animal Feeds. K. Helrick. Ed. Official method of analysis of the association of official analytical chemists 15th ed., vol. 82. Arlington, VA: Association of Official Analytical Chemists.

Bondad, M.G., Subasinghe, R.P., Arthur, J.R., 2005. Disease and health management in Asian aquaculture. Veterinary Parasitology 132, 249-272. [CrossRef]

Brown, M.E., 1957. Metabolism. In: (W. S. Hoar, Randal, D.D. (Eds.). Physiology of fishes. New York. Academic Press.

De Silva, S.S., Anderson, T.A. 1995. Fish Nutrition in Aquaculture. Chamann and Hall Aquaculture Series, UK, p. 319.

Diab, A.S., EL-Nagar, O.G., Abd-El-Hady, M.Y., 2002. Evaluation of *Nigella sativa* L. (black seeds; baraka), *Allium sativum* (garlic) and Biogen as a feed additive on growth performance of *Oreochromis niloticus* fingerlings. Veterinary Medicine Journal Suez Canal University 2, 745-753.

El-Haroun E.R., 2007. Improved growth rate and feed utilization in farmed African catfish *Clarias gariepinus* (Burchell 1822) through a growth promoter Biogen® supplementation. Journal of Fisheries and Aquatic Science 2, 319-327. [CrossRef]

Guz, L., Sopinska, A., Oniszczuk, T., 2011. Effect of *Echinacea purpurea* on growth and survival of guppy (*Poecilia reticulata*) challenged with *Aeromonas bestiarum*. Aquaculture Nutrition 6, 695-700. [CrossRef]

Halver, J.E., 1972. Fish Nutrition. Academic Press Incorporated New York 71, p. 6.

Hassan, L.G., Ngaski, M.M.A., 2007. Nutritional evaluation of Cassia siamea leaves. Journal of Chemical Society of Nigeria 32,137-143.

Hata, T., Tanaka, R., Ohmomo, S., 2010. Isolation and characterization of plantaricin ASM1: a new bacteriocin produced by *Lactobacillus plantarum* A-1. International Journal of Food Microbiology 137, 94-99. [CrossRef]

Iranmanesh, M., Ezzatpanah, H., Mojghani, N., 2014. Antibacterial activity and cholesterol assimilation of lactic acid bacteria isolated from traditional Iranian dairy products. LWT - Food Science and Technology 58, 355-359. [CrossRef]

Lashkar, B.M., Jafaryan, H., Faramarzi, M. and Adineh, H. 2011. The effects of Amax yeast fed to Persian sturgeon (*Acipencer persicus*) larvae via bioenrichment of Daphnia magna. Aquaculture Aquarium Conservation and Legislation Bioflux 4, 361-367.

Mourad, K., Nour-Eddine, K., 2006. In vitro pre-selection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin. International Journal of Probiotics and Prebiotics 1, 27-32.

Moura, M.C., 2011. *Bacillus cereus* var.toyoi and *Bacillus subtilis* C-3102 in the Nile tilapia culture of the GIFT strain-Master's degree in Fishery Engineering and Fishing Resources-Western State University of Paraná, Paraná p: 58.

NRC (National Research Council)., 2011. Nutrient requirements of fish and shrimp. Washington DC, National Academy Press p. 392.

Nwanna, L.C., Ajani, E.K., Bamidele, S.F., 2014. Use of lactic acid bacteria from Nile tilapia *Oreochromis niloticus* as probiotics for sustainable production and improvement in fish welfare. The Israeli Journal of Aquaculture-Bamidgeh 66, 1-12.

Nwanna, L.C., Lemme, A., Metwally, A., Schwarz, F., 2012. Response of common carp (*Cyprinus carpio L.*) to supplemental DL-Methionine and different feeding strategies. Aquaculture 357, 365-370. [CrossRef]

Olmedo-Sanchez, J.A., Curiel-Flores, A., Orozco, J.R., 2009. The effect of an herbal growth promoter feed additive on shrimp performance. Research Journal of Biological Science 4, 1022-1024.

Owodeinde, F.G., Ndimele, P.E., 2011. Survival, growth and feed utilization of two clariid catfish (*Clarias gariepinus,* Burchell 1822 and *Heterobranchus bidorsalis*, Geoffroy, 1809) and their reciprocal hybrids. Journal of Applied Ichthyology 27, 1249-1253. [CrossRef]

Sandre, L.C.G., Buzollo, H., Nascimento, M.T., Neira, L.M., Jomori, R.K., Carneiro, D.J., 2017. Productive performance and digestibility in the initial growth phase of tambaqui (*Colossoma macropomum*) fed diets with different carbohydrate and lipid levels. Aquaculture Reports 6, 28-34.

Sang, H.M., Fotedar, R., Filer, K., 2011. Effects of dietary mannan oligosaccharide on the survival, growth, immunity and digestive enzyme activity of freshwater crayfish, *Cherax destructor* Clark (1936). Aquaculture Nutrition 17, 629-635. [CrossRef]

Saraniya, A., Jeevaratnam, K., 2012. Molecular characterization of bacteriocinogenic *Lactobacillus* species from fermented Uttapam batter. Biosciences Biotechnology Research Asia 9, 417-421. [CrossRef]

Sayed, S.H., Zakaria, A., Mohamed, G.A., Mohamed, K. K., 2011. Use of Probiotics as growth promoter, anti-bacterial and their effects on the physiological parameters and immune response of *Oreochromis niloticus* Lin. fingerlings. Journal of Arabian Aquaculture Society 6, 201-222.

Shalaby, A.M., Khattab, Y.A., Abdel-Rahman, A.M., 2006. Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia. Journal of Venomous Animals and Toxins including Tropical Diseases 12, 172-201. [CrossRef]

Todorov, S.D., Rachman, C., Fourrier, A., Dicks, L.M.T., van Reenen, C.A., Prévost, H., Dousset, X., 2011. Characterisation of a bacteriocin produced by *Lactobacillus sakei* R1333 isolated from smoked salmon. Anaerobe 17, 23-31. [CrossRef]

Waché, Y., Auffray, F., Gatesoupe, F.J., Zambonino, J., Gayet, V., Labbé, L., 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss* fry. Aquaculture 258, 470-478. [CrossRef]

Welker, T. L., Lim, C., 2011. Use of probiotics in diets of tilapia. Journal of Aquatic Research and Development S1:014, 1-8.