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Growth performance of *Clarias gariepinus* (Burchell, 1822) Fed Diets Fortified with Lemongrass *(Cymbopogon citratus)*

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Abstract

The utilization of synthetic antibiotics in the production of food fish is a concern globally due to the development of antibiotic-resistant bacteria and the residual effects of these drugs. This study investigated the *in vitro* antimicrobial activity and growth-promoting effects of lemongrass meal (LGM) in *Clarias gariepinus* fingerlings. The fish were fed diets (40% crude protein) fortified with 0, 5, 10, and 15gLGM/kg lemongrass meal, and a 3g Oxytetracycline (OTC)/kg diet was used as the negative control for 70 days. Data on the zones of inhibition (ZI), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and optimum inclusion level was obtained. Higher ZI was obtained from OTC against *Aeromonas hydrophila*, while higher ZI was obtained from lemongrass extract against *Pseudomonas putida*. Feeding *C. gariepinus* fingerlings with10gLGM/kg diet significantly enhanced WG, SGR, and PER, compared to the control treatments. The FCR (1.40) was significantly reduced in fish fed a 10gLGM/kg diet, compared to the control and other LGM-fortified treatments. The quadratic regression analysis showed 0.83% and 0.86% as optimum inclusion levels for WG and FCR, respectively. The results from this study suggested that lemongrass possesses antibacterial properties and that the meal could be included in the fish diet at 10 g/kg as a natural growth promotant.

Keywords: Antibacterial activity, *Clarias gariepinus*, growth performance, lemongrass, oxytetracycline

Introduction

Intensification of aquaculture has led to remarkable improvements in productivity; however, this cultivation method has also been associated with diseases (Kennedy et al., 2016; Prigeon and Klesius, 2012), as a result of exposure to a variety of stressors such as sorting, size grading, transportation of fingerlings, high stocking density and poor water quality. Outbreaks of disease arising from these stressful conditions result in significant mortality and economic loss (Pridgeon et al., 2012) and, consequently, the use of synthetic antibiotics. The use of antibiotics for therapeutic / growth promotion leads to chemical residues in food fish and the development of resistant-pathogen strains (Gent et al., 2012; Santos and Ramus, 2018). The ban or regulation of the utilization of these synthetic drugs calls for a search for efficacious alternatives. Several studies have been conducted using herbal products in aquaculture and have shown the future potential of these products for enhancing the growth and health of fish (Adeniyi and Lawal, 2017; Adeniyi et al., 2017; Adeniyi et al., 2018; Saleh et al., 2014).

Lemongrass belongs to the family Poaceae. It is an aromatic perennial grass widely available in Africa (Olorunnisola et al., 2014) and is well cultivated for the medicinal value in Nigeria. Cheel et al. (2005) reported the presence of isoscoparin, swertiajaponin, and isoorientin in *Cymbopogon citratus*. Various categories of other compounds such as tannins, saponins, flavonoids, alkaloid, phenols, anthraquinones, citral, furfurol, geraniol, caffeic acid, a chlorogenic acid among others are found in lemongrass (Akhila, 2010; Bharti et al., 2013). The chemical

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composition of *C. citratus* extract varied according to geographical origin, genetic differences, the part of the plant used, method of extraction, age/stage of maturity, and the season of harvest. However, *C. citratus* contains a higher percentage (about 80%) of citral, which is a mixture of terpenoids, neral, and geranial, irrespective of geographical origin (Ewansiha et al., 2012; Nur, 2014).

Aeromonas hydrophila is ubiquitous, oxidase-positive, facultative anaerobic, glucose-fermenting, gram-negative bacteria that are native to aquatic environments. *Aeromonas* has been isolated from a variety of sources including soil, lakes, rivers, reservoirs, groundwater, and drinking water leaving the treatment plant and wound infections sustained in an aquatic environment and human stools (Anuradha et al., 2010; Newaj-Fyzul et al., 2008, Rogo et al., 2009; Saidi et al., 2013; Shayo et al., 2012; Subashkumar et al., 2006; US EPA, 2005). *Pseudomonas putida* is a rod-shaped flagellated, gram-negative bacterium found in moist soil and aquatic environments. Mortalities of about 35% in a rainbow trout culture resulting from *P. putida* infection have been reported previously (Ilhan et al., 2006). The *P. putida* was reported to be predominant among the *Pseudomonas* species isolated from wastewater discharged into the marine coastal zone with high resistance to antimicrobials (Aneta et al., 2015). Oxytetracycline is one of the antibiotics commonly used in aquaculture, and emerging antimicrobial resistance has resulted from overuse which is a public health concern (Newaj-Fyzul *et al.*, 2008; Romero et al., 2012).

Tea made from lemongrass leaves is widely used for its antifever, carminative, antiseptic, analgesic, diuretic, and stomachic, antifungal, anti-inflammatory, and antioxidant properties (Fandohan et al., 2008; Francisco et al., 2011; Mirghani et al., 2012; Negrelle and Gomes, 2007; Tatiana and Jose, 2011). African sharp-toothed catfish (*Clarias gariepinus)* is a farmed fish species of important economic value and has been widely cultured intensively in Africa, and many parts of Europe, and Asia due to fast growth, high consumer acceptance and adaptability to a wide range of environmental conditions (Al-Dohail et al., 2009; Brummett, 2008). Information regarding the use of lemongrass and the growth performance of fish is scarce. Therefore, this study investigated the antibacterial potential of lemongrass and the effect on the growth performance of *C. gariepinus*.

Materials and Methods

Plant extraction

Lemongrass was air-dried at room temperature (27°C). The lemongrass was ground into a meal (LGM) using a kitchen mill. The LGM was mixed with distilled water (1:10 w/v); then, the mixture was homogenized and left for 48 days, during which it was placed in a rotary shaker for 18 hours. Then, the mixture was centrifuged (SE-CF-TDZ-WS, Labkits, U-Therm International (Hong Kong) Limited) at 12544g for 30 minutes at room temperature. The supernatant was collected as a crude extract, filtered through Whatman No.4 filter paper, and concentrated under vacuum using a rotary evaporator (IKA® RV10 digital, Artisan Technology Group, Champaign, USA) at 90°C to reduce the content to about 1/4th. The concentrated extract was stored in a freezer prior to use (Ghassan et al., 2012).

Phytochemical and antimicrobial screening

The extract was screened qualitatively for the presence of reducing sugars, terpenoids, alkaloids, cardiac glycosides, flavonoids, saponins, and tannins as described by Sofowora (1993) and Trease and Evans (1989). 100 µL of the extract and Oxytetracycline (OTC) at 10 mg/mL were screened to determine the zone of inhibition (ZI) against *Aeromonas hydrophila* and *Pseudomonas putida* using the agar well diffusion method (CLSI, 2012). The *A. hydrophila* and *P. putida* were sub-cultured from the preserved slants for 24 hours prior to use. The 24-hour old test organisms were standardized to the 0.5 McFarland standards (10^6 CFU/mL) as described by CLSI (2012). Then, 100µl of the standardized cell suspension was spread onto Mueller-Hinton agar (Oxoid limited, Hampshire, United Kingdom) in 4mm deep plates using three replicates. Four wells were bored in each plate with a sterile 6mm diameter cork-borer. Then, 100 µL of the lemongrass crude extract at 10 mg/mL was introduced into the wells, allowed to stand at room temperature for about 30 minutes under aseptic conditions, which ensured diffusion of the extracts into the agar before microbial growth. The plates were then incubated right side up at 37°C for 24h. The controls were also set up in parallel using distilled water in the well and Oxytetracycline (OXY 200 WSP, Kepro, Deventer, Holland). The inoculated plates were observed for ZI diameter (mm).

Diet preparation

Lemongrass meal (LGM) was incorporated into five isonitrogenous (≈40%) and isocaloric (≈393 kcal/100g gross energy) diets (Table 1) at 0, 5, 10, 15g/kg diet (Ahmad and Abdel Tawwab, 2011; Bello et al., 2012), while 3g oxytetracycline (OTC)/kg diet was used as a negative control. Other feed ingredients were ground using a hammer mill and thoroughly mixed with the LGM at the specified inclusion levels to create the five diets. Each diet was formed into pellets using a pelleting machine (Shuangying SYSLJ-1, Henan, China) with 2 mm die size. The pellets were air-dried, hand crumbled into smaller sizes, packed in airtight polythene bags, labeled, and then stored in a dry environment at room temperature throughout the experimental period.

The test ingredient and diets were analyzed for proximate composition (AOAC, 2005). Briefly, the moisture content was estimated by drying the samples to a constant weight at 105°C in a drying oven (Mini/50/SS, Genlab, England) and calculated from the loss in weight. Nitrogen content was measured using an automated digestor (8 Holes, Foss Tecator digestor, Denmark) and Kjeltec auto distillation unit (Kjeltec 8200, Denmark). Then, the crude protein was estimated by multiplying the nitrogen content by 6.25. Crude lipids were determined by petroleum

Table 1. Ingredients and proximate composition (g/kg) of the experimental diets fed to *Clarias gariepinus* for 12 weeks

	Diets (g/kg)				
Ingredients	**0.0**	**3 OTC**	**5.0 LGM**	**10.0 LGM**	**15.0 LGM**
Fish meal	242.0	242.0	242.0	242.0	242.0
Groundnut cake	243.0	243.0	243.0	243.0	243.0
Soybean meal	240.0	240.0	240.0	240.0	240.0
Cornmeal	190.0	187.0	185.0	180.0	175.0
Soybean oil	15.0	15.0	15.0	15.0	15.0
DCP	10.0	10.0	10.0	10.0	10.0
Premix*	20.0	20.0	20.0	20.0	20.0
Table salt	5.0	5.0	5.0	5.0	5.0
Starch	35.0	35.0	35.0	35.0	35.0
Oxytetracycline	-	3.0	-	-	-
LGM**	-	-	5.0	10.0	15.0
Total	100.0	100.0	100.0	100.0	100.0
Proximate composition (%)					
Moisture	6.53	6.55	6.57	6.56	6.54
Crude protein	40.63	40.56	40.60	40.53	40.46
Ether extract	6.63	6.62	6.63	6.68	6.71
Crude fiber	10.09	10.09	10.21	10.33	10.46
Ash	11.54	11.53	11.58	11.61	11.66
NFE***	24.58	24.65	24.49	24.29	24.17
Gross energy****	393.24	393.03	392.60	391.95	391.35

*Vitamin and Minerals: Vitamin A –10,000,000 I.U.; D3- 2,000,000 I.U.; E –23,000mg; K3 – 2,000 mg; B1 – 3,000 mg; B2-6,000 mg; Niacin– 50,000 mg; Calcium Pantothenate– 10,000 mg; B6 – 5,000 mg; B12- 25.0 mg; Folic acid 1,000 mg; Biotin- 50.0mg; Choline chloride – 400,000 mg; Manganese – 120,000 mg; Iron- 100,000 mg; Copper– 8,500 mg; Iodine – 1,500 mg; Cobalt-300 mg; Selenium-120 mg; Antioxidant 120,000 mg.
**Moisture content=8.75% Crude protein=8.80% Ether extract=9.8% Crude fiber=7.2% ash=11.00% NFE=54.45%
***NFE=Nitrogen free extract=100 – (crude protein + ether extract + crude fiber + ash)
****Gross Energy (kcal/100g DM) calculated using 5.65, 9.45, 4.11 kcal/g for protein, lipid, and carbohydrate respectively (NRC, 1993)
OTC: oxytetracycline; DCP: dicalcium phosphate; LGM: lemongrass meal

ether extraction in a soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA). The crude fiber was estimated from dried samples after digestion of the fat-free samples using an acid and base. Ash content was estimated by combusting the dry samples in an electric muffle furnace (Shanghai Changji Geological instrument equipment Co Ltd, China) at 550°C for 6 h. The gross energy in the tested diets was calculated according to the NRC (1993).

Fish feeding and culture technique

Fingerlings of *C. gariepinus* were obtained from a fish hatchery in Ilorin and then acclimated to laboratory conditions for two weeks, during which they were fed with a commercial fish feed. Then, the fingerlings (n=225, 3.56±0.2) were distributed into fifteen 50-L plastic tanks in a completely randomized design. The fish were fed diets at a 5% body weight daily ration the daily ration was divided in two and fed to the fish twice at 8.30-9.00 and 16.30-17.00 h for 70 days. The fish tanks were cleaned regularly and completely renewed at three-day intervals with borehole water throughout the experimental period. The water temperature was monitored before the morning feed ration daily while pH and dissolved oxygen (DO) were determined biweekly using a mercury-in-glass thermometer, Hanna pH meter (pHep, HI98107, USA) and AMSTAT dissolved oxygen meter (DO-Temp., AMT07, C. V. Java Multi Mandiri, Indonesia), respectively. The range of water temperature, pH, and DO were 24.2–26.5°C, 6.8-7.2, 4.5–5.2 mg/L, respectively. The fish in each tank were monitored daily for mortality, and dead fish were removed, counted, and recorded. Batch weights as well as the weight of randomly sampled fish from each tank were taken fortnightly to assess fish growth and adjust the feed ration. The feed intake for each fish was calculated from the amount of feed consumed by the group of fish in each tank.

Fish growth performance and survival parameters

At the end of the feeding trial, the following parameters for fish growth and nutrient utilization were calculated:

i. Weight Gain (WG, g)= Final weight (FW) – Initial weight (IW)

ii. Relative Growth Rate (RGR, %)=WG / IW x 100
iii. Specific Growth Rate (SGR, %/day)=100 x (Ln FW- Ln IW) / 70
iv. Feed Conversion Ratio (FCR)=Feed intake / WG
v. Protein Efficiency Ratio (PER)= WG / Protein intake
vi. Nitrogen metabolism (Nm)=70 x (0.549) x (IW + FW) / 2 (Nwanna, 2003)
vii. Survival (%)=100 x (Number of surviving fish / Initial number of fish stocked).

Determination of some biometric indices

Following the feeding trial, four fish were sampled from each replicate and sacrificed on ice. All fish were weighed, and then the total and standard lengths of the fish were measured. The fish were then dissected; the gonads, liver, and spleen were removed and weighed. These metrics were used to calculate the following indices. I thought this suppose to be:

i. Condition factor (CF)=(Weight of fish (g) / Standard length^3 (cm)) x 100
ii. Hepatosomatic index (HI):=Weight of liver (g) / Weight of fish (g)
iii. Gonadosomatic index (GI)=Weight of gonad (g) / Weight of fish (g)
iv. Spleen-somatic index (SI)=Weight of spleen (g) / Weight of fish (g).

The experimental protocols were performed in accordance with the guidelines of the International (2010/63/EU) and College of Agriculture (KWASU/COA/SEC/2015002), Kwara State University, Malete Institutional rules on animal experimentation and biodiversity rights.

Statistical analyses

All of the statistical analyses were done using a Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Version 23, Armonk, NY, USA). The data were analyzed using a one-way analysis of variance, and the Duncan multiple range test at $p<0.05$. The optimum inclusion level of LGM with respect to WG and FCR was determined using quadratic regression analysis.

Results

Phytoconstituents and antibacterial activity of lemongrass

The qualitative phytochemical screening showed that the lemongrass contained reducing sugar, tannin, phlobatannin, terpenoids, and flavonoids. Moreover, higher ZI was obtained from OTC against *A. hydrophila*, and higher ZI was obtained from lemongrass extract against *P. putida* (Figure 1).

Growth performance and nutrient utilization

The growth performance and nutrient utilization of *C. gariepinus* fingerlings fed diets containing different levels of LGM are shown in Table 2. Fish fed diets containing 10gLGM/kg diet had significantly higher ($p<0.05$) WG and RGR compared to those fed the positive (0.0g LGM) and negative (3gOTC/kg diet) control diets. The SGR of the fish fed with diets containing 5–10gLGM/kg diet were significantly promoted, compared to the control treatments. The FCR (1.40) was significantly reduced in fish fed the 10gLGM/kg/diet, compared to the control and other LGM-fortified treatments. Also, significantly higher ($p<0.05$) PER, Nm, and survival were exhibited in fish fed 10gLGM, compared to those fed other diets. The relationships between the dietary inclusion levels of LGM with respect to WG ($Y=-12.135x^2 + 20.089x + 28.84$) and FCR ($Y=0.260x^2 - 0.448x + 1.628$) are shown in Figure 2 and Figure 3, respectively. These relationships showed 0.83% (8.3g/kg diet) and 0.86% (8.6g/kg diet) as the optimum dietary inclusion levels for WG and FCR, respectively.

Biometric indices

Feeding the fingerlings of *C. gariepinus* with diets containing LGM failed to alter CF, GI, SI, and HI (Table 3) significantly ($p>0.05$).

Discussion

This study showed the antimicrobial activity of lemongrass against *A. hydrophila* and *P. putida*. Castro et al. (2008) similarly observed the antimicrobial activity of *Cariniana legalis*, *Croton floribundus*, *Euglenia florida,* and *Myrcia velutina* against *A. hydrophila*. While OTC exhibited a higher ZI against *A. hydrophila*, the value obtained for lemongrass against the two microbes was higher than 15 mm. The ZI of ≥15 mm indicated susceptibility of these microbes to the herbal product (Tkachenko et al., 2016). Considerable antimicrobial potential for the *Mucuna* seeds, *Ficus putima* leaves, tamarind pulp, and leaf extracts against *A. hydrophila* or *P. putida* were similarly reported (Adeniyi et al., 2017; Marimuthu et al., 2015). The antimicrobial activity of lemongrass against these microbes might be due to the phytochemical components (tannin, terpenoids, flavonoids) of this plant, and the antibacterial effects of this plant extract could be attributed to the phytochemicals (Pakravan et al., 2011; Rahman et al., 2010). The mechanisms involved in the antibacterial activities of these phytochemicals were associated with the formation of complexes in the protein, inactivation of the microbial adhesions and enzymes, disruption of the microbial membrane, and inhibition of the synthesis of nucleic acids (Kumar and Pandey, 2013; Ulanowska et al., 2006).

The best growth-promoting effects of LGM on *C. gariepinus* in this study were expressed at 10 g/kg at the dietary level. While lemongrass was a widely used herb in traditional medicine, to the best of my knowledge, it has not been used for aquaculture previously. However, various other herbal additives have been utilized in fish culture and have exhibited great potential. The inclusion of guava leaf meal at 5 g/kg in the diet of *Labeo rohita* (Giri et al., 2015), cumin seed meal at 10 g/kg diet of *Oreochromis mossambicus* (Yilmaz et al., 2013), 10 g garlic or onion meal/kg diet in sea bass (Saleh et al., 2014), 10 g/kg cotton leaf meal (Adeniyi and Lawal, 2017), and 10-20

Table 2. Growth performance and nutrient utilization of *Clarias gariepinus* fed diets fortified with varying levels of lemongrass meal

	Diets (g/kg)				
Parameters	**0.0**	**3 OTC**	**5 LGM**	**10 LGM**	**15 LGM**
Initial Weight (g)	3.55±0.03	3.57±0.03	3.53±0.03	3.53±0.03	3.53±0.01
Final Weight (g)	33.00±0.22^{c}	36.51±0.52^{b}	36.13±1.31^{b}	43.22±0.66^{a}	34.24±0.64^{bc}
Weight gain (g)	29.47±0.25^{c}	32.98±0.55^{b}	32.60±1.33^{b}	39.68±0.69^{a}	30.71±0.64^{bc}
Relative growth rate (%)	934.49±14.13^{c}	1024.5±23.66^{b}	1023.28±43.97^{b}	1223.64±29.23^{a}	968.23±20.56^{c}
Specific growth rate (%/day)	1.15±0.01^{c}	1.14±0.01^{c}	1.20±0.02^{b}	1.29±0.01^{a}	1.17±0.01^{bc}
Feed intake (g)	47.66±0.93^{b}	50.83±1.64^{b}	49.29±1.33^{b}	55.30±0.33^{a}	47.85±1.27^{b}
Feed conversion ratio	1.62±0.02^{a}	1.54±0.06^{ab}	1.52±0.02^{b}	1.40±0.03^{c}	1.56±0.01^{ab}
Protein efficiency ratio	1.50±0.02^{b}	1.60±0.06^{b}	1.65±0.02^{b}	1.79±0.03^{a}	1.61±0.01^{b}
Nitrogen metabolism	842.39±4.43^{c}	924.09±11.32^{b}	914.63±29.69^{b}	1077.96±14.64^{a}	870.98±22.73^{b}
Survival (%)	86.67±3.33^{bc}	90.33±1.92^{b}	88.67±2.22^{bc}	95.67±2.88^{a}	83.33±3.85^{c}

Means along the same row with similar superscripts are not significantly different (p>0.05)

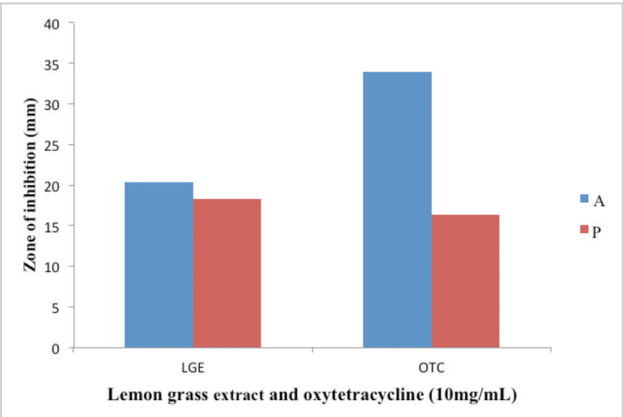


Figure 1. Zone of inhibition for lemongrass extract (LGE) and oxytetracycline (OTC) against *Aeromonas hydrophila* (A) and *Pseudomonas putida* (P)

g/kg tamarind leaf and pulp meal (Adeniyi et al., 2018), in *C. gariepinus* among others, enhanced growth performance and nutrient utilization of the fishes.

The enhanced growth and nutrient utilization obtained in the LGM-enriched diets might be due to the phytocomponents and the antimicrobial activity of this additive, which might have induced the growth of beneficial gut microflora and the digestibility of nutrients with the resulting growth promotion. Earlier studies of fish indicated that the phytoadditives stimulated digestion (Adeniyi et al., 2018; Bhosale et al., 2010; El-Dakar et al., 2015). Gut microflora has been associated with the digestive activity of fish (Bairaji et al., 2002; Gutowska et al., 2004). The antimicrobial activities of lemongrass might have reduced the pathogenic gut microflora and enhanced the colonization of the beneficial gut flora, which promoted digestion of feed, absorption of nutrients, higher growth performance, and improved the survival rate of fish fed the LGM-fortified diets.

However, the results from this study showed that dietary LGM above 10 g/kg reduced growth performance while the quadratic regression analysis estimated the optimum inclusion level for maximum growth and feed utilization to be 0.83%, 0.86% (8.3 g, 8.6 g/kg) in the diet of *C. gariepinus*. Recent studies on the estimated optimum inclusion levels of 17.2g tamarind leaf meal, 30.2 g/kg tamarind pulp meal/kg diet (Adeniyi et al., 2018), 12 g clove basil extract/kg diet (Abdel Tawwab et al., 2018), and 1.79% *Aloe vera* polysaccharide crude extract (Gabriel et al., 2019) in *C. gariepinus* were higher than the levels obtained in this present study. The diminishing growth-promoting effect of LGM in this study suggested a higher content of antinutritional factors at a higher level, which reduced feed digestibility, utilization, and hence growth. Similar results were reported in fish fed diets containing cinnamon (Ahmad et al., 2011), caraway seeds (Ahmad and Abdel-Tawwab, 2011), and cotton leaves (Adeniyi and Lawal, 2017). The reduction in growth performance at the higher dietary inclusion of herbal additives in fish might be ascribed to the formation of antinutritional factor-protein complexes, reduction in feed palatability, intake, reduction in gut protein availability, and digestion as well as inhibition of digestive enzymes (Becker and Makkar, 1999; Mandal and Ghosh, 2010; Omnes et al., 2017).

The health status of fish is often measured by organ-somatic indices and condition factors (Fox et al., 1997). Measurement of the organ-somatic indices were important for assessing the quality and the safety of the food, and the environment that the fish were exposed to, because of the significant roles of these organs in food absorption, metabolism, as well as the synthesis

Table 3. Biological indices of *Clarias gariepinus* fed varying levels of lemongrass meal

	Diets (g/kg)				
Parameters	**0.0**	**3 OTC**	**5.0 LGM**	**10.0 LGM**	**15 LGM**
Condition factor	1.007±0.063	1.090±0.113	1.056±0.038	1.124±0.013	1.052±0.792
Hepatosomatic index	0.013±0.007	0.008±0.000	0.009±0.004	0.008±0.002	0.008±0.050
Gonadosomatic index	0.007±0.002	0.006±0.002	0.009±0.006	0.006±0.006	0.006±0.002
Spleenosomatic index	0.002±0.001	0.003±0.001	0.002±0.001	0.002±0.001	0.002±0.000

Means without superscript are not significantly different (p>0.05)

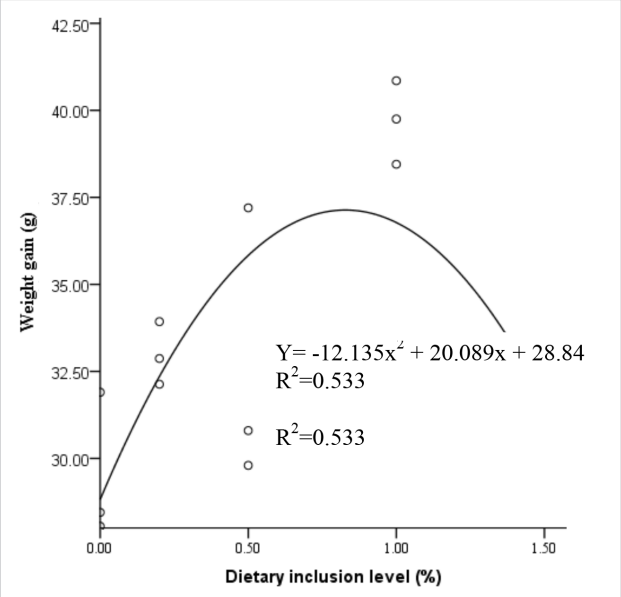


Figure 2. The relationship between the dietary levels of lemongrass meal, the control diets, and weight gain for *Clarias gariepinus*

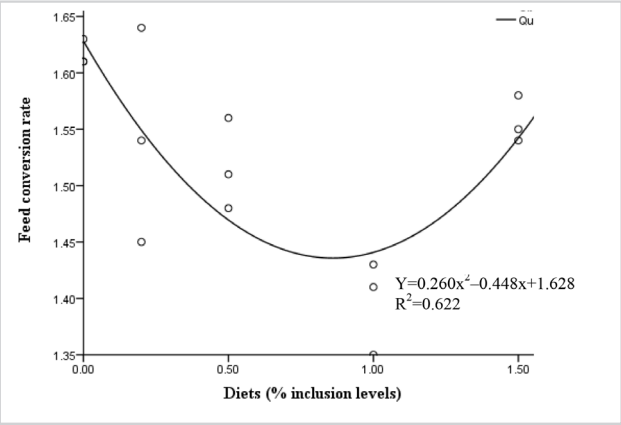


Figure 3. The relationship between the feed conversion ratio of *Clarias gariepinus* fed dietary levels of lemongrass meal and the control diets

and secretion of enzymes (Abdel-Hameid, 2007; McLaughlin, 1983). The spleen plays an essential role in the immune system of fish, especially in the production of blood cells and melano-macrophages (Anderson, 1974; Kumaran et al., 2010), while a positive correlation between the spleen-somatic index has been reported (Wiens and Vallejo, 2010). A significant increase or reduction of these indices in fish could be a reflection of the presence of antinutritional compounds or toxins in the diet at a quantity high enough to disrupt weight and the physiological status of the organs. The non-significant alteration observed for these indices in the fish fed the LGM-fortified diets might evince the safety of this herbal product. Similar observations were reported on the organ-somatic indices of fish fed with herbal additives (Adeniyi et al., 2017; Bello et al., 2012; Cho, 2011; El-Dakar et al. 2015). However, dietary inclusion of some plant-based products significantly reduced (Adesina, 2017; Tulli et al., 2012; Yilmaz et al., 2013; Yilmaz et al., 2017) or increased (Akerman et al., 2003; Prusty et al., 2007; Zheng et al., 2009) some of these indices.

Conclusion

Lemongrass meal has demonstrated *in vitro* antibacterial properties when included in the normal diet of *Clarias gariepinus* at 10 g/kg. Moreover, this supplement enhanced the growth performance and nutrient utilization without significant variation in the organ-somatic indices. Further research into the effects of lemongrass on the histopathological changes and the immune responses of fish are still required.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Kwara State University (Protocol No: KWASU/COA/2015002).

Informed Consent: N/A.

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Conflict of Interest: The author have no conflicts of interest to declare.

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References

Abdel-Hameid, N.A.H., 2007. Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* iuveniles. Turkish Journal of Fisheries and Aquatic Sciences 7, 131-138.

Abdel-Tawwab, M., Adesina, I., Jenyo-Oni, A., Ajani, E.K., Emikpe, B.O., 2018. Growth, physiology, antioxidants and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection. Fish and Shellfish Immunology 78, 346-354. [CrossRef]

Adeniyi, O.V., Lawal, A.O., 2017. Growth performance of African catfish fed diet supplemented with *Gossypium herbacium* powder as dietary feed additive. Journal of Agriculture and Ecology Research International 11, 1-7. [CrossRef]

Adeniyi, O.V., Olaifa, F.E., Emikpe, B.O., 2017. Effect of *Tamarindus indica* (Linn, 1753) pulp and leaf- fortified diets on experimental *Aeromonas hydrophila* infection in *Clarias gariepinus* (Burchell, 1822). Bulletin of Animal Health and Production in Africa 65, 623-634.

Adeniyi, O.V., Olaifa, F.E., Emikpe, B.O., 2018. Growth performance and nutrient digestibility of *Clarias gariepinus* (Burchell 1822) fed diets fortified with *Tamarindus indica* pulp and leaf meal. Asian Fisheries Science 31, 17-31.

Adesina, S.A., 2017. Assessment of organosomatic indices and histopathological changes in vital organs of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower (*Helianthus annuus*) seed meal. Ife Journal of Science 19, 85-98. [CrossRef]

Ahmad, M.H., Abdel-Tawwab M., 2011. The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization and whole body composition of Nile tilapia, *Oreochromis niloticus* (L) fingerlings. Aquaculture 314, 110-114. [CrossRef]

Ahmad, M.H., ElMesallamy, A.M.D., Samir, F., Zahran, F., 2011. Effect of cinnamon (*Cinnamomum zeylanicum*) on growth performance, feed utilization, whole-body composition, and resistance to *Aeromonas hydrophila* in Nile tilapia. Journal of Applied Aquaculture 23, 289-298. [CrossRef]

Akerman, G., Amcoff, P., Tjarnlund, U., Fogelberg, K., Torrissen, O., Balk, L., 2003. Paraquat and menadione exposure of rainbow trout (*Oncorhynchus mykiss*): Studies of effects on the pentose phosphate shunt and thiamine levels in liver and kidney. Chemico-Biological Interactions 142, 269-283. [CrossRef]

Akhila A.S., 2010. Essential Oil Bearing Plants: The Genus *Cymbopogon*. In: Anand Akhila, Broca Raton, F. L. (Eds): CRC Press Taylor & Francis Group, 123-136. [CrossRef]

Al-Dohail, M.A., Hashim R., Paiko M.A., 2009. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African catfish (*Clarias gariepinus*, Burchell 1822) fingerling. Aquaculture Research 40, 1642-1652. [CrossRef]

Anderson, D.P., 1974. Fish Immunology. Snieszko, S.F. (Ed). T.F.H Publications, 239.

Aneta, L., Ewa, K., Wojciech, A., Katarzyna, T., Sylwia F., 2015. Antimicrobial resistance of *Pseudomonas* spp. isolated from wastewater and wastewater-impacted marine coastal zone. Environmental Science and Pollution Research 22, 19823-19834. [CrossRef]

Anuradha, K., Foo, H. L., Mariana, N. S., Loh, T. C., Yusoff, K., Hassan, M. D., Sasan, H., Raha, A. R., 2010. Live recombinant *Lactococcus lactis* vaccine expressing aerolysin genes D1 and D4 for protection against *Aeromonas hydrophila* in tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology 109, 1632-1642. [CrossRef]

AOAC (Association of Official Analytical Chemistry) International. 2005. Official Methods of Analysis. 18th Edition. AOAC International, Gaithersburg, Maryland: USA.

Bairaji, A., Ghosh, K., Sen, S. K., Ray, A. K., 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International 10, 109-121. [CrossRef]

Becker, K., Makkar, H.P.S., 1999. Effects of dietary tannic acid and quebracho tanninon growth performance and metabolic rates of common carp (*Cyprinus carpio* L.). Aquaculture 175, 327-335. [CrossRef]

Bello, O.S., Olaifa, F.E., Emikpe, B.O., 2012. The effect of walnut (*Tetracarpidium conophorum*) leaf and onion bulb residues on the growth performance and nutrient utilization of *Clarias gariepinus* juveniles. Journal of Agricultural Sciences 4, 205-213.

Bharti, S.K., Kumar, A., Prakash, O., Sharma, N.K., Krishnan, S., 2013. Essential Oil of *Cymbopogon citrates* against diabetes: Validation by *in vivo* experiments and computational Studies. Scientific Reports 2, 1-9.

Bhosale, S.V., Bhilave, M.P., Nadaf, S.B., 2010. Formulation of fish feed using ingredients from plant sources. Research Journal of Agricultural Sciences Sci 1, 284-287.

Brummett, R.E., 2008. *Clarias* catfish: Biology, ecology, distribution and biodiversity. In: Ponzoni R. W., Nguyen N. H. (Eds), Proceedings of a Workshop on the Development of a Genetic Improvement Program for African catfish *Clarias gariepinus*. World Fish Center Conference Proceedings, The World Fish Center, Penang, Malaysia, 64-72.

Castro, S.B.R., Leal, C.A.C., Freire F.R., Carvalho, D.A., Oliveira, D.F., Figueiredo, H.C.P., 2008. Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. Brazilian Journal of Microbiology 39, 756-760. [CrossRef]

Cheel, J., Theoduloz, C., Rodríguez, J., Schmeda-Hirschmann, G., 2005. Free Radical Scavengers and Antioxidants from Lemongrass *(Cymbopogon citratus* (DC.) Stapf.). Journal of Agricultural and Food Chemistry 53, 2511-2517. [CrossRef]

Cho, S.H., 2011. Effects of putative growth or health-enhancing dietary additives on juvenile olive flounder, *Paralichthys olivaceus*, performance. Journal World Aquaculture Society 42, 90-95. [CrossRef]

Clinical and Laboratory Standards Institute (CLSI), 2012. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute's Twenty-Second Information Supplement 32, 44-160.

El-Dakar, A.Y., Sakr, E.M., Toutou, M.M., 2015. Possibility of using basil (*Ocimum basilicum*) supplementation in gilthead sea bream (*Sparus aurata*) diet. Egyptian Journal of Aquatic Research 41, 203-210. [CrossRef]

Ewansiha, J.U., Garba, S.A., Mawak, J.D., Oyewole O.A., 2012. Antimicrobial activity of *Cymbopogon citratus* (lemon grass) and its phytochemical Properties. Frontiers in Science 2, 214-220. [CrossRef]

Fandohan, P., Gnonlonfin, B., Laleye, A., Gbenou, J. D., Darboux, R., Moudachirou, M., 2008. Toxicity and gastric tolerance of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Ocimum basilicum* in Wistar rats. Food Chemical Toxicology 46, 2493-2497. [CrossRef]

Francisco, V., Figueirinha, A., Neves, B.M., Garcia-Rodríguez, C., Lopes, M.C., Cruz, M.T., Batista, M.T., 2011. *Cymbopogon citratus* as source of new and safe anti-inflammatory drugs: bio-guided assay using lipopolysaccharide-stimulated macrophages. Journal of Ethnopharmacology 133, 818-827. [CrossRef]

Fox, H.E., White, S.A., Koa, M.F., Fernald, R.D., 1997. Stress and dominance in a social fish. Journal of Neuroscience 16, 6463-6469. [CrossRef]

Gabriel, N.N., Wilhelm, M.R., Habte-Tsion, H., Chimwamurombe, P., Omoregie, E., Iipinge, L.N., Shimooshili, K., 2019. Effect of dietary Aloe vera polysaccharides supplementation on growth performance, feed utilization, hemato-biochemical parameters, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings. International Aquatic Research 11, 57-72. [CrossRef]

Gent, Y., Wang, K.Y., Huang, X.L., Chen, D.F., Li, C.W., Ren, S.Y., Liao, Y.T., Zhou, C.Z.Y., Liu, Q.F., Du, Z.J., Lai, W.M., 2012. *Streptococcus agalactiae*, an emerging pathogen for cultured Ya-fish, *Schizothorax prenanti* in China. Transboundary and Emerging Diseases 59, 369-375. [CrossRef]

Ghassan, A., Harbant, S., Muhammad, S., 2012. Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. Annals of Agricultural and Environmental Medicine 19, 673-676.

Giri, S. S., Sen, S.S., Chi, C., Kim, H.J., Park, S.C., Sukumaran, V., 2015. Effect of guava leaves on the growth performance and cytokine gene expression of Labeo rohita and its susceptibility to Aeromonas hydrophila infection. Fish and Shellfish Immunology 46, 217-224. [CrossRef]

Gutowska, M.A., Drazen, J.C., Robison, B.H., 2004. Digestive chitinolytic activity in marine fishes of Monterey Bay, California. Comparative Biochemistry and Physiology Part A 139, 351-358. [CrossRef]

Ilhan, A., Sevki, K., Erol, C., 2006. *Pseudomonas putida* infection in rainbow trout. Aquaculture, 261, 850-855. [CrossRef]

Kennedy, D.A., Kurath, G., Porito, I.I., Purcell, M.K., Read, A.F., Winto, J.R., Wargo, A.R., 2016. Potential drivers of virulence evolution in aquaculture. Evolutionary Applications 9, 344-354. [CrossRef]

Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal 29, 162750. [CrossRef]

Kumaran, S., Deivasigamani, B., Alagappan, K.M., Sakthivel M., 2010. Infection and immunization trials of Asian seabass (*Lates calcarifer*) against fish pathogen *Vibrio anguillarum*. Journal of Environmental Biology 31, 539-541.

Mandal, S., Ghosh, K., 2010. Inhibitory effect of *Pistia* tannin on digestive enzymes of Indian major carps: an *in vitro* study. Fish Physiology and Biochemistry 36, 1171-1180. [CrossRef]

Marimuthu, M., Ilansuriyan, P., Karthikeyan, K., 2015. Effect of two different germplasm of *Mucuna pruriens* seed extract against some fish pathogens. International Journal of Pharmacological Research 5, 163-166.

McLaughlin, P.A., 1983. Internal Anatomy. In: Bliss, D. E., Mantel, T. H. (Eds.). The biology of Crustacea. Academic Press, New York, 5, 1-52.

Mirghani, M.E.S., Liyana, Y., Parveen, J., 2012. Bioactivity analysis of lemon grass (*Cymbopogon citratus*) essential oil. International Food Research Journal 19, 569-575.

Negrelle, R. R., Gomes, E. C., 2007. *Cymbopogon citratus* (DC) Stapf: Chemical composition and biological activities. Revista Brasileira De Plantas Medicinais 9, 80-92.

Newaj-Fyzul, A., Mutani, A., Ramsubbag, A., Adesiyun, A., 2008. Prevalence of bacteria pathogens and their antimicrobial resistance in tilapia and their pond water in Trinidad. Zoonoses Public Health 55, 206-213. [CrossRef]

NRC (National Research Council). 1993. Nutrient Requirement of Fish. Committee on Animal Nutrition, Board on Agriculture. Washington, DC, USA: National Academy Press, 123.

Nur, A.B., 2014. Effect of different types of solvent on extraction of phenolic compounds from *Cosmos caudatus*, 1-16. Accessed at http://umpir.ump.edu.my/4390/1/.pdf. 5 April, 2014.

Nwanna, L.C., 2003. Nutritional value and digestibility of shrimp head waste meal by African catfish, *Clarias gariepinus* (Burchell, 1822). PhD Thesis, Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria.

Olorunnisola, S.K., Asiyanbi, H.T., Hammed, A.M., Simsek, S., 2014. Biological properties of lemon grass: An overview. International Food Research Journal 21, 443-462.

Omnes, M.H., Goasduff, J.L., Delliou, H.L., Bayon, N.L., Quazuguel, P., Robin, J.H., 2017. Effects of dietary tannin on growth, feed utilization and digestibility and carcass composition in juvenile European seabass (*Dicentrarchus labrax* L.). Aquaculture Reports 6, 21-27. [CrossRef]

Pakravan, S., Hajimoradloo, A., Ghorbani, R., 2011. Effect of dietary willow herb, *Epilobium hirsutum*, extract on growth performance, body composition, haematological parameters and *Aeromonas hydrophila* challenge on common carp, *Cyprinus carpio*. Aquaculture Research 42, 1-9. [CrossRef]

Prigeon, J.W., Klesius, P.H., 2012. Major bacterial diseases in aquaculture and their vaccine development. CAB Reviews 7, 1-16. [CrossRef]

Pridgeon, J.W., Yildirim-Aksoy, M., Klesius, P.H., Srivastava, K.K., Reddy, P.G., 2012. Attenuation of a virulent *Aeromonas hydrophila* with novobiocin and pathogenic characterization of thenovobiocin-resistant strain. Journal of Applied Microbiology 113, 1319-1328. [CrossRef]

Prusty, A.K., Sahu, N.P., Pal, A.K., Reddy, A.K., Kumar, S., 2007. Effect of dietary tannin on growth and haemato-immunological parameters of *Labeo rohita* (Hamilton) fingerlings. Animal Feed Science Technology 136, 96-108. [CrossRef]

Rahman, M.A., Rana, M.S., Zaman, M.M., Uddin, S.A., Uddin, S.A., Akter, R., 2010. Antioxidant, antibacterial and cytotoxic of the methanol extract of *Urtica crenulata*. Journal of Scientific Research 2, 169-177. [CrossRef]

Rogo, L.D., Attah, M., Bawa, E., Aishatul, A.M., 2009. Antimicrobial susceptibility pattern of *Aeromonas hydrophila* among patient presented with diarrhoea attending ABUTH Zaria and AKTH Kano, Nigeria. World Journal of Biological Research 2, 1-7. [CrossRef]

Romero, J., Feijoo, C.G., Navarrete, P., 2012. Antibiotics in Aquaculture-Use, Abuse and Alternatives. Health and Environment in Aquaculture. Edmir D.C., Gianmarco S.D. and Reinaldo J.S. (Eds.). Chapter 6, 159-198. [CrossRef]

Saidi, N., Lagha, R., Abdallah, F.B., Rokbani, K.B., Bakhrouf, A., 2013. Slime producing, heavy metals and antibiotics resistance in *Aeromonas hydrophila* isolated in Tunisia. African Journal of Microbiology Research 7, 5697-5608. [CrossRef]

Saleh, N.E., Micheal, F.R., Toutou, M.M., 2014. Evaluation of garlic and onion powder as phytoadditives. Egyptian Journal of Aquatic Research 41, 211-217. [CrossRef]

Santos I., Ramus, R., 2018. Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. International Journal of Antimicrobial Agents 52, 135-143. [CrossRef]

Shayo, S.D., Mwita, C.J., Hosea, K., 2012. Ulcerative *Aeromonas* infections in tilapia (Cichlidae: Tilapini) from Mtera hydropower Dam, Tanzania. Open Access Scientific Reports 1, 115.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 150.

Subashkumar, R., Thayunmanavan, T., Vivekanandhan, G., Lakshmanaperumasamy, P., 2006. Occurrence of *Aeromonas hydrophila* in acute gastroenteristic among children. Indian Journal of Medical Research 123, 61-66.

Tatiana F.B., Jose M.S. 2011. Lemongrass and citral effect on cytokines production by murine macrophages. Journal of Ethnopharmacology 137, 909-913. [CrossRef]

Tkachenko H., Buyun L., Lyudmyta B., Terech-Majewska G., Osadowski Z., 2016. *In vitro* antimicrobial activity of ethanolic extracts obtained from *Ficus* spp leaves against the fish pathogen *Aeromonas hydroplila*. Archives of Polish Fisheries 24, 219-230. [CrossRef]

Trease, G. E., Evans, W. C., 1989. Pharmacognsy, 11th Edition. Brailliar Tiridel Canada, Macmillian publishers, 176-180.

Tulli, F., Chini Z.G., Giorgi, G., Poli, B.M., Tibaldi, E., Tredici, M.R., 2012. Effect of the inclusion of dried *Tetraselmis suecica* on growth, feed utilization, and fillet composition of European sea bass juveniles fed organic diets. Journal of Aquatic Food Product Technology 21, 188-197. [CrossRef]

Ulanowska, K., Tkaczyk, A., Konopa, G., Wegrzyn, G., 2006. Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. Archives of Microbiology 184, 271-278. [CrossRef]

United State Environmental Protection Agency (U.S. EPA). 2005. *Aeromonas* detection what does it means. http/www.Epagor/safe water urenr/dateAeromonas.html (Access date: May 3, 2017).

Wiens, G.D., Vallejo, R.L., 2010. Temporal and pathogen-load dependent changes in rainbow trout (*Oncorhynchus mykiss*) immune response traits following challenge with biotype 2 *Yersinia ruckeri*. Fish and Shellfish Immunology 29, 639-647. [CrossRef]

Yilmaz, S., Sebahattin, E., Nergiz, S., 2013. Dietary supplementation of cumin (*Cuminum cyminum*) preventing streptococcal diseases during first-feeding of Mozambique tilapia (*Oreochromis mossambicus*). Journal of Bioscience and Biotechnology 2, 117-124.

Yilmaz, E., Coban, D., Bayar, F., 2017. The effects of the fig *(Ficus carica)* extract and onion (*Allium capa*) extract addition to *Cyprinus carpio* feeds in different amount on the growth performance. Ege Journal of Fisheries and Aquatic Sciences 34, 321-326. [CrossRef]

Zheng, Z.L., Tan, J.Y.W., Liu, H.Y., Zhou, X.H., Xiang, X., Wang, K.Y., 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). Aquaculture 292, 214-218. [CrossRef]