ISTANBUL UNIVERSITY CERRAHPAŞA

Wound-healing Potentials of Dietary *Lactobacillus plantarum* and *Psidium guajava* Leaf Meal in *Clarias gariepinus* Juveniles

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Abstract

This study aimed to assess healing processes in juvenile *Clarias gariepinus (C. gariepinus)* (Burchell, 1822) fed varying levels of *Lactobacillus plantarum (L. plantarum)* (strain 762) and *Psidium guajava (P. guajava) (Linneaus)* meal. Eighty-four *C. gariepinus* with mean body weight of 125 g were analyzed. The fish were selected using a completely randomized design. They were distributed into 21 aquaria with 40 L water capacity. The adopted wound model was a standard 10-mm^2 incision made on lateral and caudal regions. The fish were fed with 42% crude protein diet at 0, 40, 60, and 80 cells$^{-1000\ mL}$ and g$^{-1000\ g}$ of *L. plantarum* and guava leaf meals, respectively. Wound closures were observed daily for quantitative measurements. Healing rates were measured on the regions at 0, 3, 6, 9, and 12 days. Data were analyzed using descriptive statistics and analysis of variance at a $p<0.05$. The results showed better healing rate at lateral parts across treatments with complete healing recorded on the 12th day. The fastest healing rates and percentage were noted in fish fed LPA-basal diet+40 cells$^{-1000\ mL}$ *L. plantarum* per kg of feed and GMC-basal diet+80 g$^{-1000\ g}$ guava meal per kg of feed. These results reveal that *L. plantarum* at lower and *P. guajava* at higher concentrations led to faster healing than the control.

Keywords: *Clarias gariepinus, Lactobacillus plantarum, Psidium guajava,* wound-healing

Introduction

Several commercial fish species have been intensively cultured in enclosed or narrow spaces, such as tanks, cages, and ponds under high density (Kaileh et al., 2007). This practice has led to negative effects on cultured fish species, including African catfish. However, the defensive mechanism of African catfish, which gives it its venomous characteristics, is attributed to the presence of a single, sharp, and stout sting in front of the soft-rayed portion of the pectoral fins (Weston et al., 2005). Consequently, African catfish uses the mechanism of their pectoral and dorsal well-developed articulated stings to inflict severe wounds into their victims (Junqueira, 2006). Often, their cannibalistic characteristic is displayed in circumstances of hunger-induced stress and overcrowding. Moreover, the impaired skin is infected with opportunistic microorganisms, leading to delayed wound healing (Weber et al., 2012).

With the intensification of aquaculture to meet global food consumption, high stocking density is necessitated in ponds for optimum catfish production. Consequently, fish incur wounds or injuries, which predispose them to infection and death (Weber et al., 2012). Presently, emphases are placed on feeds that have both growth-promoting and wound healing potential (Castillo et al., 2014). However, in most countries, the role of medicinal plants is highly important in primary healthcare. Anti-inflammatory and wound healing properties have been revealed in several plants including *Tamarindus indica* pulp (Adeniyi et al., 2018), *Mallotus oppositifolius, Momordica charantia* (Agyare et al., 2014), and *Phyllanthus muellerianus* (Boakye et al., 2018).

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The guava tree, *Psidium guajava* (*P. guajava*) Linnaeus in the family Myrtaceae, is a tropical hardwood plant that can reach a height of 10 m (Martins et al., 2009). It is a native of Mexico and extends throughout South America, Europe, Asia, and Africa. It is used in the treatment of fever, pain, dysentery, respiratory disorders, gastroenteritis, diarrhea, diabetes, and hypertension (Martins et al., 2009). Guava leaves are also used in the treatment of wounds and ulcers and relief of toothache and rheumatic pain (Gutiérrez et al., 2008). However, the significant role of *P. guajava* in aquaculture in relation to wound healing has not been explored.

Similarly, *Lactobacilli* are normal intestinal microflora of animals and humans, which are classified as probiotics. They are Gram-positive lactic acid bacteria and nonpathogenic (Martin et al., 2007). Their culture supernatants have been shown to possess wound healing and antimicrobial properties (Bleau et al., 2005). They usually enhance energy and are known to relieve common cold, prevent cancers, and reduce cholesterol level (Reid and Bruce, 2006). Likewise, they enhance the inflammatory response during tissue repair in animals and stimulate proliferation of embryonic cells (Brackett and Halper, 2005). However, fermented dairy products containing *Lactobacillus* have been used in traditional medicine in the modulation of microbial ecology to prevent infections caused by pathogenic bacteria, stimulation of the immune system, and treatment of gastrointestinal disorders (Chen et al., 2009). *Lactobacilli* are also known to be therapeutic, aiding in the treatment of urinary tract infections and diarrhea, irritable bowel syndrome, and atopic eczema (SenthiKumar et al., 2013). However, the significant role of *Lactobacillus plantarum* (*L. plantarum*) as probiotics in relation to wound healing in aquaculture is yet to be explored.

Wound healing in humans and animals involves the process of inflammation, cell proliferation, and contraction of the collagen lattice. However, this process may be disrupted by the presence of oxygen free radicals or microbial infection (Gonçalves et al., 2008). In view of these, this study aimed to evaluate the wound healing potential of *L. plantarum* and guava (*P. guajava*) leaf meal at different inclusions levels in the diets of juvenile African catfish.

Materials and Methods

Ethical statement

The experimental procedure was approved by the University of Ibadan Ethical Committee for Animal Use in Research (UI/ACUREC/App/2017/0028). This was in conformity with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-83) and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Probiotic and plant additive diet preparations

Fresh guava leaves (*P. guajava*) were collected and identified in the Forestry Research Institute of Nigeria with voucher number 110937. After harvesting, the leaves were washed with clean water, air dried for 21 days, and processed into meal. Samples of lactic acid bacteria (LAB) were collected from fermented cow milk (wara). LAB were identified using physiological and biochemical tests. These were further confirmed through molecular characterization as *L. plantarum* (strain 762) (Agaliya and Jeevaratnam, 2013).

Fish feed ingredients were purchased from a reputable fish feed store, and basal diet of 42% crude protein was prepared using the Pearson square method. The single-cell proteins of *L. plantarum* (strain 762) and *P. guajava* leaf meal were added to the experimental feed relative to each treatment. The dry feed ingredients were well grinded, mixed, and pelletized with water and a binding agent (starch). The extruded pellets of 2 mm in size were sun-dried and stored in neoplastic bags made in Nigeria until experimental use.

The ingredients used included fish meal, groundnut cake, soybean meal, wheat middlings, yellow maize, Guinea corn, common salt, palm oil, and vitamin and mineral premix. Eight experimental diets were prepared, including two control diets, by incorporating *L. plantarum* and *P. guajava* leaf meal at different concentrations into the diets as follows: T_m1 and T_m2 were control diet comprising basal diet of 42% crude protein level. T_m3, T_m4, and T_m5 consisted of basal diet with additional 40 cells$^{-1000\ Ml}$ (LPA), 60 cells$^{-1000\ Ml}$ (LPB), and 80 cells$^{-1000\ Ml}$ (LPC) of *L. plantarum* per kg of feed, respectively. T_m6, T_m7, and T_m8 consisted of basal diet with additional 40 g$^{-1000\ g}$ (GMA), 60 g$^{-1000\ g}$ (GMB), and 80 g$^{-1000\ g}$ (GMC) of guava meal per kg of feed, respectively.

Experimental setup

The weight of the fish was obtained in each treatment at day 0 and day 12 of the experiment. The experiment was conducted at the Department of Aquaculture and Fisheries Management Wet Laboratory of the University of Ibadan. Ninety-six *C. gariepinus* juveniles (12 for each treatment) with average body weight of 125±0.2 g were randomly selected from each replicate treatment. They were selected from previously fed fish with experimental diets for 84 days. Fish were randomly distributed into seven treatment groups. Four fish per replicate were distributed into rectangular plastic aquaria from Nigeria with dimensions of 50×34×27 cm and 40 L water capacity. Twenty-four plastic aquaria were used for the experimental setup. Fish were acclimatized for 2 weeks after the feeding experiment and confirmed to be of good general health based on a complete physical examination before the commencement of the experiment.

Wound creation

The caudal and lateral areas of the fish were aseptically prepared. These processes were conducted by swabbing the two areas with absorbent cotton wool (Vandana Surgi Pharma, Bijaynagar, India) immersed in absolute ethanol (99%, Fred Holmberg & Co AB, Brenntag Nordic, Sweden) and placed on sterilized slabs. These two areas were selected to assess the differences in the healing rates on the fish bodies. The epidermal

and sub epidermal tissues of *Clarias gariepinus* (*C. gariepinus*) at various body parts are known to have different thicknesses, hence different healing rates on fish bodies (Kurokawa et al., 1993). Sterilized surgical blades (Kiato, Himachal Pradesh, India) were used to make an incision. A standard template (a thick measuring stick, drawing the site on a well-uniform 10-mm^2 quadrant) was placed on the fish, and surgical cuts of 10 mm^2 were made on the fish toward the dermis to the lateral part, above the pelvic fin, and around the caudal region (Bello et al., 2013). The fish were returned to the aquaria and fed to satiation for 12 days (Abo et al., 2004; Bell, 2002).

Wound healing assessment

The wounds inflicted were photographed using a phone camera (Nokia Asha 210, Texas, USA). Wound closure was observed on a daily basis for visual assessment and quantitative measurements. Measurement of the wound area in two-dimensional healing was performed using a measuring ruler (Beginner dual-scale ruler, Zhejiang, China) at 0, 3, 6, 9, and 12 days. Healing percentage, daily healing rates, and change in wound area were calculated as follows:

$$\%\ \text{Healing} = \frac{\text{Change in wound area (area of regenerated tissue)}}{\text{(Initial wound area)}} \times 100$$ (Ammar et al., 2015)

$$\text{Daily healing rates} = \frac{\text{Total percentage of area healed}}{\text{Number of days measurement was obtained}}$$ (Bell, 2002)

Change in wound area (mm)=Initial wound area on 0^{th} day-wound area left on nth day (n=3,6,9,12)

Skin biopsy was performed in the treatment groups (caudal and lateral) at 3, 6, and 12 days for routine histological processing for microscopic examination (Abramczuk, 1972).

Statistical analysis

This experiment was subjected to one-way analysis of variance using the Statistical Package for Social Sciences (2006, version 20.0). The individual means were compared using the Duncan multiple range test.

Results and Discussion

Histological examination of juvenile African catfish *(C gariepinus)* fed *L. plantarum* and guava *(P. guajava)* leaf meal at various inclusion levels

Figure 1 revealed hyperemia and scab tissue formation at the caudal region on the 3^{rd} day. These were characterized by granulation tissue and inflammatory cells on the epidermis. However, Figure 2 revealed wound healing at the lateral region after 3 days with intense hyperemia and inflammatory response on

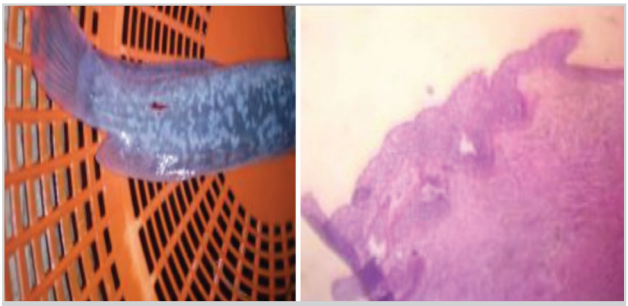


Figure 1. Wound healing at the lateral region after 3 days with intense hyperemia and inflammatory response on the epidermis (insert, HE 400×)

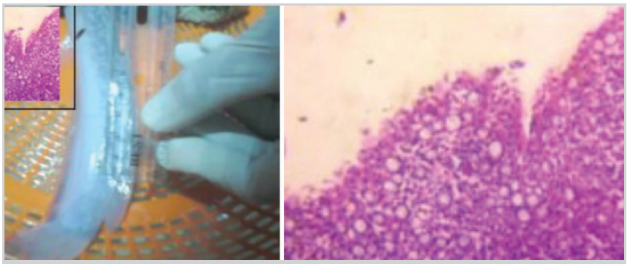


Figure 2. Wound healing at the lateral region after 3 days with intense hyperemia and inflammatory response on the epidermis (insert, HE 400×)



Figure 3. Wound healing at the caudal region after 6 days with reduced surfaceand proliferation of keratinocytes on the epidermis (insert, HE 400×)

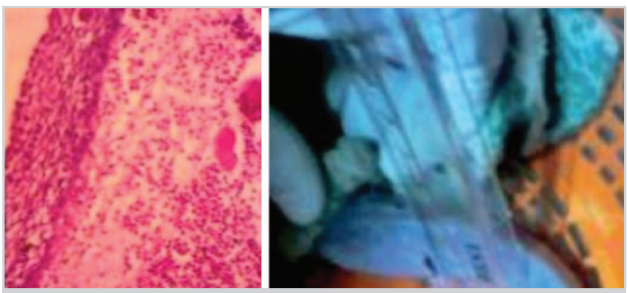


Figure 4. Wound healing at the lateral region after 6 days with reduced surface area on gross examination and appearance of normal skin morphology (insert, HE 400×)

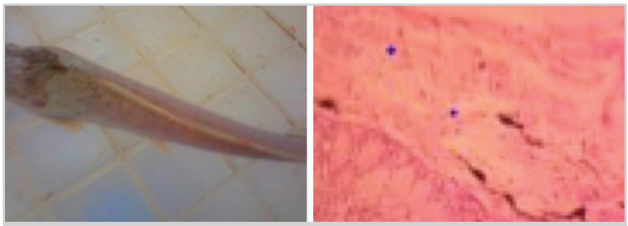


Figure 5. Completely healed wound at the caudal and lateral regions on day 12 on gross and microscopic examinations (insert, HE 400×)

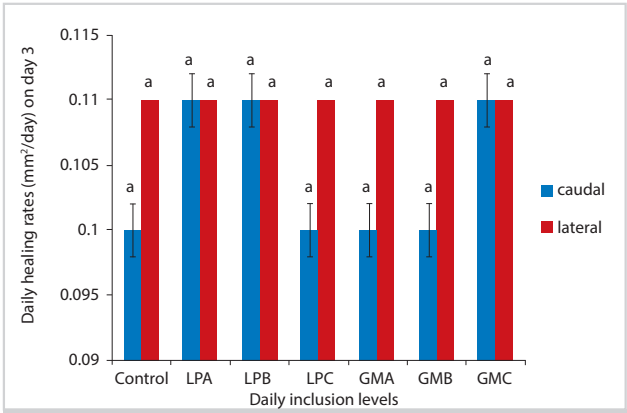


Figure 6. Daily healing rates of juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* diets at various levels on day 3

Key: Control, basal diet of 42% crude protein; LPA, basal diet+40 cells^{-1000} mL *L. plantarum* per kg of feed; LPB, basal diet +60 cells^{-1000} mL; LPC, basal diet +80 cells^{-1000} mL *L. plantarum* per kg of fish; GMA, basal diet+ 40 g^{-1000} g guava meal per kg of feed; GMB, basal diet + 60 g^{-1000} g guava meal per kg of feed; GMC, basal diet+ 80 g^{-1000} g guava meal per kg of feed

the epidermis. Figure 3 revealed reduced surface keratinocytes on the epidermis of the caudal region on the 6th day, while Figure 4 revealed wound healing with grossly reduced surface area at the lateral region and appearance of normal skin morphology on the 6th day. Figure 5 revealed completely healed wound at the caudal and lateral regions on the 12th day.

Daily healing rates of juvenile *C. gariepinus* fed *L. plantarum* and guava *(P. guajava)* leaf meal after 12 days of wound incision.

Figures 6-9 show the daily healing rates of juvenile *C.* gariepinus after 12 days of wound incision. In Figures 6-8, the daily healing rates of fish treated with LPA, LPB, and GMC were significantly higher ($p<0.05$) than those of fish treated with control, LPC, GMA, and GMC at the caudal regions after 3, 6, and 9 days. On the lateral region, there was no significant difference ($p>0.05$) in all treatment groups after 3, 6, and 9 days. Figure 9 presents complete healing after 12 days with no significant difference ($p>0.05$) on the caudal region in all treatment groups. There was complete healing at the lateral region in all treatments groups after 12 days with no significant difference ($p>0.05$).

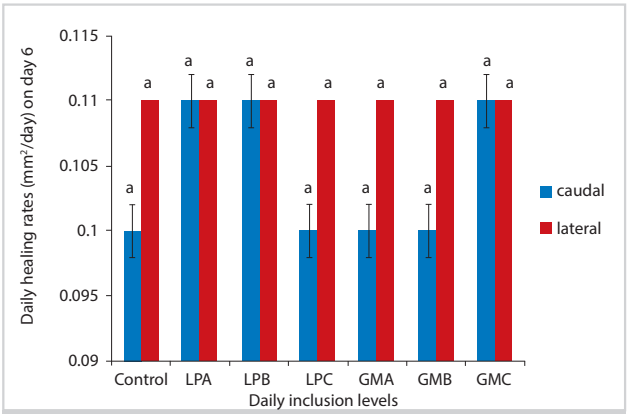


Figure 7. Daily healing rates (mm^2/day) of juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* diets at various levels on day 6

Key:Control, basal diet of 42% crude protein; LPA, basal diet+40 cells^{-1000} mL L. plantarum per kg of feed; LPB, basal diet +60 cells^{-1000} mL; LPC, basal diet +80 cells^{-1000} mL L. plantarum per kg of fish; GMA. basal diet+ 40 g^{-1000} g guava meal per kg of feed; GMB, basal diet + 60 g^{-1000} g guava meal per kg of feed; GMC, basal diet+ 80 g^{-1000} g guava meal per kg of feed

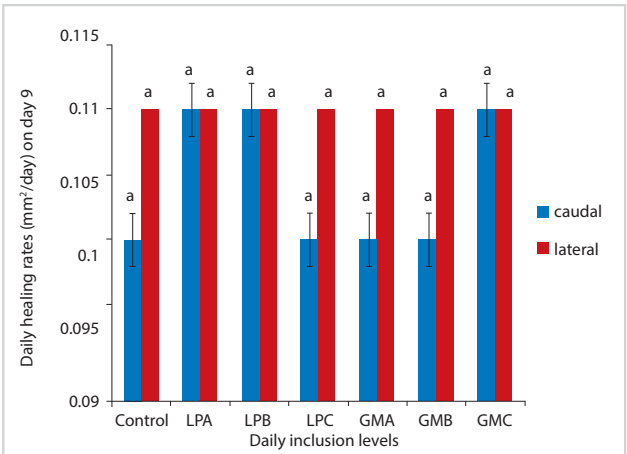


Figure 8. Daily healing rates (mm^2/day) of *C. gariepinus* fed *L. plantarum* and *P. guajava* diets at various levels on day 9

Key: Control, basal diet of 42% crude protein; LPA, basal diet+40 cells^{-1000} mL *L. plantarum* per kg of feed; LPB, basal diet +60 cells^{-1000} mL; LPC, basal diet +80 cells^{-1000} mL *L. plantarum* per kg of fish; GMA, basal diet+ 40 g^{-1000} g guava meal per kg of feed; GMB, basal diet + 60 g^{-1000} g guava meal per kg of feed; GMC, basal diet+ 80 g^{-1000} g guava meal per kg of feed

Healing percentage (%) of juvenile African catfish *(C. gariepinus)* fed *L. plantarum* and guava *(P. guajava)* leaf meal at various inclusion levels.

Tables 1-4 show the healing percentage (%) and change in wound area at the caudal and lateral regions of juvenile African catfish (*C. gariepinus*) fed *L. plantarum* and guava (*P. guajava*) leaf at various inclusion levels. Table 1 shows a significant difference ($p<0.05$) at the caudal region with basal diet +80 cells$^{-1000\ mL}$ *L. plantarum* per kg of fish (80 LPc) inclusion level

with 25.2±19.8% healing compared with the lateral region (14.3±3.6%) and control (10.0±2.5%).

The wound healing percentage was higher ($p<0.05$) at the lateral and caudal regions in fish fed inclusion basal diet+ 80 $g^{-1000\ g}$ guava meal per kg of feed (80 PGLM) (84.8±1.4% and 78.6±2.3%) compared to the control at the lateral and caudal regions (53.8±9.3% and 42.2±2.4%) on the 6th day (Table 2). There was no significant difference ($p>0.05$) at the caudal and lateral regions between all inclusions and control on the 9th day (Table 3). Complete healing in all treatment groups at the caudal and lateral regions was noted on the 12th day (Table 4).

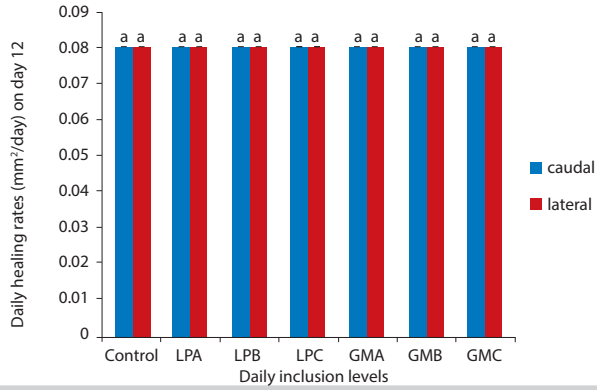


Figure 9. Daily healing rates (mm²/day) for juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* diets at various levels on day 12
Key: Control, basal diet of 42% crude protein; LPA, basal diet+40 $cells^{-1000}$ mL *L. plantarum* per kg of feed; LPB, basal diet +60 $cells^{-1000}$ mL; LPC, basal diet +80 $cells^{-1000}$ mL *L. plantarum* per kg of fish; GMA, basal diet+ 40 g^{-1000} g guava meal per kg of feed; GMB, basal diet + 60 g^{-1000} g guava meal per kg of feed; GMC, basal diet+ 80 g^{-1000} g guava meal per kg of feed

This study assessed the wound healing performance of *L. plantarum and P. guajava* diets at varying levels on juvenile *C. gariepinus*. Procedurally, there are four steps involved in wound healing. These include hemostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). It was observed in this experiment that fish treated with the control, *L. plantarum* diet, and *P. guajava* leaf meal showed fast healing rates at all levels after 12 days of wound creation. Nonetheless, the highest change in wound area (mm²) and healing percentage (%) were noted in fish fed dietary *L. plantarum* (40 LPc) and dietary *P. guajava* (80 PGLM) compared to the control and other treatment groups. In similar studies, doses of *Lactobacillus fermentum* LP3 and *L. plantarum* AT4 administered to animals (e.g., mice and rabbits) and humans helped in accelerating healing of burns and leg ulcers (Peral et al., 2009; Weston et al., 2005). In another study, it was reported that *Lactobacillus rhamnosus* Gorbach-Goldin strain (G-G strain) and *Lactobacillus gasseri* strain ATCC 33323 hastened the healing of gastric ulcers in rats. The mechanism of wound healing in animals may be due to the production of immunoglobulin (IgG, IgM, and IgA) (Lahteinen et al., 2014). Moreover, they help in the absorption of antigens released from dead microbes and likewise stimulate cell-mediated immunity (Dongarra et al., 2013). According to Rask et al. (2013), during wound healing, probiotics induce macrophages, lymphocytes, and natural killer cells. During this process, degranulation of heterophils and oxidative burst regulation occur to hasten wound healing (Rask et al., 2013). This may have probably enhanced the wound healing process of fish fed dietary probiotics in this present study (Rask et al., 2013).

However, the healing mechanism of probiotics, such as *L. plantarum*, may be influenced by the presence of plantaricin and bacteriocin. This was substantiated with the study conducted by Umeh et al. (2005). They observed an inhibitory activity of

Table 1. Changes in wound area of juvenile African catfish *(C. gariepinus)* fed with *L. plantarum* and guava *(P. guajava)* leaf meal at various levels on day 3

	CA			LA		
Treatments	**3 days Initial wound area (mm²)**	**Change in wound area (mm²)**	**% Healing**	**3 days Initial wound area (mm²)**	**Change in wound area (mm²)**	**% Healing**
Control	10.0	1.0±0.0	10.0±2.5^{d}	10.0	2.0±0.0	16.6±3.2^{b}
40 LPc	10.0	2.0±0.1	18.3±5.8^{b}	10.0	2.0±0.0	16.6±4.5^{b}
60 LPc	10.0	1.0±0.04	14.0±3.6^{c}	10.0	2.0±0.2	14.8±8.4^{c}
80 LPc	10.0	2.0±0.2	25.2±19.8^{a}	10.0	1.0±0.0	14.3±3.6^{c}
40 PGLM	10.0	1.0±0.1	8.4±8.0^{e}	10.0	1.0±0.0	13.3±2.2^{c}
60 PGLM	10.0	1.0±0.1	11.8±5.1^{d}	10.0	2.0±0.0	20.0±0.0^{a}
80 PGLM	10.0	1.0±0.0	13.3±3.0^{c}	10.0	1.0±0.0	13.4±4.1^{c}

Means with same letter in column are not significantly different ($p>0.05$)
Key: Control, basal diet of 42% crude protein; 40 LPc, basal diet+40 $cells^{-1000\ mL}$ *L. plantarum* per kg of feed; 60 LPc, basal diet +60 $cells^{-1000\ mL}$; 80 LPc, basal diet +80 $cells^{-1000\ mL}$ *L. plantarum* per kg of fish; 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; CA: caudal area; LA: lateral area

Table 2. Changes in wound area of juvenile African catfish *(C. gariepinus)* fed with *L. plantarum* and guava *(P. guajava)* leaf meal at various levels on day 6

	CA			LA		
Treatments	**6 days Initial wound area (mm²)**	**Change in wound area (mm²)**	**% Healing**	**6 days Initial wound area (mm²)**	**Change in wound area (mm²)**	**% Healing**
Control	10.0	4.0±0.02^{b}	42.2±2.4^{c}	10.0	5.0±0.1^{b}	53.8±9.3^{c}
40 LPc	10.0	8.0±0.01^{a}	79.3±1.4^{a}	10.0	8.0±0.1^{a}	80.0±5.6^{b}
60 LPc	10.0	5.0±0.06^{b}	50.2±6.5^{b}	10.0	8.0±0.0^{a}	75.6±4.0^{b}
80 LPc	10.0	8.0±0.04^{a}	79.5±4.2^{a}	10.0	9.0±0.0^{a}	84.3±0.8^{a}
40 PGLM	10.0	7.0±0.04^{a}	74.0±4.3^{a}	10.0	8.0±0.0^{b}	82.0±2.8^{a}
60 PGLM	10.0	8.0±0.02^{a}	78.6±2.3^{a}	10.0	8.0±0.0^{b}	84.1±4.2^{a}
80 PGLM	10.0	7.0±0.03^{a}	74.1±2.9^{a}	10.0	9.0±0.0^{a}	84.8±1.4^{a}

Means with same letter in column are not significantly different (p>0.05)
Key: Control, basal diet of 42% crude protein; 40 LPc, basal diet+40 cells$^{-1000\ mL}$ *L. plantarum* per kg of feed; 60 LPc, basal diet +60 cells$^{-1000\ mL}$; 80 LPc, basal diet +80 cells$^{-1000\ mL}$ *L. plantarum* per kg of fish; 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; CA: caudal area; LA: lateral area

Table 3. Changes in wound area of juvenile African catfish *(C. gariepinus)* fed with *L. plantarum* and guava *(P. guajava)* leaf meal at various levels on day 9

	CA			LA		
Treatments	**9 days Initial wound area (mm²)**	**Change in wound area (mm²)**	**% Healing**	**9 days Initial wound area (mm²)**	**Change in wound area (mm²)**	**% Healing**
Control	10.0	9.0±0.0^{b}	94.2±1.4^{ab}	10.0	10.0±0.0	100.0±0.0
40 LPc	10.0	10.0±0.0^{a}	99.2±1.4^{a}	10.0	10.0±0.0	99.2±1.4
60 LPc	10.0	10.0±0.0^{a}	95.0±2.5^{ab}	10.0	10.0±0.0	100.0±0.0
80 LPc	10.0	9.0±0.0^{b}	94.2±1.4^{ab}	10.0	10.0±0.0	99.2±1.4
40 PGLM	10.0	9.0±0.2^{b}	91.7±1.4^{b}	10.0	10.0±0.0	100.0±0.0
60 PGLM	10.0	9.0±0.2^{b}	90.8±1.4^{b}	10.0	10.0±0.0	100.0±0.0
80 PGLM	10.0	9.0±0.1^{a}	95.8±5.2^{ab}	10.0	10.0±0.0	100.0±0.0

Means with same letter in column are not significantly different (p>0.05)
Key: Control, basal diet of 42% crude protein; 40 LPc, basal diet+40 cells$^{-1000\ mL}$ *L. plantarum* per kg of feed; 60 LPc, basal diet +60 cells$^{-1000\ mL}$; 80 LPc, basal diet +80 cells$^{-1000\ mL}$ *L. plantarum* per kg of fish; 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; CA: caudal area; LA: lateral area

L. plantarum on the bacteria that often complicate wounds and burns in animals. The above mentioned study agrees with other reports that there was faster tissue repair with the treatments that had probiotics inclusions at various levels compared with the control (Nowroozi et al., 2004). Faster wound healing observed in fish fed dietary *L. Plantarum* at all levels compared with control might have been enhanced by nonspecific immune activity in *L. plantarum* (Salvador et al., 2012).

Likewise, the healing observed in fish fed dietary *P. guajava* at various inclusion levels in this study may be due to the presence of phytochemical constituents (Setufe et al., 2018a; 2018b). These include saponins, tannins, terpenoids, alkaloids, cardiac glycosides, and phenols in *P. guajava*, which may have influenced the fast healing rate of the experimental fish. The anti-inflammatory potential of *P. guajava* leaf meal might have further enhanced the fast wound healing activity in experimental fish compared with the control fed basal diet (Szynanski et al., 2006). Mbuh et al. (2008) and Ogbonnia et al. (2008) also reiterate the fact that tannins react with proteins to induce tanning effect in animals, as this proline-rich protein complexes help in the synthesis of cell protein essential for the treatment of inflammation and tissue wounds. The faster wound healing process at the lateral region of juvenile *C. gariepinus* than at the caudal region might be due to the movement of migratory fronts (epidermis from wound edges) on the 6th and 9th days. The thicker epidermis at the lateral region might have hastened wound closure of fish compared

Table 4. Changes in wound area of juvenile African catfish *(C. gariepinus)* fed with L. plantarum and guava *(P. guajava)* leaf meal at various levels on day 12

	CA			LA		
Treatments	**12 days Initial wound area (mm^2)**	**Change in wound area (mm^2)**	**% Healing**	**12 days Initial wound area (mm^2)**	**Change in wound area (mm^2)**	**% Healing**
Control	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0
40 LPc	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0
60 LPc	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0
80 LPc	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0
40 PGLM	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0
60 PGLM	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0
80 PGLM	10.0	10.0±0.0	100.0±0.0	10.0	10.0±0.0	100.0±0.0

Means with same letter in column are not significantly different (p>0.05)
Key: Control, basal diet of 42% crude protein; 40 LPc, basal diet+40 $cells^{-1000\ mL}$ *L. plantarum* per kg of feed; 60 LPc, basal diet +60 $cells^{-1000\ mL}$; 80 LPc, basal diet +80 $cells^{-1000\ mL}$ *L. plantarum* per kg of fish; 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; CA: caudal area; LA: lateral area

with that at the caudal region, resulting in the visible fast wound healing at the lateral region (Guerra et al., 2008).

Optimal intensification of wound healing can only occur when biophysiological processes occur sequentially at a particular time and duration. Otherwise, healing may be impaired or delayed (Mathieu et al., 2006). According to a study conducted by Akanmu et al. (2016), *Lactobacillus fermentum* enhanced wound healing in *Heterobranchus bidorsalis* juveniles. On this premise, the faster healing rate at the lateral region might have also been due to cell differentiation and concentration of tissue-specific stem cells within the region compared with the wound at the caudal region of fish (Venkaiah and Lakshmipathi, 2000).

Conclusion

The inclusions of dietary *L. plantarum* and dietary *P. guajava* leaf meal in the diet of juvenile *C. gariepinus* enhanced wound healing at dietary inclusion of LPA-basal diet+40 $cells^{-1000\ mL}$ *L. plantarum* per kg of feed and GMC-basal diet+ 80 $g^{-1000\ g}$ guava meal per kg of feed compared to the control.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of University of Ibadan (date: January 2017; no: 0028).

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