Acta Biologica 26/2019 | www.wnus.edu.pl/ab | DOI: 10.18276/ab.2019.26-01 | strony 5–17

Growth performance, gut morphometry and innate immune profiles of common carp, *Cyprinus carpio* juveniles fed diet fortified with *Mitracarpus scaber* leaves extract and its susceptibility to pathogenic bacteria, *Aeromonas hydrophila*

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Keywords: Common carp, feed supplements, growth performance, gut morphometry, immunostimulants

Abstract

Mitracarpus scaber, an endemic medicinal plant to Nigeria, Africa, has medicinal value. In the present study, *Mitracarpus scaber* leaves extract (MSLE) was fed to common carp, *Cyprinus carpio* to evaluate its effect on growth performance, nutrient utilization, gut morphometry, and innate immunity parameters. Four isonitrogenous diets (32% crude protein) containing 0.0, 5, 10, or 15 g MSLE/kg diet were fed to fish (7.52 ±0.23 g) for 12 weeks. After the feeding trial, fish were exposed to pathogenic bacteria (*Aeromonas hydrophila*) for 14 days. Growth performance, nutrient utilization, and feed intake were significantly improved with increasing MSLE levels up to 10 g/kg diet. Similarly, fish fed MSLE diets increased significantly intestinal villi length/width, and absorption area. Furthermore, activities of respiratory burst, lysozyme, catalase, and superoxide dismutase were significantly higher in fish fed diets containing MSLE levels, and their highest values were obtained at fish fed 15 g MSLE/kg diet. After bacterial challenge, fish mortality was lowest (8.45 ±1.30%) in fish fed 15 g MSLE/kg diet, whereas highest mortality (52.50 ±4.56%) was observed with fish fed the control diet. The present study conjured that MSLE inclusion in fish diets with optimum level of 10 g/kg diet stimulated significantly the performance, nutrient utilization, modified gut morphometry, and innate immune response of common carp. Also, its inclusion protected fish against pathogenic bacteria, *A. hydrophila* infection.

Szybkość wzrostu, morfometria jelit i wrodzony profil odpornościowy młodocianych osobników karpia *Cyprinus carpio* karmionych dietą wzbogaconą ekstraktem z liści *Mitracarpus scaber* oraz ich podatność na bakterię chorobotwórczą *Aeromonas hydrophila*

Słowa kluczowe karp, wzbogacanie diety, szybkość wzrostu, morfometria jelit, immunostymulanty

Streszczenie *Mitracarpus scaber*, endemiczna roślina lecznicza z Nigerii w Afryce, ma wartość leczniczą. W niniejszym badaniu ekstrakt z liści *Mitracarpus scaber* (MSLE) podawano karpiowi, *Cyprinus carpio*, aby ocenić jego wpływ na szybkość wzrostu, wykorzystanie składników odżywczych, morfometrię jelit i parametry odporności wrodzonej. Cztery izoazotowe diety (32% surowego białka) zawierające 0,0; 5, 10 lub 15 g diety MSLE/kg podawano młodym rybom (7,52 ±0,23 g) przez 12 tygodni. Po tym okresie ryby były narażone na bakterie chorobotwórcze (*Aeromonas hydrophila*) przez 14 dni. Szybkość wzrostu, wykorzystanie składników odżywczych i spożycie paszy zwiększały się wraz ze wzrostem poziomu MSLE do wartości 10g/kg diety. Ryby ze wzbogaconą dietą znacznie zwiększyły stosunek długości/szerokości kosmków jelitowych i obszar wchłaniania. Ponadto wielkość wybuchu tlenowego, oraz aktywność lizozymu, katalazy i dysmutazy ponadtlenkowej były znacznie wyższe u ryb karmionych pokarmem wzbogaconym o MSLE. Najwyższe wartości wyżej wspomnianych parametrów uzyskano u ryb karmionych pokarmem wzbogaconym o 15 g MSLE/kg. Po wprowadzeniu bakterii śmiertelność ryb była najniższa (8,45 ±1,30%) u ryb karmionych 15 g diety MSLE/kg, podczas gdy najwyższą śmiertelność (52,50 ±4,56%) obserwowano u ryb karmionych dietą kontrolną. W niniejszym badaniu wykazano, że włączenie MSLE w ilości 10 g/kg diety znacząco stymuluje wydajność, wykorzystanie składników odżywczych, modyfikuje morfometrię jelit i wrodzoną odpowiedź immunologiczną karpia. Włączenie do diety MSLE chroniło ryby przed infekcją bakteriami chorobotwórczymi, *A. hydrophila*.

Introduction

Fish are considered as ones of the highly nutritional sources for animal proteins. It has high-quality protein, vitamins and minerals, and unsaturated fatty acids. The knowledge of nutritional, and health benefit of fish has resulted into its consumption (Adeshina, Jenyo-Oni, Emikpe, Ajani, Abdel-Tawwab, 2018b); therefore, creating mammoth gap between the demand, and supply for fish. In recent times, aquaculture has been augmenting shortage between the demand, and supply of fish, following the shortage of supply from captured fisheries. However, diseases are impeding the success of aquaculture industry.

Series of researches have been focused on use of phytobiotics to combat fish diseases, and improve their immunity (Abdel-Tawwab, 2010, 2012, 2015, 2016; Abdel-Tawwab, Abbass, 2017; Abdel-Tawwab, Ahmad, 2009; Guardiola et al., 2016; Van Doan, Hoseinifar, Dawood, Chitmanat, Tayyamath, 2017; Hoseinifar, Dadar, Khalili, Cerezuela, Esteban, 2017a; Hoseinifar et. al. 2017b; Adeshina, Adewale, Tiamiyu, 2017; Abdel-Tawwab, Adeshina, Jenyo-Oni, Ajani, Emikpe, 2018a). This is because medicinal plants such as *Mitracarpus scaber* are generally regarded as safe (GRAS), have no residual effect, environmental eco-friendly among other factors. *M. scaber* belongs to the family Rubiaceae with about 30 different species. However, in Nigeria, *M. scaber* is the commonest species. The leaves of this plant are used to treat many diseases in both man and animal. It has been reported to have antimicrobial and antimycotic properties such as gallic acid, 3,4,5-trimethoxybenzoic acid, 4-Methoxyacetophenone, 3,4,5-trimethoxyacetophenone, n-octane, 2-hexanol, p-cymene, α and β pinene etc (Owolabi, Arhewoh, Innih, Anaka, Monyei, 2014). It is claimed that this plant has also antibacterial and antifungal activities (Bisignano et al., 2000; Abere, Onwukaeme, Eboka, 2007; Anejionu et al., 2011).

Fish production is hindered by disease outbreaks of aeromonoid caused by *Aeromonas hydrophila* infection. The disease is causing severe loss in fish farming. Some of the signs of aeromonoid include furunculosis, hemorrhage, and red sore disease, among others, leading to mass mortality of fish, and economic losses (Shoko, Limbu, Mgaya, 2016; Talpur, 2014; Tan et al., 2018). The common way of controlling *A. hydrophila* infection is using antibiotics, which has been discouraged due to evolving disease resistance, environmental degradation among others. Therefore, there is need to focus on developing feed supplements as substitutes to chemotherapeutics for control, and management of bacterial diseases in order to sustain environmentally eco-friendly aquaculture.

Common carp is one of the cultured fish species in tropical Africa, and widely accepted (Adeshina, Jenyo-Oni, Emikpe, Ajani, 2018a). To large extent, it is important to develop feed supplements as a replacement to the use of synthetics drugs to promote, and sustain responsible aquaculture. Therefore, the present study was carried out to evaluate the application of *Mitrascarpus scaber* leaf extract (MSLE) in practical diets for common carp as growth, nutrient utilization, and immune parameters. Fish resistance to pathogenic bacteria, *A. hydrophila*, was also investigated.

Materials and Methods

Plants collection and identification

The fresh leaves of *M. scaber* were collected from Ile-Apa community, and authenticated in the Herbarium Unit, Forest Research Institute of Nigeria, Ibadan, Nigeria.

Preparation of experimental diets

The leaves were dried at 35°C, milled into fine powder and 5 grams were extracted in ethyl-acetate (50 mL) using hot method for 4 hr. The chemical was eliminated in a rotary evaporator at 45°C. The extracts were then stripped into sterilized bottles, and stored at 20°C until use. Four isonitrogenous diets (32% crude protein) were formulated with fish meal (72%), toasted soybean (46.2%), and white maize (9.3%) (CFA, 1974; Drury et al., 1967) to contain 0.0, 5, 10, or 15 g MSLE/kg diet (Table 1).

Table 1. Ingredients and proximate chemical composition (%; on dry matter basis) of the experimental diets containing graded levels of Mitrascarpus scaber leaves extract (MSLE)

Ingredients	MSLE levels (g/kg diet)			
	0.0 (control)	5.0	10.0	15.0
1	2	3	4	5
Fish meal	252.4	252.4	252.4	252.4
Soybean	232.4	232.4	232.4	232.4
Maize	465.2	460.2	455.2	450.2
Starch	15.0	15.0	15.0	15.0
Vegetable oil	15.0	15.0	15.0	15.0
Premix[a]	20.0	20.0	20.0	20.0
MSLE	0.0	5.0	10.0	15.0
Total	1000.0	1000.0	1000.0	1000.0

1	2	3	4	5
Proximate composition (%)				
Crude protein	32.01	32.04	32.22	32.23
Moisture	9.17	8.53	8.83	9.17
Ether extract	9.39	9.55	9.48	9.90
Ash	8.35	8.78	8.55	8.90
CHO(%)[b]	28.18	28.10	28.45	27.27
Fibre (%)	12.90	13.00	12.47	12.53

[a] Premix = HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A, 4,000,000 International Unit (IU); vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K3, 1,600 mg; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,800 mg; vitamin B12, 3 mcg; Nicotinic acid 18,000 mg; Pantothenic acid, 8,000 mg; Folic acid, 800 mg; Biotin, 100 mcg; Choline chloride 120,000 mg; Iron, 8,000 mg; Copper, 800 mg; Manganese, 6,000 mg; Zinc, 20,000 mg; Iodine, 400 mg; Selenium, 40 mg; Vitamin C C(coated), 60,000 mg; Inositol, 10,000 mg; Colbat, 150 mg; Lysine, 10,000 mg; Methionine, 10,000 mg; Antioxidant, 25,000 mg.

[b] CHO = Carbohydrate.

Experimental procedure and design

Common carp, *C. carpio* juveniles were obtained from a reputable farm, and acclimated to lab conditions for 14 days during which fish fed a commercial diet (32% crude protein). Fish (7.52 ±0.23 g) were randomly allotted into 12 aquaria (50 L) at a density of 20 fish/aquarium. The fish were fed on one of the formulated diets up to nearly satiation two times every day at 08:00 am and 06:00 pm for 84 days. Throughout the experiment, water from tanks was replaced every 3-day intervals. Water samples were collected from the rearing tanks to monitor water quality. Water temperature ranged from 23.23 to 25.17°C, dissolved oxygen from 5.80 to 6.66 mg/l, conductivity from 0.36 to 0.90 $ms.cm^{-1}$, nitrite from 0.95 to 1.00 ppm, nitrate ranged from 33.35 to 53.20 ppm, and pH from 7.15 to 7.89 measured with mercury thermometer, Winkler method, commercial kits (Colombo nitrate, nitrite, and pH test kit) respectively.

Growth performance and nutrient utilization

Fish per each tank were collected, counted, and group-weighed biweekly and at the end of the feeding trial on a digital ScoutPro sensitive scale (Model: KD-200-110, USA). Parameters of growth performance and nutrients utilization were calculated as:

$$\text{Body weight gain}\left(\text{g}\right) = W_1 - W_0;$$

$$\text{Specfic growth rate}\left(\text{SGR};\ \%\text{g / day}\right) = \frac{\text{Ln}W_1 - \text{Ln}W_0}{\text{Length of the culture period}} \times 100;$$

$$\text{Feed intake} = \text{summation of feed fed to fish in each tanks / fish No.};$$

$$\text{Feed conversion ratio}\left(\text{FCR}\right) = \frac{\text{Dry weight of feed fed}\left(\text{g}\right)}{\text{Fish weight gain}\left(\text{g}\right)};$$

$$\text{Protein efficiency ratio}\left(\text{PER}\right) = \frac{\text{Wet body weight gain}\left(\text{g}\right)}{\text{Crude protein fed}};$$

$$\text{Where protein fed} = \frac{\text{Protein}(\%)\text{in diet} \times \text{diet consumed}}{100};$$

$$\text{Survival rate}(\%) = \frac{\text{Final number of fish stocked}}{\text{Initial number of fish stocked}} \times 100.$$

Where: W_1 = final mean weight; W_0 = initial mean weight; L_1 = final mean length; L_0 = initial mean length.

Gut morphometry

Three fish from each tank were collected and anthesized with buffered tricaine methane sulfonate (30 mg/L). Fish intestine was taken for histological examination. The guts were prepared on slides as described by Drury, Wallington and Roy (1967). The guts were excised, and cut with a sharp scalpel blade into small pieces, and fixed by immersing in modified Bouin's fluid made up of picric acid (300 mL), formalin (100 mL), and 1% tricarboxylic acid (20 mL) according to the methods of Eyarefe, Emikpe and Arowolo (2008), followed by the dehydration of the tissues in 70, 80, 90, 95%, and absolute ethanol. The sections were prepared (4 mm thickness), followed by hydration in absolute ethanol, 95, 90, 80, and 70% ethanol. Villus length and width (mm), depth of the crypt (DC, mm), villus width (VW, mm), and area of absorption (AA, mm^2) were Measurements of taken using light microscope (HE x40) (Olympus CX21, Japan) in triplicates with a micrometer rule (Eyarefe et al., 2008; Fox et al., 1997; Bello et al., 2012).

$$\text{Area of absorption}\left(\text{mm}^2\right) = \text{Villus length}(\text{mm}) \times \text{Villus width}(\text{mm})$$

Innate immunity assays

Three fish from each tank was collected, and anthesized with buffered tricaine methane sulfonate (30 mg/L). Blood collected from the caudal vein and used to determined innate immune parameters within the aid of Randox® Laboratories diagnostic kits (Crumlin, County Antrim, UK). Blood of fish from each experimental unit was pooled together. Superoxide dismutase (SOD) activity was determined by ferricytochrome-C method. Xanthine oxidase was used as the source of superoxide radicals. The quantity of enzyme required to produce 50% inhibition of ferricytochrome-C was taken as one activity unit (McCord, Trouslade, Ryu, 1984). The rate of H_2O_2 at 240 nm was used to estimate the catalase (CAT) activity (Aebi, 1984). To determine the lysozyme activities in the fish sera, *Micrococcus luteus* (0.60 mg/mL) was spread in agarose gel (1%), and phosphate buffer (pH 6.2, 50 mM) (Difco BD Co, Franklin Lakes, NJ). Nutrient Agar prepared plates were dug to have wells of 6 mm diameter using cork-borer. The wells were filled with fish sera (25 µL/well), and incubated (25°C) for 20 hr. Then, the lysozyme activity was estimated from a standard curve prepared with lysozyme from chicken egg white (Abdel-Tawwab et al., 2018a).

Respiratory burst activity (RBA) was measured with the aid of Randox® Laboratories diagnostic kits (Crumlin, County Antrim, UK) as described by Chiu, Guu, Liu, Pan, Cheng (2007). Nitroblue tetrazolium (NBT) assay was used to measure the RBA which indicates the quantity

of intracellular oxidative free radicals (Grinde, 1989; Secombes, 1990). Microplate reader (Optica, Mikura Ltd, UK) was used evaluate the NBT reduction.

Bacterial challenge test

The susceptibility of common carp, *C. carpio* was examined using pathogenic bacteria (*A. hydrophila*) after the 12-week feeding trial. The A. hydrophila (ATCC 4356) isolate was pobtained from Department of Microbiology Laboratory, University of Ibadan, Nigeria. The LD50 (lethal dose) of the pathogenic bacteria was determined. Bacterial isolates were grown on nutrient broth plus yeast extract, and incubated at 35°C for 24 h to revive the bacteria. Ten (10) fish from each experimental unit were exposed to *A. hydrophila*, which were grown on nutrient broth plus yeast extract for 24 hour at 35°C in an incubator. Bacterial cells were then centrifuged at 3000 g for 30 min to form pellets. The pellets were resuspended in 1.0 mL of 0.1% peptone water. For the challenge test, fish from each treatment were collected, and 20 fish (10 fish per tank) were transferred into two other tanks previously filled with dechlorinated freshwater as two replicates. Fish were challenged with a 0.1-mL dose of *A. hydrophila* (5×10^5 CFU/mL) by intraperitoneal injection, and were returned to the experimental setup (Adeshina et al., 2018b; Abdel-Tawwab et al., 2018a). Fish were fasted for 24 h before infection, and refeeding with the corresponding experimental diets 12 h later. All fish groups were kept under observation for 14 days to record any abnormal clinical signs and daily mortality.

Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) using IBM statistical package (SPSS version 20) to determine differences among the treatments in all parameters. Individual means were separated using Duncan multiple range test. Quadratic regression was used to determine the optimum level of extract for weight gain. All data were presented as means ± SD, and were declared significant at $P < 0.05$ according to Dytham (2011).

Results

Growth performance

The growth and nutrients utilization of common carp, *C. carpio*, fed diets fortified with *M. scaber* leaves extract showed significant improvements when compared to the control group ($P < 0.05$; Table 2). Diets fortification with higher MSLE (10 g/kg diet) gave higher performance in terms of final weight, body weight gain, SGR, feed intake, and protein efficiency ratio, while least ones were recorded in fish fed the control diet. There were no significant difference was noticed in the fish survival ($P > 0.05$). Fish growth was significantly improved by dietary MSLE over that fed the control diet ($P < 0.05$).

Table 2. Growth performance and nutrients utilization of *Cyprinus carpio* fed diets fortified with *Mitrascarpus scaber* extract for 84 days

Parameters	MSLE levels (g/kg diets)			
	0.0	5.0	10.0	15.0
Initial weight (g)	7.52 ±0.23^{a}	7.51 ±0.20^{a}	7.52 ±0.14^{a}	7.54 ±0.09^{a}
Final weight (g)	20.86 ±1.50^{b}	22.61 ±2.03^{b}	25.27 ±2.10^{a}	25.70 ±1.33^{a}
Weight gain (g)	13.34 ±1.83^{c}	15.10 ±1.70^{b}	17.75 ±2.17^{a}	18.16 ±1.15^{a}
Specific growth rate (%g/day)	1.21 ±0.03^{b}	1.31 ±0.12^{b}	1.44 ±0.22^{a}	1.46 ±0.16^{a}
Feed intake (g)	32.13 ±0.12^{c}	33.04 ±0.20^{b}	33.13 ±0.05^{b}	33.54 ±0.11^{a}
Feed Conversion Ratio	2.41 ±0.09^{a}	2.18 ±0.08^{b}	1.87 ±0.09^{c}	1.85 ±0.05^{c}
Survival rate (%)	96.77 ±5.77^{a}	95.00 ±8.66^{a}	98.33 ±2.89^{a}	98.33 ±2.89^{a}

Different superscripts in the same row are statistically significant different between means at $P < 0.05$.

Intestine morphometry

The inclusion of MSLE in diets for common carp, *C. carpio* increased significantly villi length/width and absorption area of the intestine ($P < 0.05$). The increase in the intestine morphometry was observed with the increase in MSLE levels in diets; however, fish fed the basal diet had lowest villi length/width and area of absorption. Similarly, there was a progressive increase in the cryptal depth of the fish fed diets fortified with MSLE ($P < 0.05$; Table 3).

Table 3. Changes in morphometry of the intestine of *Cyprinus carpio* juveniles fed *Mitrascarpus scaber* extract based diets for 84 days

MSLE levels (g/kg)	Villi length (mm)	Villi width (mm)	Cryptal depth (mm)	Area of absorption (mm^2)
0	0.32 ±0.03^{c}	0.21 ±0.06^{c}	0.14 ±0.01^{b}	0.07 ±0.03^{c}
5	0.54 ±0.08^{b}	0.30 ±0.04^{b}	0.19 ±0.04^{a}	0.16 ±0.03^{b}
10	0.63 ±0.02^{a}	0.33 ±0.02^{b}	0.21 ±0.01^{a}	0.21 ±0.02^{a}
15	0.64 ±0.20^{a}	0.35 ±0.04^{a}	0.24 ±0.03^{a}	0.22 ±0.04^{a}

Different superscripts in the same column are statistically significant different between means at $P < 0.05$.
Note: area of absorption (mm^2) = Villi length (mm) × Villi width (mm).

Innate immune parameters

The innate immune profile of fish fed diets supplemented with MSLE is shown in Table 4. The results indicated that supplementation of MSLE to common carp, *C. carpio* enhanced significantly the immunity parameters ($P < 0.05$). It was noticed that fish fed diet containing 10 g MSLE/kg diet had highest RBA (154.31 ±8.01), lysozyme activity (10.41 ±1.45), catalase (1.32 ±0.10 mg/g protein), and SOD (1.37 ±0.06 mg/g protein), and their least values were observe with fish fed the control diet (121.02 ±8.24, 9.53 ±1.20, 1.24 ±0.02 mg/g protein, and 0.43 ±0.01 mg/g protein, respectively). Furthermore, fish resistance against bacterial infection was improved. The post-challenge mortality of the fish infected with *A. hydrophila* is also shown in Table 4. There was a significant reduction in the post-challenge mortality ($P < 0.05$). Highest mortality (52.50 ±4.56%) was recorded in fish fed the control diet, while least one (8.45 ±1.30%) was observed in fish fed a diet containing 10 g MSLE/kg diet.

Table 4. Changes in respiratory burst activity (RBA), lysozyme activity, Catalase, Superioxide dismutase (SOD) and post-challenge mortality of *Cyprinus carpio* juveniles fed *Mitrascarpus scaber* extract based diets for 84 days

MSLE levels (g/kg diet)	RBA (mg/mL)	Lysozyme (U/mg protein)	Catalase (mg/g protein)	SOD (mg/g protein)	Post-challenge mortality (%)
0	121.02 ±8.24^{b}	9.53 ±1.20^{a}	1.24 ±0.02^{b}	0.43 ±0.01^{c}	52.50 ±4.56^{a}
5	128.16 ±5.92^{b}	9.58 ±1.11^{a}	1.27 ±0.05^{a}	0.84 ±0.03^{b}	33.21 ±4.21^{b}
10	143.29 ±6.11^{ab}	10.27 ±0.98^{a}	1.30 ±0.12^{a}	1.32 ±0.01^{a}	15.17 ±2.50^{c}
15	154.31 ±8.01^{a}	10.41 ±1.45^{a}	1.32 ±0.10^{a}	1.37 ±0.06^{a}	8.45 ±1.30^{d}

Values are represented as mean ± standard deviation of triplicates; different superscripts in the same column are statistically significant different between means at $P < 0.05$.

Discussion

The inclusion of medicinal plants in fish diets has been studied, and positive results have been documented (Abdel-Tawwab, Ahmad, 2009; Abdel-Tawwab, Ahmad, Seden, Sakr, 2010; Abdel-Tawwab, 2018a; Abdel-Tawwab, 2012, 2015, 2016; Guardiola et al., 2016; Abdel-Tawwab, Abbass, 2017; Adeshina et al., 2017; Hoseinifar et al., 2017a, 2017b; Van Doan et al., 2017; Adeshina et al., 2018b). Their phytobiotics activity has made them suitable substitutes for antibiotics, growth promoters, and immune boosters. The present study, fish fed diets fortified with MSLE showed improved growth, and utilized the given diets efficiently when compared to the groups fed the basal diet. The higher growth observed herein might be associated with the higher feed consumed by fish fed MSLE-supplemented diets. The presence of antimicrobial, and antimycotic properties such as gallic acid, 3,4,5-trimethoxybenzoic acid, 4-Methoxyacetophenone, 3,4,5-trimethoxyacetophenone, n-octane, 2-Hexanol, p-Cymene, α and β pinene, eugenol etc might assist in reducing pathogenic organisms in fish gut; hence, promoting the colonization of beneficial bacteria that enhanced diets digestibility. The presence of the secondary metabolites MSLE may be stimulated its immunostimulant properties. The present of eugenol, and p-cymene in the plants could be responsible for it acceptability by the fish (Adeshina et al., 2018b). The results of the present study are in agreement with previous studies, which fed fish on phytobiotics additives (Abdel-Tawwab et al., 2018a; Sogbesan, Ahmed, Ajijola, 2017; Offor et al., 2014; Adewole, Faturoti, 2017; Harikrishnan, Balasundaram, Heo, 2011). Other studies (Adeshina et al., 2018b; Abdel-Tawwab et al., 2018a; Sogbesan et al., 2017) reported that fish fed varying inclusion levels of plants extracts had higher growth performance that the growth fed the basal diet. The present study revealed that the increase in fish weight with the increase in the MSLE levels in diets more than the control diet. Thus, such increase in the body weight of the fish fed MSLE-based diets suggests better ingestion, digestion activity, and nutrient absorption.

One of the tools that researchers have adopted in assessing the nutrients absorption in fish is the examination of gut morphometry. The improvements observed in villi length/width, cryptal depth, and area of absorption of common carp, *C. carpio* juveniles fed MSLE-based diets revealed the improvements of nutrients utilization by fish. These increases in gut morphometry in the present study supported the increase in fish weights better than observation in the control group especially fish fed 10 g MSLE/kg diet. These results are in concomitant with previous studies (Zhou et al., 2010; Zahran, Risha, AbdelHamid, Mahgoub, Ibrahim, 2014; Zhang et al., 2010). Furthermore, it has been reported that inducement of intestinal morphology increases nutrient

utilization ability of the fish leading to increasing fish growth, and feed utilization (Dimitroglou et al., 2010; Zhou, Buentello, Gatlin, 2010; Zahran et al., 2014). In similar studies, Abdel-Tawwab et al. (2018a) and Adeshina et al. (2018b) found significant increases in villi length/width and areas of absorption of African catfish, *Clarias gariepinus* fed diets fortified with plants extracts.

The fish survival in all treatments was higher than 95% which validated that MSLE has no toxic effect on fish. This result is in agreement with the earlier findings revealing the successful usage of aromatic plants in fish diets (Ainsworth, 1992; Saeidi, Adel, Caipang, Dawood, 2017; Awad, Austin, 2010; Abdel-Tawwab, Sharafeldin, Mosaad, Ismaiel, 2015; Brum et al., 2017; Abdel-Tawwab et al., 2018b).

The innate immune response of common carp, *C. carpio* fed MSLE-enriched diets improved significantly ($P < 0.05$) better than fish fed the basal diet. Higher RBA, lysozyme, SOD, and catalase activities may be attributed to the bioactive ingredients of MSLE. These active ingredients perform chemopreventive, free radical scavenging, and antimycotic activities (Adeshian et al., 2018a; Abdel-Tawwab et al., 2018a). Also, several phenolic compounds with antioxidant properties are presence in MSLE. Reports have shown that RBA and lysozyme activities are important immune parameters, which play a significant role as defense mechanism against many invading agents (Adel et al., 2015; Jayashree, Subramanyam, 1999). As revealed in the present study, MSLE stimulates respiratory burst, and lysozyme activities. Moreover, dietary MSLE enhanced the fish resistance against *A. hydrophila* infection. Abdel-Tawwab et al. (2018a) reported that disease challenge trial is a critical way to investigate the effectiveness of phytobiotics on the immune system of fish. The reduction in post-challenged fish mortality observed in the present study is associated with immunomodulatory effect of MSLE. These results may be because this plant has antibacterial and antifungal activities (Bisignano et al., 2000; Abere et al., 2007; Anejionu et al., 2011). Furthermore, usefulness of MSLE active ingredients is their hydrophobicity and phagocytic activities (Chou, Hung, Lin, Lee, Leu, 2010; Zahran et al., 2014). Several studies indicated that feed supplemented with various medicinal plants improved their resistance against pathogens bacteria (Adeshian et al., 2018a; Abdel-Tawwab, 2012, 2015, 2016; Abdel-Tawwab, Abbass, 2017; Abdel-Tawwab, Ahmad, 2009; Abdel-Tawwab et al., 2010; Guardiola et al., 2016; Van Doan et al., 2017; Hoseinifar et al., 2017a, 2017b; Adeshina et al., 2017; Abdel-Tawwab et al., 2018a; Owolabi et al., 2014; Irobi, Daramola, 1993).

Conclusions

Growth performance and nutrient utilization of common carp fed MSLE-fortified diets were significantly improved over the control diet with optimum level of 10 g MSLE/kg diet. The present study proffers a new outlook for the use of *M. scaber* as phytobiotics to boost fish growth, and immunity. Furthermore, MSLE inclusion in the diet of common carp, *C. carpio,* could be used to enhance intestinal morphology, and resistance against bacterial infection with *A. hydrophila*.

Ethical statement

The protocol of the study was subjected to ethical consideration and was approved by Animal Care Use and Research Ethics Committee, University of Ibadan, Ibadan, Oyo state, Nigeria with reference number UI-ACUREC/App/03/2017/008.

Conflict of interest

We declared that there are no conflict of interests.

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Cite as: Adeshina, I., Emikpe, B.O., Jenyo-Oni, A., Ajani, E.K., Abubakar, M.I. (2019). Growth performance, gut morphometry and innate immune profiles of common carp, *Cyprinus carpio* juveniles fed diet fortified with *Mitracarpus scaber* leaves extract and its susceptibility to pathogenic bacteria, *Aeromonas hydrophila*. *Acta Biologica*, *26*, 5–17. DOI: 10.18276/ab.2019.26-01.