Umma *et al.*
The Journal of Basic and Applied Zoology (2023) 84:23
https://doi.org/10.1186/s41936-023-00345-7

The Journal of Basic and Applied Zoology

RESEARCH

Open Access

Relationship pattern of enteric bacterial load and assessed micronutrients in the gut of *Clarias gariepinus* fish sampled in the Ibadan municipal zone

Samuel Bem Umma[1*], Adams Ovie Iyiola[2] and Ibrahim Adeshina[3]

Abstract

Background Farmed fish are faced with factors like microflora and micronutrients that could impact their prime health. There is no clear understanding of some specific bacterial flora amidst micronutrients in the gut of the African sharp-tooth catfish (*Clarias gariepinus*); hence, this study investigated the encountered bacterial flora, micronutrients and their interactions with the aid of standard microbiological procedures and atomic absorption spectroscopy.

Results The bacterial counts in the Ibadan municipal zones were significantly different (P<0.05) across each sampling week, with prevalence ranging from 0.40 ± 0.1 in Ibadan North to 0.10 ± 0.00 in Ibadan Northwest. The pattern of encountered bacterial flora indicated Ibadan North (*Enterobacter* species: $3.70 \pm 0.07 \times 10^6$ CFU/g) as the prevalent. The assessed gut micronutrients were significantly different (*P*<0.05) across the farm areas; however, sodium was not significantly different (*P*<0.05) in Ibadan Northeast (0.97 ± 0.02) and Ibadan Southwest (0.98 ± 0.01), manganese in Ibadan North (10.50 ± 0.07) and Ibadan Northeast (11.00 ± 0.71), cobalt in Ibadan North (0.00 ± 0.00), Ibadan Northeast (0.00 ± 0.00) and Ibadan Southeast (0.00 ± 0.00). Zinc (92.55 ± 0.01 mg/kg), chromium (13.11 ± 0.22 mg/kg), cadmium (0.82 ± 0.00 mg/kg) and cobalt (6.94 ± 0.22 mg/kg) were predominantly high in Ibadan Northwest, sodium (0.98 ± 0.01 mg/kg) in Ibadan Southwest, lead (4.37 ± 0.01 mg/kg) and copper (34.10 ± 0.07 mg/kg) in Ibadan Southeast. The gut bacterial were positively correlated with manganese, iron, zinc, nickel, potassium and sodium; however, they were negatively correlated with copper, lead, chromium, cadmium and cobalt.

Conclusions Therefore, the present study identified some fish farm areas in the Ibadan municipal zones with prevalent gut bacterial load; this is possibly due to the state of the culture environment and also the interactions of the gut bacterial flora with micronutrients, which could be a potential health indicator.

Keywords *Clarias gariepinus*, Micronutrients, Gut bacterial flora, Prime health, Ibadan municipal zones

*Correspondence:
Samuel Bem Umma
umma@fuwukari.edu.ng
Full list of author information is available at the end of the article

Background

Fish is one of the man's most important sources of high-quality animal protein, providing approximately 17% of the animal protein consumed by the world's population, especially from farmed fish (Food & Agriculture Organization, 2018). It is considered to be a central component of man's diet due to its superior protein quality. However, farmed fish are faced with many factors that could have an effect on their health status and the efficient utilization of ingested nutrients. Many local aqua-feed producers pay little attention to the micronutrient requirements of fish. According to Lilly et al. (2017), micronutrients are vital in fish food for good health. However, inadequate intake of one or more nutrients can result in malnutrition, thereby altering the gut chemistry and flora distribution pattern in fish. The deficiency of nutrients in feed can result in stress and growth retardation in fish (Bertucci et al., 2019; Shefat & Karim, 2018). The nutrient requirements vary and can be determined by various factors such as environmental conditions, sex and the stage of the life cycle (Michael et al., 2017).

In recent times, there has been increasing awareness of the role of some specific nutrients, their mechanism of action and the etiological effect of their deficiencies on fish (Brown et al., 2002; Shefat & Karim, 2018). Studies have shown that high levels of some micronutrients can result in contamination in much edible fish species (Kamaruzzaman et al., 2010). Since there is no single blueprint for the alimentary canal of fish species, variations in the biological system permit each species to develop its kind of adaptive digestive system and resident microflora regardless of environmental parameters (Roeselers et al., 2011; Egerton et al., 2018; Izah et al., 2022). The fish gut system accommodates important biological and chemical factors such as gut flora and micronutrients; hence, their resultant interaction would either benefit or affect the prime health of the fish.

Enteric bacteria belong to the family Enterobacterialceae and are normal resident bacterial in the gut of many animals, including fish and humans. Some of the bacterial species could be pathogenic, causing disease in fish species, thereby undermining the economic purpose of fish farming (Faruk et al., 2004), while some could have a mutual relationship in the fish gut without causing health problems to their host (Canny & McCormick, 2008). In recent times, research has been focused on the association between gut bacterial to meet the resurfacing needs of farmed freshwater fish for their health benefits (Egerton et al., 2018). However, a clear understanding of the roles that specific gut microflora play amidst macronutrient composition in the gut of fish is still lacking. Therefore, this study is aimed at investigating the relationship pattern of the bacterial flora and micronutrients in the gut of the African sharp-tooth catfish (*Clarias gariepinus*) as a reference for other future studies.

Methods

Sample collection

Thirty farms were selected in Ibadan municipal zones (IMZ) comprising of Ibadan North, Ibadan Northeast, Ibadan Northwest, Ibadan Southwest and Ibadan Southeast local government areas, and denoted with the alphabets A & B, C, D, E and F, respectively (Fig. 1). Five farms from each of the municipal zones were purposively selected based on their sizes and culture facility. The selected farms had concrete tanks ranging from 5 to 20 units in number. Twenty samples of *Clarias gariepinus* with an average weight of 595 ± 82.56 g were randomly collected weekly from the selected farms in each of the locations for six weeks. The total numbers of fish collected per week were twenty ($n = 20$), and at the end of the sampling period (June–July), a total of one hundred and twenty fish ($n = 120$) samples were collected.

The fish were sampled from the ponds using a scoop net and anesthetized with sodium bicarbonate-buffered tricaine methane sulfonate (MS222; 30 mg/L, Syndel, Ferndale, Washington, USA) as described by Adeshina et al. (2021). Thereafter, fish were placed one at a time on a clean board and their gut was excised using a sterile surgical blade, and the content from each gut was sample aseptically divided into two portions and placed in 20-mL sterile sampling bottles previously autoclaved at 121 °C for 15 min as described by Oscroft and Correy (1991). The samples were subsequently transferred separately in aseptic conditions sealed with nylon bags to avoid external contact as described by Kim et al. (2007), for microbial and micronutrient analysis in the Departments of Microbiology and Animal Science, University of Ibadan, respectively. The total number of samples for microbial analysis was 120 in numbers, while the samples for micronutrient analysis on the other hand were pooled, with twenty gut ($n = 20$) content per week from the sampled farms during the six-week sampling period.

Microbiological analysis

The culture media used for the microbial analysis were prepared according to the manufacturer's instructions (Oxoid Ltd.). Plate count agar (PCA), nutrient agar (NA) and eosin methyl blue agar (EMBA) were sterilized after preparations by autoclaving as previously mentioned.

Briefly, as described by the American Public Health Association (1978), PCA was prepared by adding 17.5 g (g) to 1 L (L) of distilled water and dissolved by boiling with frequent stirring in an Erlenmeyer flask and then distributed into Petri dishes. The NA was prepared by suspending 20.5 g of the agar in 1 L of distilled water,

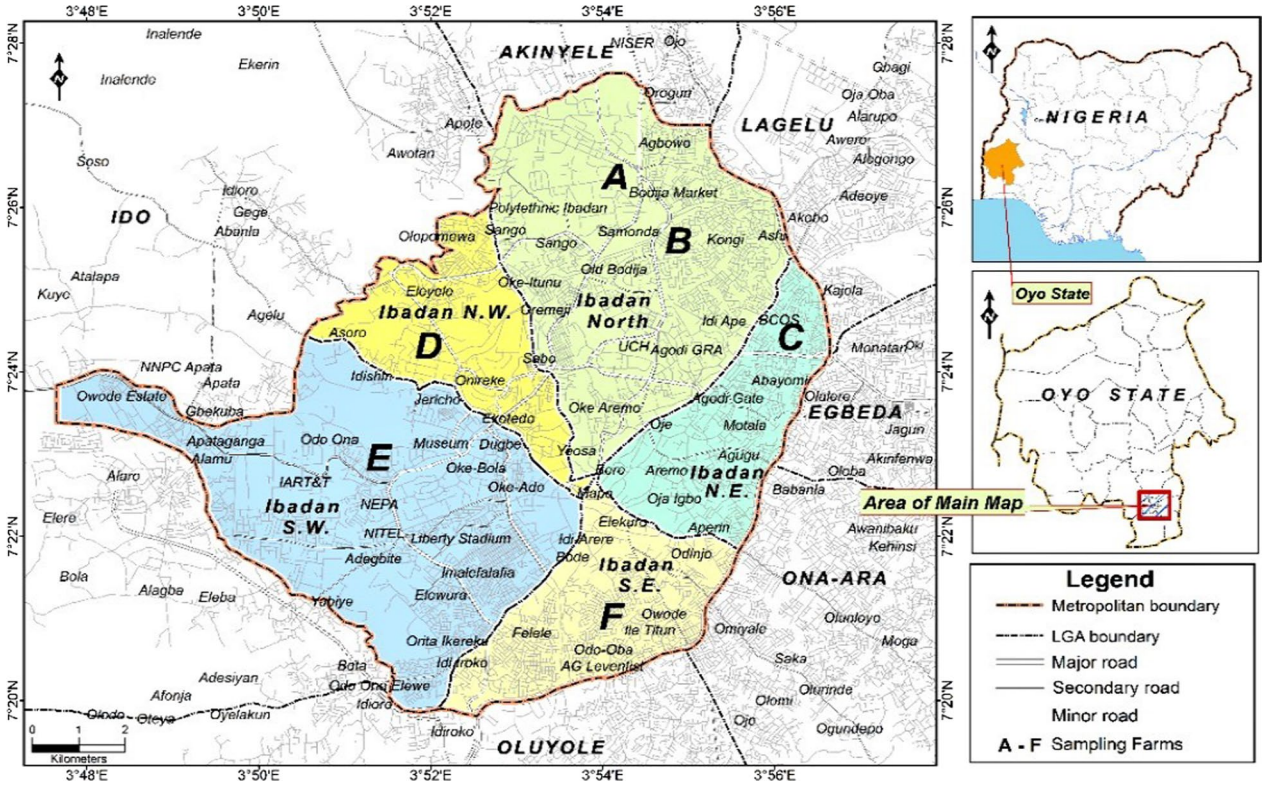

Fig. 1 Map of Ibadan municipal zones showing the sampled farm areas

boiled and then sterilized, and the EMBA was prepared by suspending 37.5 g of the agar in 1 L of distilled water, boiled and then sterilized.

The fish gut flora was assessed for ten bacterial species including *Escherichia coli*, *Pseudomonas, Bacillus, Flavobacterus, Enterobacter, Klebsiella, Aeromonas, Proteus, Salmonella* and *Shigella* species by placing 1 g of each homogenized gut samples in 10 ml (mL) of normal saline, using tenfold serial dilutions, and an aliquot from the dilution factor (10^{-6}) was used. Subsequently, 1 mL from the selected dilutions was placed into Petri dishes with already solidified molten agar and swirled properly. The inoculated plates were then incubated at 37 °C for 48 h to check for bacterial growth.

The bacterial colonies were counted after incubation and further subcultured to obtain a pure culture. Isolates from the pure culture were further identified using standard biochemical tests, namely gram staining, catalase, motility, oxidase, fermentation of sugar and indole production.

Briefly, gram staining was carried out by a 24-h culture of the isolates, after which a smear of each was made on a grease-free slide and heat-fixed properly. Crystal violet which is the primary stain was added to each slide and allowed to stay for 60 s after which it was washed off using clean running water. Grams of iodine were added as mordent and after 60 s washed off. The slides were decolorized using 95% ethanol to remove excess stains and rinse with water. The slides were counterstained using a secondary stain called Safranin and rinsed with clean running water. The slides were air-dried and observed under a microscope (X100 objective, oil immersion) for morphological characteristics as described by Herzog et al. (1998). The motility test was achieved by using an already prepared sulfideindole motility medium. The inoculum was then stabbed into the center of the semisolid agar deep at about half an inch to check for bacterial movement through growth zones from the inoculation line (Tille & Forbes, 2014). Catalase test was carried out using 2 mL of hydrogen peroxide (H_2O_2) solution placed inside the test tube. Colonies from the culture were picked using a sterile glass rod after a 24-h test and immersed in H_2O_2 and then observed for bubbling of gas (Sapkota, 2020). An oxidase test was carried out using tetramethyl-*p*-phenylenediamine-dihydrochloride substrate to soak filter paper. Sterile distilled water was used to moisten the paper, and the colony was picked using a sterile platinum loop and observed for any color change from deep blue or purple between 10 and 30 s (Shield & Cathcart, 2010). For the fermentation of sugar, an inoculation needle was

used to touch the top of each of the isolated colonies; the triple sugar iron agar (TSI) was inoculated at 37 °C in ambient air for 24 h, and the reaction was observed for color change. A lactose-positive color change from red to yellow indicated a pH change to acidity. A positive glucose color change to yellow indicated that the bacterium fermented glucose (Cappuccino & Sherman, 2008). For the Indole test, the inoculum was placed in a sterile test tube with prepared 4 mL tryptophan broth and incubated for 24 h, followed by introducing 0.5 mL of Kovac's reagent in the culture broth and observing for the presence of ring (Kar et al., 2017).

Micronutrient analysis

The collected samples from the fish gut were assessed for eleven micronutrients including potassium (K), sodium (Na), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd) and cobalt (Co) according to the Association of Official Analytical Chemists (2000). The instrumentation for the determination of the micronutrients in the samples included: analytical balance, laboratory microwave oven (equipped with 100 mL digestion container), dispenser (micropipette and tips), volumetric flasks, polyethylene tubes, atomic absorption spectrometer (PerkinElmer Analyst 700 model), auto-sampler, air, acetylene and cathode lambs. The laboratory glass wares for the analysis were cleansed using 10% $HN0_3$, rinsed with distilled water and then air-dried.

Briefly, 10 g of the pooled specimen ($n=20$) each per week was properly homogenized and thermally dried in batches at 105 °C for 12 h using a laboratory microwave oven until a constant weight of 0.05% was obtained. The dried sample was ground for better homogeneity and sieved with a 0.5 mm diameter mesh size sieve. The sample was then ashed for about 8 h until a gray–white ash residue was obtained in a programmed furnace, with a temperature increase from room temperature to 450 °C.

The ash residues were then dissolved in 5 mL and 0.5 mL of 70% nitric acid (HNO_3 v/v) and 30% H_2O_2 v/v, analytical grades, respectively (Sigma-Aldrich, St. Louis, Mo, USA). The samples were then digested using a microwave oven. Afterward, cooled samples were filtered using Whatman filtered paper, then transferred into a volumetric flask and made up to 25 mL with 3% HNO_3. The digestion blanks were also prepared without the sample material, and the standard operating parameters for the working elements (Table 1) were then set according to the recommendations of the manufacturer (PerkinElmer, PA, USA). The cathode lambs for the elements were connected and the AAS instrument equipped with flame was switched on. The instrument was adjusted to zero versus water, followed by measurement of the absorbance of the standard solutions and the reagent blanks. All the samples and standards were run in replicates.

Table 1 Operational wavelength and slit width for the assessed micronutrients in the gut of *Clarias gariepinus* sampled across farms in the Ibadan municipal zones

Parameters	Wavelength (nm)	Slit width
K	766	0.7
Na	589	0.7
Mn	279	0.7
Fe	248	0.7
Cu	324	0.7
Zn	213	0.7
Pb	485	0.7
Ni	232	0.7
Cr	350	0.7
Cd	228	0.7
Co	241	0.7

The external calibration curve was calculated for the micronutrients using the data obtained from AAS measurements. The number of micronutrients in the reagent blanks and sample solutions was calculated using the equations below:

$$C_{\mathrm{bl}} = A_{\mathrm{bl}} \times K \times V_{\mathrm{o}} \tag{1}$$

where C_{bl} = weight of micronutrient (μg) in the reagent blank solutions (V_{o}), A_{bl} = absorbance of the reagent blank solution (milli-absorbance units), K = slope of the standard curve (μg)/mL per milli-absorbance units) and V_{o} = total volume of the reagent blank solution (mL).

$$C_{\mathrm{s}} = A_{\mathrm{s}} \times V_l \times f \tag{2}$$

where C_{s} = weight of micronutrients (μg) in the sample (V_1), A_{s} = absorbance of the sample solution (milli-absorbance units), V_l = total volume of the sample solution (mL) and f = dilution factor.

$$C = (C_{\mathrm{s}} - C_{\mathrm{bl}})/m \tag{3}$$

where C = concentration of micronutrients in the sample (μg/g) and m = weight of the sample (g).

Statistical analysis

Data analysis was carried out with the aid of Minitab®19.0 statistical software at $\alpha_{0.05}$. One-way ANOVA was used to define the differences and follow-up with the Tukey pairwise comparison test. Correlation analysis was used to establish the relationship between bacterial load and nutrients. The linear regression model was used to determine the relationship between micronutrients from the sampled farms during the weeks of study

$$Y = a + bX \quad (4)$$

where Y = micronutrients (dependent variable), a = constant, b = regression coefficient and X = weeks of study (independent variables).

The linear regression model was also used to determine the relationship between bacterial counts from the sampled farms during the weeks of study.

$$Y = a + bX \quad (5)$$

where Y = bacterial counts (dependent variable), a = constant, b = regression coefficient and X = weeks of study (independent variables).

Results

Microbiological analysis

Mean bacterial count in the gut of Clarias gariepinus sampled across farms in the Ibadan municipal zones

The values of the mean bacterial count (10^6 coliform-forming units per gram, CFU/g) of the gut of *Clarias gariepinus* ($n = 120$) from the selected farms in each of the Ibadan municipal zones are presented in Table 2. There were significant differences ($P < 0.05$) across farms per week (one-way ANOVA) during the study. The values ranged from 0.21 ± 0.01 (C: Ibadan Northeast and D: Ibadan Northwest) to 0.28 ± 0.01 (A & B: Ibadan North), with pooled StDev of 0.02 in week 1, 0.21 ± 0.01 (C: Ibadan Northeast) to 0.39 ± 0.01 (A & B: Ibadan North), with pooled StDev of 0.01 in week 2, 0.14 ± 0.07 (D: Ibadan Northwest) to 0.40 ± 0.01(A & B: Ibadan North), with a mean total of 0.24 ± 0.10 in week 3, 0.10 ± 0.00 (D: Northwest) to 0.31 ± 0.01 (A & B: Ibadan North), with pooled StDev of 0.01 in week 4, 0.18 ± 0.08 and 0.18 ± 0.01 (C: Ibadan Northeast and E: Ibadan Southwest, respectively) to 0.26 ± 0.01 (F: Ibadan Southeast), with pooled StDev of 0.01 in week 5, and 0.19 ± 0.01 (C: Ibadan Northeast) to 0.32 ± 0.01 (A & B: Ibadan North), with pooled StDev of 0.01 in week 6, respectively.

Distribution of bacterial isolates in the gut of sampled Clarias gariepinus across farms in the Ibadan municipal zones

In Table 3, there were significant differences ($P < 0.05$) among the isolated bacterial in all the farm locations (one-way ANOVA). The distribution of bacterial isolates in the gut of *Clarias gariepinus* ($n = 120$) across the sampled areas ranged from 0.00 ± 0.00 (*Salmonella* sp.) to 3.70 ± 0.07 (*Enterobacter* sp.) in A & B (Ibadan North) with pooled StDev of 0.24, 0.20 ± 0.07 (*Aeromonas* sp.) to 2.20 ± 0.10 (*Enterobacter* sp.) in C (Ibadan Northeast) with pooled StDev of 0.08, 0.10 ± 0.00 (*E. coli.*) to 3.30 ± 0.07 (*Enterobacter* sp.) in D (Ibadan Northwest) with pooled StDev of 0.05, 0.10 ± 0.00 (*Klebsiella* sp. and *Aeromonas* sp., respectively) to 3.30 ± 0.10 (*E. coli*) in E (Ibadan Southeast) with pooled StDev of 0.05 and 0.10 ± 0.00 (*Aeromonas* sp.) to 1.65 ± 0.01 (*Enterobacter* sp.) in F (Ibadan Southeast) with pooled StDev of 2.86.

Assessed micronutrients in the gut of sampled Clarias gariepinus across farms in Ibadan municipal zones

The mean value of the micronutrients (mg/kg) in the gut of *Clarias gariepinus* ($n = 20$) pooled per week as presented in Table 4 was significantly different ($P < 0.05$) across the farm locations, ranged from 0.68 ± 0.01 (D: Ibadan Northwest) to 2.31 ± 0.01 (A & B: Ibadan North) for K with pooled StDev of 0.01, 0.29 ± 0.01 (F: Ibadan Southeast) to 0.98 ± 0.00 (D: Ibadan Northwest) for Na with pooled StDev of 0.01, 2.02 ± 0.01 (F: Ibadan Southeast) to 10.5 ± 0.07 (A & B: Ibadan North) for Mn with pooled StDev of 0.32, 1.94 ± 0.01 (F: Ibadan Southeast) to 9.03 ± 0.01 (A & B: Ibadan North) for Fe with pooled StDev of 0.03, 7.22 ± 0.01(A: Ibadan North) to 34.10 ± 0.07 (F: Ibadan Southeast) for Cu with pooled StDev of 0.05, 12.41 ± 0.01(E: Ibadan Southwest) to 92.55 ± 0.01 (D: Ibadan Northwest) for Zn with pooled StDev of 0.09, 0.45 ± 0.02 (A & B: Ibadan North) to 4.37 ± 0.01(F: Ibadan Southeast) for Pb with pooled StDev of 0.01, 4.95 ± 0.01 (D: Ibadan Northwest) to

Table 2 Mean bacterial count (10^6 CFU/g) in the gut of *Clarias gariepinus* sampled across farms in the Ibadan municipal zones

IMZ	A & B	C	D	E	F	Pooled StDev
Week 1	0.28 ± 0.05^{a}	0.21 ± 0.01^{b}	0.21 ± 0.01^{b}	0.23 ± 0.01^{b}	0.22 ± 0.22^{b}	0.02
Week 2	0.39 ± 0.01^{a}	0.21 ± 0.01^{c}	0.27 ± 0.01^{b}	0.22 ± 0.01^{c}	0.28 ± 0.001^{b}	0.01
Week 3	0.40 ± 0.1^{a}	0.24 ± 0.01^{b}	0.14 ± 0.07^{c}	0.21 ± 0.01^{bc}	0.22 ± 0.01^{bc}	0.05
Week 4	0.31 ± 0.01^{a}	0.18 ± 0.02^{c}	0.10 ± 0.00^{d}	0.19 ± 0.01^{c}	0.23 ± 0.01^{b}	0.01
Week 5	0.21 ± 0.01^{ab}	0.18 ± 0.08^{b}	0.24 ± 0.01^{ab}	0.18 ± 0.01^{b}	0.26 ± 0.01^{a}	0.04
Week 6	0.32 ± 0.01^{a}	0.19 ± 0.01^{d}	0.22 ± 0.01^{c}	0.21 ± 0.01^{c}	0.25 ± 0.01^{b}	0.01

The means that do not share a letter across the rows are significantly different ($P < 0.05$)

IMZ Ibadan municipal zones, *A & B* farms located in Ibadan North, *C* farms located in Ibadan Northeast, *D* farms located in Ibadan Northwest, *E* farm located in Ibadan Southwest, *F* farms located in Ibadan Southeast

Table 3 Distribution of the isolated bacterial species (10^6 CFU/g) in the gut of *Clarias gariepinus* sampled across farms in the Ibadan municipal zones

Parameters	*E. coli*	*Pseudomonas*	*Bacillus*	*Flavobacterus*	*Enterobacter*	*Klebsiella*	*Aeromonas*	*Proteus*	*Salmonella*	*Shigella*	Pooled StDev
A & B	2.00 ± 0.71^{b}	1.40 ± 0.16^{cd}	1.80 ± 0.07^{bc}	1.05 ± 0.01^{de}	3.70 ± 0.07^{a}	1.05 ± 0.01^{de}	0.15 ± 0.01^{fg}	0.60 ± 0.07^{ef}	0.00 ± 0.00^{g}	0.30 ± 0.16^{fg}	0.24
C	1.25 ± 0.01^{c}	1.30 ± 0.16^{c}	1.90 ± 0.07^{b}	0.55 ± 0.01^{f}	2.20 ± 0.10^{a}	$0.80 \pm 0.10d^{e}$	0.20 ± 0.07^{g}	0.65 ± 0.01^{ef}	0.65 ± 0.01^{ef}	0.90 ± 0.07^{d}	0.08
D	0.10 ± 0.00^{g}	1.10 ± 0.07^{d}	1.45 ± 0.01^{b}	1.15 ± 0.01^{d}	3.30 ± 0.07^{a}	1.30 ± 0.07^{c}	0.00 ± 0.00^{g}	0.55 ± 0.01^{f}	0.80 ± 0.10^{e}	0.55 ± 0.01^{f}	0.05
E	3.30 ± 0.10^{a}	0.75 ± 0.02^{e}	2.20 ± 0.07^{b}	1.05 ± 0.01^{d}	1.80 ± 0.10^{c}	0.10 ± 0.00^{g}	0.10 ± 0.00^{g}	0.45 ± 0.02^{f}	0.35 ± 0.02^{f}	0.15 ± 0.01^{g}	0.05
F	0.20 ± 0.07^{a}	1.00 ± 0.00^{a}	1.40 ± 0.07^{a}	0.90 ± 0.07^{a}	1.65 ± 0.01^{a}	1.25 ± 0.01^{a}	0.10 ± 0.00^{a}	0.20 ± 0.07^{a}	0.40 ± 0.07^{a}	4.30 ± 9.06^{a}	2.86

The means that do not share a letter across the rows are significantly different ($P < 0.05$)

IMZ, Ibadan municipal zones; A & B, farms located in Ibadan North; C, farms located in Ibadan Northeast; D, farms located in Ibadan Northwest; E, farms located in Ibadan Southwest; F, farms located in Ibadan Southeast

Table 4 Mean values of micronutrients in the gut of *Clarias gariepinus* sampled across farms in Ibadan municipal zones

Nutrients (mg/kg)	A & B	C	D	E	F	Pooled StDev	DRH
K	2.31 ± 0.01^{b}	2.38 ± 0.01^{a}	0.68 ± 0.01^{e}	2.28 ± 0.01^{c}	0.97 ± 0.01^{d}	0.01	3.58^{b}
Na	0.44 ± 0.02^{c}	0.97 ± 0.02^{a}	0.71 ± 0.01^{b}	0.98 ± 0.01^{a}	0.29 ± 0.01^{d}	0.01	1.97^{c}
Mn	10.50 ± 0.07^{a}	11.00 ± 0.71^{a}	4.50 ± 0.07^{c}	8.30 ± 0.07^{b}	2.02 ± 0.01^{d}	0.32	$2–5^{a}$
Fe	9.03 ± 0.01^{a}	2.80 ± 0.07^{d}	3.74 ± 0.01^{c}	4.06 ± 0.01^{b}	1.94 ± 0.01^{e}	0.03	$1–2^{a}$
Cu	7.22 ± 0.01^{e}	28.70 ± 0.07^{c}	16.15 ± 0.01^{d}	32.24 ± 0.01^{b}	34.10 ± 0.07^{a}	0.05	$2–5^{a}$
Zn	73.20 ± 0.07^{b}	46.10 ± 0.16^{c}	92.55 ± 0.01^{a}	12.41 ± 0.01^{e}	18.60 ± 0.10^{d}	0.09	$15–20^{a}$
Pb	0.45 ± 0.02^{e}	1.54 ± 0.01^{c}	1.43 ± 0.01^{d}	1.75 ± 0.01^{b}	4.37 ± 0.01^{a}	0.01	
Ni	5.18 ± 0.01^{d}	26.60 ± 0.07^{a}	4.95 ± 0.01^{e}	11.97 ± 0.01^{c}	22.60 ± 0.07^{b}	0.05	
Cr	0.05 ± 0.01^{e}	6.75 ± 0.02^{b}	13.11 ± 0.01^{a}	1.88 ± 0.01^{c}	1.61 ± 0.01^{d}	0.01	0.005^{a}
Cd	0.00 ± 0.00^{e}	0.10 ± 0.00^{c}	0.82 ± 0.01^{a}	0.48 ± 0.01^{b}	0.02 ± 0.01^{d}	0.01	
Co	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	6.94 ± 0.01^{a}	3.65 ± 0.01^{b}	0.00 ± 0.00^{c}	0.01	0.0001^{a}

The means that do not share a letter across the rows are significantly different ($P < 0.05$)

Values with (a) were cited by Prashanth et al. (2015); (b) cited by Raman (2017); and (c) cited by American Heart Association (2020)

DRH, Daily requirements in humans; K, potassium; Na, sodium; Mn, manganese; Fe, iron; Cu, copper; Zn, zinc; Pb, Lead; Ni, nickel; Cr, chromium; Cd, cadmium; and Co, cobalt; A & B, farms located in Ibadan North; C, farms located in Ibadan Northeast; D, farms located in Ibadan Northwest; E, farm located in Ibadan Southwest; + F, farms located in Ibadan Southeast

26.60 ± 0.07(C: Ibadan Northeast) for Ni with pooled StDev of 0.05, 0.05 ± 0.01 (A & B: Ibadan North) to 13.11 ± 0.01 (D: Ibadan Northwest) for Cr with pooled StDev of 0.01, 0.00 ± 0.00 (A & B: Ibadan North) to 0.82 ± 0.00 (D: Ibadan Northwest) for Cd with pooled StDev of 0.01 and 0.00 ± 0.00 (A & B: Ibadan North, C: Ibadan Northeast and F: Ibadan Southeast, respectively) to 6.94 ± 0.22 (D: Ibadan Northwest) for Co with pooled StDev of 0.01.

Relationship between micronutrients, bacterial counts (BC) and weeks (Wks) of study for the gut samples of Clarias gariepinus sampled across farms in Ibadan municipal zones

The Pearson correlation coefficient between micronutrients, bacterial counts and weeks across the sampled farms is presented in Table 5. A positive correlation implied that an increase in one factor increased the other while a negative correlation implied that an increase in one, posed an inhibiting effect on the other. On a relationship guide for positive and negative correlation of

Table 5 Correlation coefficient between the weeks of study, micronutrients and bacterial counts in the gut of *Clarias gariepinus* sampled across farms in Ibadan municipal zones

	Mn	Fe	Cu	Zn	Pb	Ni	Cr	K	Na	Cd	Co	BC	Wks
Mn	1	.543	− .315	.122	− .691	− .020	− .196	.807	.454[a]	− .268	− .298	.533	− .786
Fe	.543	1	− .820	.453[a]	− .621	− .660	− .344	.460[a]	− .114	− .194	− .124	.487	− .724
Cu	− .315	− .820	1	− .859	.712	.758	− .128	− .023	.220	− .082	− .140	− .273	.783
Zn	.122	.453[a]	− .859	1	− .578	− .604	.602	− .224	− .080	.363[a]	.400[a]	.209	− .655
Pb	− .691	− .621	.712	− .578	1	.568	− .126	− .298	− .209	− .200	− .176	− .041	.822
Ni	− .020	− .660	.758	− .604	.568	1	− .135	.155	.122	− .516	− .577	.076	.321
Cr	− .196	− .344	− .128	.602	− .126	− .135	1	− .418[a]	.303	.746	.722	− .067	− .051
K	.807	.460[a]	− .023	− .224	− .298	.155	− .418[a]	1	.475	− .390[a]	− .388[a]	.626	− .454[a]
Na	.454[a]	− .114	.220	− .080	− .209	.122	.303	.475	1	.335	.312	.244	− .112
Cd	− .268	− .194	− .082	.363[a]	− .200	− .516	.746	− .390[a]	.335	1	.989	− .343	.189
Co	− .298	− .124	− .140	.400[a]	− .176	− .577	.722	− .388[a]	.312	.989	1	− .287	.184
BC	.533	.487	− .273	.209	− .041	.076	− .067	.626	.244	− .343	− .287	1	− .502
Wks	− .786	− .724	.783	− .655	.822	.321	− .051	− .454[a]	− .112	.189	.184	− .502	1

Values with alphabets across the rows are significant at $P < 0.05$ level

K, Potassium; Na, sodium; Mn, manganese; Fe, iron; Cu, copper; Zn, zinc; Pb, lead; Ni, nickel; Cr, chromium; Cd, cadmium; Co, cobalt; BC, bacterial counts; and Wks, weeks of study

0.01 to 1.0 and − 0.01 to − 1.0, a very high positive relationship was observed between manganese and potassium ($r=0.807$), cadmium and cobalt ($r=0.989$), while a very high negative relationship was observed between iron and copper ($r=-0.820$), and copper and zinc ($r=-0.859$). A high positive relationship was observed between copper and lead ($r=0.712$), copper and nickel ($r=0.758$), and zinc and chromium ($r=0.602$), while a high negative relationship was observed between manganese and lead ($r=-0.691$), iron and lead ($r=-0.621$), iron and nickel ($r=-0.660$), iron and weeks of study ($r=-0.724$), copper and weeks of study ($r=-0.783$), zinc and nickel ($r=-0.604$), and zinc and weeks of study ($r=-0.655$). A moderate positive relationship was observed between manganese and iron ($r=0.543$), manganese and sodium ($r=0.454$), manganese and bacterial counts ($r=0.533$), iron and zinc ($r=0.453$), iron and potassium ($r=0.460$), iron and bacterial counts ($r=0.487$), zinc and iron ($r=0.453$), and zinc and cobalt ($r=0.400$), and a moderate negative relationship between zinc and lead ($r=-0.578$). A low positive relationship was observed between copper and sodium ($r=0.220$), zinc and cadmium ($r=0.363$), and zinc and cobalt ($r=0.400$) while a negative relationship was observed between manganese and cadmium ($r=-0.268$), manganese and cobalt ($r=-0.298$), iron and chromium ($r=-0.344$), copper and manganese ($r=-0.315$), copper and bacterial counts ($r=-0.273$), and zinc and potassium ($r=-0.224$). A very low positive relationship was observed between manganese and zinc ($r=0.122$), while a very low negative relationship was observed between manganese and nickel ($r=-0.020$), iron and sodium ($r=-0.114$), iron and cobalt ($r=-0.124$), iron and cadmium ($r=-0.194$), manganese and chromium($r=-0.196$),copper and chromium ($r=-0.128$), copper and potassium ($r=-0.023$), copper and cadmium ($r=-0.082$), and copper and cobalt ($r=-0.140$).

Relationship between macronutrients across weeks of study in the gut of Clarias gariepinus sampled across farms in Ibadan municipal zones

The regression analysis between micronutrients in the gut of sampled *Clarias gariepinus* and weeks of study across farms in Ibadan municipal zones is presented in Table 6. The relationship between the micronutrients in the gut across the weeks of study was not significantly different ($P>0.05$) as presented by the one-way ANOVA, although there were some interactions. In farms A & B, Nickel had an inverse relationship across the weeks while the other micronutrients had a direct relationship across the weeks of study. In farm C, zinc ($b=0.02$), chromium ($b=0.003$) and sodium ($b=0.001$) had a direct relationship while other macronutrients had an indirect relationship across the weeks of study. In farm D, iron ($b=-0.001$), zinc ($b=-0.001$), lead ($b=-0.001$) and potassium ($b=-0.002$) had an inverse relationship while other macronutrients had a direct relationship across the weeks of study. In farm E, zinc ($b=0.001$), lead ($b=0.001$) and nickel ($b=0.001$) had a direct relationship while other macronutrients had an indirect relationship across the weeks of study. In farm F, manganese ($b=0.001$), lead and cadmium ($b=0.001$) had a direct relationship while other macronutrients measured had an indirect relationship across the weeks of study.

Table 6 Relationship between the micronutrients in the gut of sampled *Clarias gariepinus* and weeks of study across farms in Ibadan municipal zones

Farms/micronutrients	A & B		C		D		E		F	
	b-value	*P*-value	*b*-value	*P*-value	*b*-value	*P*-value	*b*-value	*P*-value	*b*-value	*P*-value
Manganese	0.01	0.71	− 0.10	0.71	0.01	0.72	− 0.01	0.70	0.001	0.71
Iron	0.003	0.22	− 0.01	0.72	− 0.001	0.72	− 0.001	0.72	− 0.001	0.71
Copper	0.004	0.37	− 0.23	0.10	3.00	0.18	− 0.002	0.18	− 0.01	0.70
Zinc	0.01	0.72	0.02	0.75	− 0.001	0.72	0.001	0.70	− 0.04	0.25
Lead	0.003	0.62	− 0.001	0.72	− 0.001	0.72	0.001	0.72	0.00	1.00
Nickel	− 0.001	0.72	− 0.01	0.72	0.001	1.00	0.001	0.72	− 0.01	0.70
Chromium	0.001	0.72	0.003	0.62	0.001	0.72	− 0.001	0.72	− 0.001	0.69
Potassium	0.001	0.72	− 0.001	0.72	− 0.002	0.72	− 0.001	0.72	− 0.001	0.70
Sodium	0.004	0.53	0.001	0.87	0.001	0.72	− 0.001	0.72	− 0.001	0.72
Cadmium	0	0	0	0	0.003	0.22	− 0.00	1.00	0.001	0.72
Cobalt	0	0	0	0	0.004	0.37	− 0.003	0.22	0	0

b-value = regression coefficient; *P*-value = probability value and significant at $P<0.05$

A & B, Ibadan North local government area; C, Ibadan Northeast local government area; D = Ibadan Northwest local government area; E = Ibadan Southwest local government area; F = Ibadan Southeast local government area

Mean bacterial counts across weeks of study in the gut of Clarias gariepinus sampled across farms in Ibadan municipal zones

The regression analysis between bacterial counts in the gut of sampled *Clarias gariepinus* and weeks of study across farms in Ibadan municipal zones is presented in Table 7. The mean bacterial counts were observed to be not significant ($P<0.05$) across the weeks of study as observed by the one-way ANOVA. A direct (positive) relationship was observed among bacterial counts in farm F ($b=0.00$) while the relationship was indirect (negative) for farms A & B to E with $b=-0.03, -0.01, -0.01$ and -0.01, respectively, across the weeks of study.

Discussion

Microbiological analysis of isolated gut bacterial from sampled *Clarias gariepinus* across farms in Ibadan municipal zones

The species of bacterial isolated in the gut of sampled fish across farms in the Ibadan zones included those belonging to the families of Enterobacterialceae, Aeromonadaceae, Pseudomonadaceae, Bacillaceae, Flavobacterialceae and Pneumoniae. Though some could be opportunistic or harmful (Alikunhi et al., 2017), others can play significant roles in nutrition and immune defense for fish (Gomez & Balcazar, 2008; Sullam et al., 2012). The isolated gut bacterial could potentially provide baseline information on the environmental activities within the sampled zones, concerning fish health and the potential health risk to consumers. It is important to note that the investigation also focused on the gut of fish, as an easily colonized organ through water or food (Austin & Austin, 1987; Olafsen, 2001). There was a higher prevalence rate of bacterial in Ibadan North (A & B) as observed in the study, and in general, bacterial species belonging to the family of Enterobacterialceae were highest, followed by Bacillaceae. The species of microbes observed in the sampled farms could serve as an indicator of the nature and types of activities around the farms in this particular zone. It is also important to note that the presence of microorganisms belonging to the family Enterobacterialceae including *E. coli*, *Enterobacter* and *Klebsiella* species observed in this study are reported to be among the major causes of infection in humans (Oliveira et al., 2017). Similarly, Iyiola et al., (2019) reported similar occurrence of *E. coli* abundance in Aiba reservoir and was attributed to the nature of anthropogenic activities and the presence of animal and human fecal wastes. From the study, a direct (positive) relationship was observed among bacterial counts in farm F ($b=0.00$) across the weeks of study which implied that an increase was observed across the weeks of study by 0.00 units. The relationship was indirect (negative) for farms A & B to E which implied that along the weeks of study, bacterial counts in farms A & B to E decreased by 0.03, 0.01, 0.01 and 0.01 units, respectively, as presented by the b-values. Therefore, it can be said that for farm F, a general increase for bacterial count along the weeks of study was observed while in farms A & B to E, a decrease in bacterial counts along the weeks of study was recorded and was not significant ($P>0.05$).

Table 7 Relationship between the mean total bacterial counts in the gut of sampled *Clarias gariepinus* and weeks of study across farms in Ibadan municipal zones

Farms	*b*-value	*P*-value	Model
A & B	− 0.03	0.48	Y = 0.28 − 0.03X
C	− 0.01	0.32	Y = 0.23 − 0.01X
D	− 0.01	0.69	Y = 0.23 − 0.01X
E	− 0.01	0.00	Y = 0.25 − 0.01X
F	0.00	0.78	Y = 0.23 + 0.78X

b-value, regression coefficient; *P*-value, probability value and significant at $P<0.05$

Y, dependent variable (farms), X, independent variable (weeks), A & B, Ibadan North local government area, C, Ibadan Northeast local government area, D, Ibadan Northwest local government area, E, Ibadan Southwest local government area, F, Ibadan Southeast local government area

Micronutrient analysis of gut content of sampled *Clarias gariepinus* across farms in Ibadan municipal zones

Minerals or trace elements are important in the body of fish species for optimal growth and functions. They are ultimately provided by feed and water and are absorbed into the body of the fish through the gastrointestinal tract (Prabhu et al., 2019). Potassium helps in regulating body fluids, muscle contractions and nerve signals. The mean concentration recorded in the gut of fish was 1.72 (%) ± 0.21 which was lower than the daily recommended levels of 19% by Syner (2021). Although across some of the sampled farms, K levels were high but not up to the recommended levels. Na is important in humans because it improves blood pressure and the health of the heart. The mean concentration recorded was 0.68% ± 0.34 which was below the daily recommended rate of 1.97% by the American Heart Association (2020). For Zn, Fe, Cu, Mn, Cr and Co with a mean concentration levels of 48.57 ± 0.42 mg/kg, 4.31 ± 1.21 mg/kg, 23.68 ± 0.22 mg/kg, 7.27 mg/kg ± 0.72, 4.68 ± 0.21 and 2.12 mg/kg ± 0.03, respectively, were above the recommended daily limits of 15–20 mg/kg, 1–2 mg/kg, 2–5 mg/kg, 2.5 mg/kg, 0.005 mg/kg and 0.0001 mg/kg for humans as reported by Prashanth et al. (2015). Zn is essential for proliferation and metabolic activities in the cell. Iron reduces heart disorders and regulates iron and plasma iron in the body. Cu is important in the metabolism of tissues,

hemoglobin and tryptophan synthesis. Mn is an enzyme activator and is involved in the metabolism of fatty acids and cholesterol. Co plays a role in the transfer of enzymes and reduces iodine uptake by thyroids. Pb, Ni and Cd can cause toxicity in humans, affect the nervous systems and may damage vital organs in the body (Engwa et al., 2019). The correlation analysis which expresses the relationship between two variables can either a direct relationship (positive) where both variables increase alongside or indirect relationship (negative) where when one variable increases, the other decreases. It was observed from the correlation analysis that a very high positive relationship was observed between manganese and potassium ($r=0.807$), cadmium and cobalt ($r=0.989$) because the coefficient ranged from 0.8 to 1.0. A very high negative relationship was observed between iron and copper ($r=-0.820$), and copper and zinc ($r=-0.859$) because the coefficient ranged from − 0.8 to − 1.0. A high positive relationship was observed between copper and lead ($r=0.712$), copper and nickel ($r=0.758$), and zinc and chromium ($r=0.602$) because the coefficient ranged from 0.61 to 0.80, while a high negative relationship was observed between manganese and lead ($r=-0.691$), iron and lead ($r=-0.621$), iron and nickel ($r=-0.660$), iron and weeks of study ($r=-0.724$), copper and weeks of study ($r=-0.783$), zinc and nickel ($r=-0.604$), and zinc and weeks of study ($r=-0.655$) because the coefficient ranged from 0.61 to 0.80. A moderate positive relationship was observed between manganese and iron ($r=0.543$), manganese and sodium ($r=0.454$; *P*), manganese and bacterial counts ($r=0.533$), iron and zinc ($r=0.453$), iron and potassium ($r=0.460$), iron and bacterial count ($r=0.487$), zinc and iron ($r=0.453$), and zinc and cobalt ($r=0.400$) because the coefficient ranged from 0.41 to 0.60, while a moderate negative relationship between zinc and lead ($r=-0.578$) because the coefficient ranged from 0.41 to 0.60. A low positive relationship was observed between copper and sodium ($r=0.220$), zinc and cadmium ($r=0.363$), and zinc and cobalt ($r=0.400$) because the coefficient ranged from 0.21 to 0.40, while a negative relationship was observed between manganese and cadmium ($r=-0.268$), manganese and cobalt ($r=-0.298$), iron and chromium ($r=-0.344$), copper and manganese ($r=-0.315$) copper and bacterial counts ($r=-0.273$), and zinc and potassium ($r=-0.224$) because the coefficient ranged from 0.21 to 0. A very low positive relationship was observed between manganese and zinc ($r=0.122$) because the coefficient ranged from 0.01 to 0.20, while a very low negative relationship was observed between manganese and nickel ($r=-0.020$), iron and sodium ($r=-0.114$), iron and cobalt ($r=-0.124$), iron and cadmium ($r=-0.194$), manganese and chromium ($r=-0.196$), copper and chromium ($r=-0.128$), copper and potassium ($r=-0.023$), copper and cadmium ($r=-0.082$), and copper and cobalt ($r=-0.140$), because the coefficient ranged from 0.01 to 0.20. There were significant relationships ($p<0.05$) between manganese and sodium ($r=0.454$), iron and potassium ($r=0.460$), zinc and cobalt ($r=0.400$), iron and zinc ($r=$—0.820), zinc and cadmium ($r=-0.363$), chromium and potassium ($r=-0.418$), potassium and cadmium ($r=-0.390$), potassium and cobalt ($r=-0.388$), and potassium and weeks of study ($r=-0.454$) as observed by the one-way ANOVA.

Interactions between microorganisms and micronutrients in the gut of sampled *Clarias gariepinus* across the weeks of study in Ibadan municipal zones

The role of bacterial in the synthesis and uptake of micronutrients is highly unexplored, while humans and animals cannot synthesize micronutrients; they can be available to the animals through any one of these three routes: diets, gut bacterial and oral supplementation (Maynard & Weinkove, 2020). Gut bacterial are said to also obtain micronutrients (metals and vitamins) through the endogenous biosynthesis pathway (Magnusdottir et al, 2015; Biesalski, 2016; Mach & Clark, 2017; Rodionov et al., 2019). According to the reports of Rajota et al. (2017) and Hasan and Yang (2019), ingested food substance plays germane roles in influencing and shaping the pattern and composition of gut microbiota. The gut microflora of fish can be altered by the pattern of essential and nonessential metals present in them; this is as reported in another study by Wu et al., (2014), where chicken-fed nickel-supplemented feed had an increased prevalence of *E. coli* and *Enterococcus* species. Guo et al. (2014) and Dheer et al. (2015) reported an interchangeable abundance of Firmicutes and Bacteroidetes in the gut of mice when administered with arsenic in drinking water.

Farms A & B measured the highest values for potassium (2.31 ± 0.22 mg/kg), manganese (10.5 ± 0.22 mg/L) and iron (9.03 ± 0.22 mg/kg) with *b*-values of 0.001, 0.01 and 0.003, respectively. These b-values implied that along the weeks, the mean values of potassium, manganese and iron measured from farms A & B increased by 0.001, 0.01 and 0.003 units, respectively, but were not statistically different ($P>0.05$). In farm C, mean values were highest for zinc (92.55 ± 0.22 mg/kg), chromium (13.11 ± 0.22 mg/kg), cadmium (0.82 ± 0.00 mg/kg) and cobalt (6.94 ± 0.22 mg/kg) with *b*-values of − 0.001, 0.001, 0.003 and 0.004. These *b*-values implied that increase along the weeks of study, the mean values of zinc, chromium, cadmium and cobalt measured from farm C increased by 0.001, 0.003 and 0.004 units, respectively, while mean values for zinc decreased along the weeks of study by − 0.001 units. In farm D, mean values were

highest for sodium (0.98 ± 0.00 mg/kg) with a *b*-value of 0.001, which implied an increase by 0.001 units along the weeks of study in sampled farm D. In farm E, mean values were highest for copper (34.10 ± 0.22 mg/kg), lead (4.37 ± 0.22 mg/kg) and nickel (22.60 ± 0.22 mg/kg) with *b*-values of − 0.002, 0.001 and 0.001, respectively. This implied that along the weeks, the mean values of lead and nickel increased by 0.001 and 0.001, respectively, while copper decreased by 0.002 units in the sampled farm E in the weeks of study.

In this study, some of the micronutrients such as Mn, Fe, K and Na positively boost the bacterial counts (BC) in the gut of fish, thereby indicating a beneficial interaction for the gut flora to thrive. Hibberd et al. (2017) also reported in an experiment carried out on mice that a diet lacking in one vitamin A, iron, zinc or folic acid molecule perturbed the distribution and functionality of the fish gut microbes. The diminishing presence of Cu, Pb, Cr, Cd and Co in the gut as observed in this study had a depressing impact on the gut bacterial of fish. This observation agreed with an earlier study by Mach and Clark (2017), who reported that micronutrients in the gut of animals and humans also serve as substrates and cofactors for physiological processes such as DNA synthesis, repair and growth, and establishment of symbiotic associations of microflora within the gut.

Conclusions

In this present study, the fish gut microflora (total bacterial count) was seen to be more prevalent in the Ibadan North (A & B) zone and *Enterobacter* species were likewise seen to be highest in the farms in this area and also predominant in the gut samples of farmed *Clarias gariepinus* across all the IMZ. The study also noted the interactions between gut microflora and micronutrients of the farmed *Clarias gariepinus* across the sampled zones, whereby some micronutrient composition correlates positively with the assessed gut microflora. In this case, micronutrients such as Mn, Na, Zn, Fe and K were seen to influence the presence of gut microflora, while Cu, Pb, Ni, Cr, Co and Cd had negative correlations with the assessed fish gut microflora. Therefore, this study submits to the fact that there is a positive correlation between microflora and micronutrients of importance to the prime health of farmed fish. Furthermore, while hygienic culture practices are suggested, attention should also be given to the addition of some of the micronutrients in fish diets as they influence fish gut flora.

Abbreviations

IMZ	Ibadan municipal zone
Zn	Zinc
CFU/g	Coliform-forming unit per gram
Cr	Chromium
Cd	Cadmium
Co	Cobalt
Na	Sodium
Pb	Lead
Cu	Copper
Mn	Manganese
Ni	Nickel
mg/kg	Milligram per kilogram
AAS	Atomic absorption spectroscopy
PCA	Plate count agar
EMBA	Eosin methyl blue agar
BC	Bacterial counts

Acknowledgements

I sincerely appreciate the efforts of my departmental colleagues in proofreading and editing this manuscript. Thanks also go to the laboratories where I carried out this research work.

Author contributions

USB was the lead author of the manuscript, and was responsible for research conception, data analysis and report writing. IAO was the co-author of the manuscript, and was involved in research conception and data analysis. AI co-author of the manuscript, and contributed to proofreading and report writing. I ensured that all authors read and approved the manuscript.

Funding

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Fisheries and Aquaculture, Faculty of Agriculture and Life Sciences, Federal University Wukari, Wukari, Taraba State, Nigeria. [2]Department of Fisheries and Aquatic Resources Management, College of Agriculture and Renewable Resources, Osun State University, Osogbo, Nigeria. [3]Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

Received: 28 May 2021 Accepted: 27 July 2023
Published online: 03 August 2023

References

Adeshina, I., Tiamiyu, L. O., Akpoilih, B. U., Jenyo-Oni, A., & Ajani, E. K. (2021). Dietary *Mitracarpus scaber* leaves extract improved growth, antioxidants, non-specific immunity, and resistance of Nile tilapia, *Oreochromis niloticus* to *Gyrodactylus malalai* infestation. *Aquaculture, 535*(2021), 736377.

Alikunhi, N. M., Batang, Z. B., AlJahdali, H. A., & Al-Suwailema, A. M. (2017). Culture-dependent bacterial in commercial fishes: Qualitative assessment and molecular identification using 16S rRNA gene sequencing. *Saudi Journal of Biological Sciences, 24*(6), 1105–1116.

American Heart Association (2020). How much sodium should I eat per day? Available at www.heart.org/en/healthy-living/healthy-eating/eat-smart/sodium/how-much-sodium-should-i-eat-per-day.

American Public Health Association. (1978). *Standard methods for the examination of dairy products* (14th ed.). APHA Inc.

Association of Official Analytical Chemists. (2000). *Official methods of analysis*. 17th Edition. The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925. 10, 65. 17, 974.24, 992. 16.

Austin, B., & Austin, D. A. (1987). *Bacterial fish pathogen, disease of farmed and wild fish*. Ellis Horwood.

Bertucci, J. I., Blanco, A. M., Sundarrajan, L., Rajeswari, J. J., Velasco, C., & Unniappan, S. (2019). Nutrient regulation of endocrine factors influencing feeding and growth in fish. *Frontiers in Endocrinology (lausanne)*. https://doi.org/10.3389/fendo.2019.00083

Biesalski, H. K. (2016). Nutrition meets the microbiome; micronutrient and the microbiota. *Annals of the New York Academy of Sciences, 1372*(1), 53–64.

Brown, K. H., Peerson, J. M., Rivera, J., & Allen, L. H. (2002). Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: A meta-analysis of randomized controlled trials. *American Society of Clinical Nutrition, 75*(1), 1062–1071.

Canny, G. O., & McCormick, B. A. (2008). Bacterial in the intestine, helpful residents or enemies from within? *Infection and Immunity*. https://doi.org/10.1128/IAI.00187-08.:1-21

Cappuccino, J. G., & Sherman, N. (2008). *Microbiology: A laboratory manual* (8th ed.). Pearson Benjamin Cummings.

Dheer, R., Patterson, J., Dudash, M., Stachler, E. N., Bibby, K. J., Stolz, D. B., Shiva, S., Wang, Z., Hazen, S. L., Barchowsky, A., & Stolz, J. F. (2015). Arsenic induces structural and compositional colonic microbiome change and promotes host nitrogen and amino acid metabolism. *Toxicology and Applied Pharmacology, 289*, 397–408. https://doi.org/10.1016/j.taap.2015.10.020

Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, P. R. (2018). The gut microbiota of marine fish. *Frontier in Microbiology, 9*, 873.

Engwa, G., Ferdinand, P. U., & Nwalo, F. N. (2019). Mechanism and health effects of heavy metal toxicity in humans, poisoning in the modern world—New tricks for an old dog? *Ozgur Karcioglu and Banu Arslan*. https://doi.org/10.5772/intechopen.82511

Faruk, M. A. R., Sarker, M. M. R., Alam, M. J., & Kabir, M. B. (2004). Economic loss from fish diseases on rural freshwater aquaculture of Bangladesh. *Pakistan Journal of Biological Sciences, 7*(12), 2086–2091.

Food and Agriculture Organization. (2018). *The state of world fisheries and aquaculture*; *Meeting the sustainable developmental goals*. Food and Agricultural Organization of the United Nations, Rome 2018.

Gomez, G. D., & Balcazar, J. L. (2008). A review on the interactions between Gut microbiota and innate immunity of fish. *FEMS Immunology & Medical Microbiology, 52*(2), 145–154.

Guo, X., Liu, S., Wang, Z., Zhang, X., Li, M., & Wu, B. (2014). Metagenomic profiles and antibiotic resistance genes in the gut microbiota of mice exposed to arsenic and iron. *Chemosphere, 112*, 1–8. https://doi.org/10.1016/j.chemosphere.2014.03.068

Hasan, N., & Yang, H. (2019). Factors affecting the composition of the gut microbiota and its modulation. *PeerJ, 7*, e7502. https://doi.org/10.7717/peerj.7502

Herzog, C. A., Leite, C. A. P., Zaniquelli, M. E. D., & Galembeck, F. (1998). Easy polymer latex self-assembly and colloidal crystal formation: The case of poly [styrene-co-(2-hydroxyethyl methacrylate)]. *Colloids and Surfaces a: Physicochemical and Engineering Aspects, 144*(1–3), 207–217.

Hibberd, M. C., Wu, M., Rodionov, D. A., Li, X., Cheng, J., Griffin, N. W., Barratt, M. J., Giannone, R. J., Hettich, R. L., Osterman, A. L., & Gordon, J. I. (2017). The effects of micronutrient deficiencies on bacteriall species from the human gut microbiota. *Science Translational Medicine*. https://doi.org/10.1126/Scitranslmed.aa14069

Iyiola, A. O., Asiedu, B., Kolawole, A. S., & Failler, P. (2019). Assessment of water quality and bacteriological levels in Nile Tilapia (*Oreochromis niloticus*) of Aiba reservoir Nigeria West Africa. *Tropicultura, 37*(3), 1–6.

Izah, S. C., Iyiola, A. O., & Richard, G. (2022). Impacts of pollution on the hydrogeochemical and microbial community of aquatic ecosystems in Bayelsa State, Southern Nigeria. In S. Madhav, V. B. Singh, M. Kumar, & S. Singh (Eds.), *Hydrogeochemistry of aquatic ecosystems* (pp. 283–305). Wiley. https://doi.org/10.1002/9781119870562.ch13

Kamaruzzaman, B. Y., Ong, M. C., Rana, S. Z., & Joseph, B. (2010). Levels of some heavy metal in fishes from Pahang river estuary, Pahang, Malaysia. *Journal of Biological Science, 10*(2), 157–216.

Kar, J., Barrman, T. R., Sen, A., & Nath, S. K. (2017). Isolation and identification of *Escherichia coli* and *Salmonella* sp. from apparently healthy Turkey. *International Journal of Advanced Research in Biological Sciences, 4*(6), 72–78.

Kim, D. H., Brunt, J., & Austin, B. (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology, 102*, 1654–1664.

Lilly, T. T., Immaculate, J. K., & Jamila, P. (2017). Macro and relationships of selected marine fishes in Tuticorin, South East coast of India. *International Food Research Journal, 24*, 191–201.

Mach, N., & Clark, A. (2017). Micronutrient deficiencies and the human gut microbiota. *Trends in Microbiology, 25*(8), 607–609. https://doi.org/10.1016/j.tim.2017.06.004

Magnusdottir, S., Ravcheev, D., Lagard, V., & Thiele, I. (2015). Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Frontiers in Genetics, 6*, 148.

Maynard, C., & Weinkove, D. (2020). Bacterial increase host micronutrient availability: Mechanisms revealed by studies in *C. Elegans*. *Genes and Nutrition*. https://doi.org/10.1186/s12263-020-00662-4

Michael, M., Volkoff, H., Conde-Sieira, M., & Soengas, J. L. (2017). Nutrient sensing systems in fish: impact on food intake regulation and energy homeostasis. *NutrSensMech Fish, 10*, 603.

Olafsen, J. A. (2001). Interactions between fish and bacterial in marine aquaculture. *Aquaculture, 200*, 223–247.

Oliveira, R. V., Oliveira, M. C., & Pelli, A. (2017). Disease infection by *Enterobacteriaceae* family in fishes: A review. *Journal of Microbiology & Experimentation, 4*(5), 00128. https://doi.org/10.15406/jmen.2017.04000128

Oscroft, C. A., & Correy, J. E. L. (1991). *Guidelines for the preparation, storage and handling of microbiological media*. Technical Manual 33. Campden and Chorleywood Food & Drink Research Association.

Prabhu, P. A. A., Silva, M., Kröeckel, S., Holme, M., Ornsrud, R., Amlund, H., Lock, E., & Waagbo, R. (2019). Effect of levels and sources of dietary manganese on growth and mineral composition of post-smolt Atlantic salmon fed low fish meal, plant-based ingredient diets. *Aquaculture, 512*, 734287.

Prashanth, L., Kattapagari, K. K., Chitturi, R. T., Baddam, V. R. R., & Prasad, L. K. (2015). A review on the role of essential trace elements in health and disease. *Journal of Dr. NTR University of Health Sciences, 4*(2), 75–78.

Rajota, M. S. R., Shi, J., Mehwish, H. M., Zhu, J., Li, Q., Shao, D., Huang, Q., & Hui, Y. (2017). Interaction between diet composition and gut microbiota and its impact on gastrointestinal tract health. *Food Science and Human Wellness, 6*(3), 121–130.

Raman, R. (2017). What does potassium do for your body? A detailed review. www.healthline.com/nutrition/what-does-potassium-do-section1.

Rodionov, D. A., Dmitry, A., Arzamasov, A. A., Khoroshkin, M. S., Lablokov, S. N., Leyn, S. A., Peterson, S. N., Novichkov, P. S., & Osterman, A. L. (2019). Micronutrient requirements and sharing capabilities of the human gut microbiome. *Frontiers in Microbiology, 10*, 1316.

Roeselers, G., Mittge, E. K., Stephens, W. Z., Parichy, D. M., Cavanaugh, C. M., Guillemin, K., et al. (2011). Evidence for a core gut microbiota in the zebrafish. *The ISME Journal, 5*, 1595–1608. https://doi.org/10.1038/ismej.2011.38

Sapkota, R. P., Brunet, A., & Kirmayer, L. J. (2020). Characteristics of adolescents affected by mass psychogenic illness outbreaks in schools in Nepal: A case–control study. *Frontiers in Psychiatry, 11*(1), 1271.

Shefat, S. H. T., & Karim, M. A. (2018). Nutritional diseases of fish in aquaculture and their management: A review. *Acta Scientific Pharmaceutical Sciences, 2*(12), 50–58.

Shields, P., & Laura Cathcart, L. (2010). Oxidase test protocol. In *A peer-reviewed at the ASM Conference for Undergraduate Educators 2010.* American Society for Microbiology.

Sullam, E. K., Steven, D. E., Lozupone, C. A., O'Connor, M. P., Rosen, G., Knight, R., Kilham, S. S., & Russel, J. A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: A meta-Analysis. *Molecular Ecology, 21*(13), 3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.X

Syner, C. (2021). Is catfish healthy? Nutrients, benefits, and more. In S. Meeks (Ed.), *Nutrition*. Available at www.healthline.com/nutrition/is-catfish-healthy.

Tille, P. M., & Forbes, B. A. (2014). *Bailey & Scott's diagnostic microbiology* (13th ed.). Elsevier.

Wu, B., Cui, H., Peng, X., Pan, K., Fang, J., Zuo, Z., Deng, J., Wang, X., & Huang, J. (2014). Toxicological effects of dietary nickel chloride on intestinal microbiota. *Ecotoxicology and Environmental Safety, 109*, 70–76. https://doi.org/10.1016/j.ecoenv.2014.08.002

Publisher's Note