# Nile tilapia bones as eco-friendly alternative in water treatment and enhancing performance and immunity in broiler chickens

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**Abstract**

**Background:** Water pollution has become a major threat to the environment and the living so an eco-friendly bio-filter was chosen for its merits over conventional techniques.

**Aim:** Investigating the purifying activities of the Tilapia bone powder against inorganics, heavy metals, and microbial water pollutants and its impacts on performance, biochemical and antioxidant levels, cortisol and immunoglobulin concentrations, and intestinal microbiota in challenged broiler chickens.

**Methods:** The *in-vitro* activity of Tilapia bone powder was evaluated against magnesium chloride and lead nitrate using tube minimal inhibitory concentration (MIC), as well as against *Escherichia coli* O1527:H7, *Salmonella Typhimurium*, *Mycoplasma gallisepticum*, *Aspergillus niger*, *Trichophyton mentagrophytes*, and *Candida albicans* using a 96-micro-well MIC. A total of 250 1-day-old Hubbard chicks were divided into five groups on a deep litter system. Chicks were supplemented daily with Tilapia bone powder (1 g/l) for 4–6 hours from the 3rd day. Challenges were served on the 7th, 14th, 21st, 28th, and 35th days for four broiler groups using magnesium chloride (100 mg/l), lead nitrate (350 mg/l), *E. coli* ($2.4 \times 10^{12}$ CFU/ml), *S. Typhimurium* ($1.8 \times 10^8$ CFU/ml), respectively, and the 5th group was assigned as a control. A total of 2,250 samples (90 Tilapia-pollutants mixes, 480 Tilapia-microbial mixes, 240 sera, 240 intestinal swabs, and 1,200 tissue samples) were collected.

**Results:** Tilapia bone powder 1% reveals a 100% reduction in the lead after 1 hour, total and calcium hardness after 0.5 hours, as well as 100% killing efficacy against *E. coli* O1527:H7, *S. Typhimurium*, *M. gallisepticum*, *A. niger*, *T. mentagrophytes*, and *C. albicans* after 0.5, 1, 1, 1, 1, and 1 hour, respectively. Tilapia bone powder 1% treated water reveals highly significant ($p < 0.01$) increases in dissolved oxygen and declines in physicochemical and microbial parameters compared with tap water. Challenged treated broilers revealed highly significant ($p < 0.01$) increases in weight gains, performance index, body weights, carcasses, and organs weights, immunoglobulin concentrations, and antioxidant levels, as well as highly significant ($p < 0.01$) improvements in feed conversions, feed and water intakes, biochemical profile, cortisol hormone, and intestinal microbiota.

**Conclusion:** Tilapia bone powder provided significant *in-vitro* adsorptive and antimicrobial actions, as well as supported the broiler chickens to mitigate the polluted water stress accompanied by enhanced performance, carcass quality, immunity, and intestinal microbiota.

**Keywords:** Broiler chickens, Immunity, Performance, Tilapia bone waste, Water purification.

## Introduction

The major goal of public health is to produce safe water in face of the increased demands of the augmented population and development and to provide water with *ad-libitum* to birds and animals (WHO, 2018). Drinking water quality affects directly the bird's feed intake, performance, and carcass quality, and polluted water contributes to disease transmission (Li *et al.*, 2017). In recent days, many anthropogenic-sourced emergent contaminants are being directly introduced into water bodies through effluent and wastewater discharge (Goswami *et al.*, 2019). These contaminants are a current concern due to the environmental impacts related to the occurrence of these persistent substances in the environment. Epidemiological studies revealed the survival of a large number of pathogenic micro-organisms in water such as bacteria (Soliman *et al.,* 2009a) including *Salmonella, Escherichia coli, Shigella*, *Pseudomonas*, *Klebsiella*, *Staphylococcus*, and *Streptococcus*, and fungi (Soliman *et al*., 2009b)

including *Mucor*, *Candida*, and *Aspergillus*. Water biosecurity aims to reduce chemical and microbial contamination and face increased water demands (Li and Qian, 2018). Water quality has to be monitored and evaluated regularly using highly sensitive methods (Su *et al.*, 2019; Tian and Wu, 2019).

Tilapia, which originated in the Middle East and Africa, is the second-largest farmed fish. According to reports, fish production was approximately 6.5 million metric tons in 2019 and increased by 4% in 2021 (Tveteras *et al.*, 2019). Every year, the fish processing industry generates millions of tons of waste. As a result, indiscriminate waste disposal endangers the environment (de Medeiros *et al.*, 2019) if wastes are not properly managed. Recently, the reuse of waste material as a medium for water treatment has received a lot of attention. According to Food and Agriculture Organization FAO (2020), Tilapia fish (*Oreochromis niloticus*) production in the world reaches approximately 4 million tons per year. In Egypt, 1,114,265 tons of Tilapia were produced in 2020, from which around 20%–25% represent the bones (Boscolo *et al.*, 2008) and generating at least 222,853 tons of bone waste annually. Recently, various studies have been conducted on synthesizing some substances of high adsorbing and purifying capabilities from carbonated calcium phosphate bioceramic powder, such as that found in fish bones (Piencenaves, 2013).

Water purification processes usually target different pollutants like heavy metals, inorganics, solids, oils, gases, and microorganisms. Water purification processes have been carried out using approaches like porous medium filtration, gravitational sedimentation (Lin *et al.*, 2020), sand filtration (Nakazawa *et al.*, 2021), activated carbon (Singh *et al.*, 2018), flocculation and coagulation (Lapointe *et al.*, 2020), photocatalytic degradation (Hussain *et al.*, 2017), oxidation (Parra *et al.*, 2016), chlorination (Matsushita *et al.*, 2018), eco-friendly clay (Mohamed *et al.*, 2020; Soliman *et al.*, 2021d), magnetization (Soliman *et al.*, 2021c), reverse osmosis (Raval *et al.*, 2016), ion exchange (Szczepanik, 2017), and adsorption (Dotto and McKay, 2020). Some adsorbents of low cost have been tested like biochar, sawdust, feathers, cottonseed hull, and Tilapia fishbone. The analysis of Tilapia bone revealed 30% organic and 70% inorganic phases with the presence of hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$) which is a strong adsorbent (Ebrahimi *et al.*, 2013). Tilapia culture is incipient in countries like Brazil (Martins *et al.*, 2015) where the bones have been used as a dietary calcium supplement (Peng *et al.*, 2017) and to produce biochar as a promising adsorbent for improving water quality. Tilapia bone was able to produce high adsorption capacity with a mesopore distribution of the hydroxyapatite (HAp) bases for enhanced high diffusion rates and high purification actions (Modenes *et al.*, 2021).

This study aimed to investigate the *in-vitro* and *in-vivo* purifying (chelating and antimicrobial) activities of Nile Tilapia bone powder against inorganic (magnesium chloride, 100 mg/l), heavy metals (lead nitrate, 350 mg/l), and microbial (*E. coli* O1527:H7; $2.4 \times 10^{12}$ CFU/ml, *Salmonella Typhimurium*; $1.8 \times 10^8$ CFU/ml, *Mycoplasma gallisepticum* $1.5 \times 10^5$ CFU/ml, *Aspergillus niger*, *Trichophyton mentagrophytes*, and *Candida albicans*; $3.2 \times 10^9$ CFU/ml each) water pollutants. The study also aimed to evaluate the influence of water treated with Nile Tilapia bones on performance, biochemical profile, antioxidant levels, cortisol hormone, immunoglobulin concentrations, and intestinal microbiota in broiler chickens challenged once weekly with polluted water (magnesium chloride at a rate of 100 mg/l, lead nitrate at a rate of 350 mg/l, *E. coli* O1527:H7 at a rate of $2.4 \times 10^{12}$ CFU/ml, and *S. Typhimurium* $1.8 \times 10^8$ CFU/ml).

## Material and Methods

### Study location and duration

The *in-vitro* study was conducted from December 2021 to January 2022 in the Veterinary Public Health Laboratory and the *in-vivo* experiment was conducted in the poultry experimental units. The Faculty of Veterinary Medicine- Suez Canal University from February to April 2022. Water analysis (Physico-chemical and microbial), productive performance, carcass and organ weights, and biochemical, and bacteriological examinations were carried out in the Veterinary Public Health Laboratory. Antioxidants, immunoglobulin, cortisol markers, and water electrolytes were assessed in the Clinical Pathology Laboratories at the University Hospital, while water heavy metals analysis was conducted in the Toxicology laboratory- Chemistry Department- Faculty of Science- Suez Canal University. Histopathological examinations were conducted in El-Awael Laboratory- Ismailia governorate, Egypt.

### Tilapia bone powder preparation

Tilapia (*Oreochromis niloticus*) bones were obtained from the fish market in Ismailia governorate, Egypt. The bones were transferred to the laboratory as quickly as possible in a dry ice box. In the laboratory, the bones were washed carefully with distilled water, fragmented into small pieces, sun-dried for 24 hours, and finally ground into a fine powder using a granite mortar (Mortar and Pestle, Cut Out, Stone - Object, Granite - Rock, Horizontal).

The powder was sterilized and extra-dried using a hot air oven (Daihan® LabTech® Hot air Oven, LDO-080N, Indonesia) at 80℃ for 24 hours. The powder was stored in a cool dry place at 4℃ until tested in the laboratory and field conditions for its efficacy (antimicrobial and adsorption actions).

### Pollutant suspensions preparation

Magnesium chloride (HiMedia® GRM1068 Magnesium Chloride Hexahydrate, Purified, 500 g) was purchased and dissolved in distilled water at a rate of 100 mg/l. The solution was analyzed for total and calcium hardness

and revealed levels of total hardness up to 2,650 mg/l $CaCO_3$ and calcium hardness up to 1,960 mg/l $CaCO_3$ compared to the normal MPL < 75 mg/l and <60 mg/l, respectively.

Lead nitrate (HiMedia GRM724 Lead (II) Nitrate, Purified, 500 g) was purchased and dissolved in distilled water at a rate of 350 mg/l. The solution was analyzed for lead levels and revealed levels up to 20 mg/l compared to the normal MPL < 15 µg/l.

### Bacterial isolates propagation

*Escherichia coli* O157: H7 suspension ($1.8 \times 10^3$ CFU/ml) was purchased from Animal Health Research Institute- Ismailia- Egypt. The bacterial suspension was propagated as recommended by Soliman *et al*. (2021b) into Falcon tubes set with 9 ml of MacConkey broth (Thermo Scientific™ Oxoid™ MacConkey Broth, CM0505, 500 g) at 44℃. 10 microliters from tubes developing the yellow color were dropped aseptically onto eosin methylene blue agar (EMB, Modified Levine Eosin Methylene Blue Thermo Scientific™ Oxoid™, CM0069B, 500 g) and incubated at 37℃/24 hours. *Escherichia coli* typical colonies (metallic green) were counted, picked up, and resuscitated into Falcon tubes set with 9 ml tryptone soy broth (Thermo Scientific™ Oxoid™ Tryptone Soya Broth, CM0129, 500 g) until obtaining $2.4 \times 10^{12}$ CFU/ml suspension.

*Salmonella Typhimurium* lyophilized vial ($3.4 \times 10^2$ CFU) was purchased from Animal Health Research Institute- Cairo. The powder was resuscitated as recommended by Soliman *et al*. (2021) into Falcon tubes set with 9 ml tetrathionate broth pre-enrichment (Thermo Scientific™ Oxoid™ Tetrathionate Broth Base, CM0029, 500 g) and incubated at 37℃/24 hours. 10 microliters from the pre-enrichment tubes were dropped aseptically onto CHROMagar™ *Salmonella* plates (BD BBL™ CHROMagar™ *Salmonella* READY-TO-USE Plated Media) and incubated at 37℃/24 hours. The typical pink colonies were counted, picked up, and resuscitated into Falcon tubes set with 9 ml tryptone soy broth (Thermo Scientific™ Oxoid™ Tryptone Soya Broth, CM0129, 500 g) until obtaining $1.8 \times 10^8$ CFU/ml suspension.

*Mycoplasma gallisepticum* clinical isolates were obtained kindly free of charge from Animal Health Research Institute- Ismailia. The isolates were pre-enriched into Falcone tubes set with 9 ml buffered peptone water (ABI® Phosphate Buffered Saline, PBS, $10 \times$ Concentrated, Sterile, pH 7.4, without $CaCl_2$ and $MgCl_2$, 500 ml) and incubated at 37℃/24 hours. 10 µl from the pre-enrichment tubes were dropped aseptically onto *Mycoplasma* agar base (HIMEDIA® PPLO Agar Base, M266, 500 g) and incubated at 37℃/24 hours. Typical *Mycoplasma* fried egg colonies were counted, picked up, and resuscitated into Falcon tubes set with 9 ml tryptone soy broth (Thermo Scientific™ Oxoid™ Tryptone Soya Broth, CM0129, 500 g) until obtaining $1.5 \times 10^5$ CFU/ml suspension.

### Fungal isolates propagation

*Aspergillus niger*, *T. mentagrophytes*, and *C. albicans* clinical isolates were obtained Kindley from Animal Health Research Institute- Cairo onto malt extract broth (HIMEDIA® Malt Extract Broth Base, M255-500 g). The isolates were pre-enriched as recommended by Soliman *et al*. (2021) into tryptone soy broth (Thermo Scientific™ Oxoid™ Tryptone Soya Broth, CM0129, 500 g) at 37℃/24-72 hours. One ml was transferred aseptically into enrichment Falcone tubes set with 9 ml of malt extract broth (HIMEDIA® Malt Extract Broth Base, M255-500 g) and incubated at 37℃/24–72 hours. 10 µl from the enrichment tubes were dropped aseptically onto malt extract agar (RPI®; Research Products International, Malt Extract Agar, M21500- 1 kg), and at 37℃/72 hours.

The growing colonies were identified via the morphological appearance, stained with lactophenol cotton blue stain (Hardy Diagnostics® Lactophenol Cotton Blue, Z68, 15 ml), and confirmed by microscopic examinations using a transmitted light microscope (CARL ROCHE® Transmitted light-emitting diodes (LED) light microscope Binocular, OBS-106, 40×–100×, 400×, 4-way, Germany). The colonies were counted, picked up, and resuscitated into Falcon tubes set with 9 ml malt extract broth providing $3.2 \times 10^9$ CFU/ml suspensions for each of the three organisms.

### In-vitro adsorption activities of tilapia bone powder

One milliliter of Tilapia bone suspension (1 g Tilapia bone powder/l distilled water) was added to three triplets of pre-set 1-l capacity glass conical flasks for each magnesium chloride (100 mg/l) and lead nitrate (350 mg/l). The flask mixes were mixed in a vortex (Vortex Mixer XH-D, 2,800r/m, 30W, Bowel and disk shapes) and after specified intervals of 0.25, 0.5, 1.0, 2.0, and 4.0 hours, water samples were collected and analyzed for the approximate levels of total and calcium hardness measured by mg/l $CaCO_3$ and lead concentrations expressed by mg/l.

The water samples for lead concentrations analysis were preserved using 0.5 ml nitric acid and the lead concentrations (mg/l) were quantified using atomic absorption spectrophotometer (American Public Health Association; APHA, 2012). The total and calcium hardness levels (mg/l $CaCO_3$) were estimated by analyzing the water samples using the ethylene diamine tetra-acetic acid (EDTA) titrimetric method against EDTA solution 0.01 mol/l with aerochrome black-T and merixide powder as pH color indicator, respectively.

The final measured levels of total and calcium hardness, and lead were compared with the initial concentrations at the dissolving time to calculate the reduction percentages in the concentrations concerning exposure times.

### In-vitro antimicrobial activities of tilapia bone powder

The *in-vitro* antibacterial actions of Tilapia bone powder against *E. coli* O1527:H7 at a rate of $2.4 \times 10^{12}$ CFU/ml, *S. Typhimurium* at a rate of $1.8 \times 10^8$ CFU/ml, and *M.*

*gallisepticum* at a rate of $1.5 \times 10^5$ CFU/ml, as well as antifungal actions against *A. niger*, *T. mentagrophytes*, and *C. albicans* at a rate of $3.2 \times 10^9$ CFU/ml each were evaluated using a 96-micro-well plate minimal inhibitory concentration technique as recommended by Soliman *et al*. (2016) and Elshikh *et al*. (2016).

Tilapia bone powder was dissolved in distilled water at a rate of 1% suspension. The Tilapia bone suspension 1% was distributed at a rate of 100 µl on the 96 wells of the microplate. Ten microliters from the bacterial and fungal suspensions were aseptically inoculated as follows: *E. coli* O157: H7 $(2.4 \times 10^{12}$ CFU/ml) suspension to each well of columns 1:2 (16 wells), *S. Typhimurium* $(1.8 \times 10^8$ CFU/ml) suspension to each well of columns 3:4 (16 wells), *M. gallisepticum* $(1.5 \times 10^5$ CFU/ml) suspension to each well of columns 5:6 (16 wells), *A. niger* $(3.2 \times 10^9$ CFU/ml) suspension to each well of columns 7:8 (16 wells), *T. mentagrophytes* $(3.2 \times 10^9$ CFU/ml) suspension to each well of columns 9:10 (16 wells), and *C. albicans* $(3.2 \times 10^9$ CFU/ml) suspension to each well of columns 11:12 (16 wells).

At specified intervals of 0.25, 0.5, 1.0, 2.0, and 4.0 hours, 10 µl of Tilapia bone-bacterial and fungal mixes were transferred aseptically into 15-ml Falcon tubes set with 10 ml buffered peptone water (Thermo Scientific™ Oxoid™ Buffered Peptone Water, CM0509B, 500 g). The resuscitation tubes were vortexed and incubated at 37℃/2 hours. Ten microliters from the Falcon tubes were aseptically dropped onto eosin methylene blue, *Mycoplasma* agar base, CHROMagar, and malt extract agar and incubated at 37℃/24 hours. The growing colonies of *E. coli* (metallic green), *M. gallisepticum* (fried egg)*, S. Typhimurium* (pink), *A. niger*, *T. mentagrophytes*, and *C. albicans* were counted using the Dark-field colony counter (R164109 Reichert-Jung Quebec Darkfield 3325 Colony Counter). The antimicrobial efficacies were judged based on the killing percentages of Tilapia bone powder suspension, these killing percentages were calculated based on the number of surviving colonies concerning the initial counts and expressed as a percentage (%) using the following formula:

$$\frac{\text{The initial microbial count - Number of Surviving organisms}}{\text{The initial microbial count}} \times 100$$

### The in-vivo activity of tilapia bone powder
### Experimental birds' housing microclimate

A total of 250 1-day-old classic Hubbard chicks were purchased from the Ismailia-Egypt Company at Abousweer city- Ismailia, Egypt. Chicks on their arrival were weighed ($W_0$) and then divided into five groups, 50 birds each (Five replicates of ten birds) into research units supplied with a deep litter system of hay following Soliman and Hassan (2020). The units were adjusted with some biosecurity measures to maintain the broilers' health after Soliman and Abdallah (2020).

These measures included dry cleaning of the units using brooms followed by wet cleaning using water under pressure with quaternary ammonium compound and water again. The disinfection action of the units was carried out in three stages at 24 hours intervals: the first using hypochlorite, the second using sodium hydroxide 5% solution, and the third using formaldehyde spray, the units were then closed for 24 hours, and the latter opened for ventilation. The units were supplied at the entrance area with foot dip filled with commercial carbolic acid 5%, break-back traps for rodent control, fly-proof nets, prohibition for the introduction of wild birds, divided doors for optimizing the restricted access concept, secured food storage areas, and sufficient roomy space for optimizing traffic control inside the units during daily activities such as observation, feeding, and watering of broilers.

The units' floors were lined with a thin film of superphosphate at a rate of 0.5 g.m$^{-2}$ before the hay litter usage after Soliman *et al*. (2018) for optimizing litter abiotic conditions such as acidic pH, reduced ammonia levels, and reduced microbial development. The research units were provided with blue LED lamps of 20 watts and 2,250 lumen to serve for a continuous lighting regimen (23 hours of lighting and one hour of darkness; 23 l: 1D) after Soliman and Hassan (2019). The ventilation act of the units was adjusted based on cross-negative ventilation using *V*-shaped side-wall windows to serve as air inlets and suction fans on the crossing walls to serve as air outlets with extra ceiling fans to enhance the maximum ventilation act. The units were provided with halogen heaters (Halogen-4 tubes- 2,400 watt heater, Bravo BR-4T Heater 4 halogen Candles) for maintaining the temperature as recommended by Soliman *et al*. (2021a).

### Experimental birds' management

The microclimatic temperature was adjusted and maintained for brooding (The 1st week of the fattening cycle) at 35℃ in the five rooms by turning on the halogen heaters before the chicks' arrival to achieve the requested temperature. Later, the heaters' working hours were reduced gradually to contribute to a non-significant decline in the microclimatic temperature at a rate of 3.5℃ weekly until optimized at 25℃ by the end of the 3rd week.

Chicks were given *ad libitum* access to clean tap water that was dechlorinated previously via exposure to sunlight. Broilers were provided with standard corn–soybean ration to meet their nutritional requirements after National Research Council; NRC, (1994) and Applegate and Angel (2014) modifications. The corn-soybean ration was purchased from Veterinary Clinic in Ismailia, Egypt (El-Eman Company, El-Sharkia, Egypt). The starter ration was provided from day 1 to 14 days old and was based on 22.5% protein, 2,910 kcal energy, 3.78% crude fiber, and 5.38% fat. The grower ration was provided from 15 days old until the end of the fattening cycle (39 days) and was

based on 21% protein, 3,110 kcal energy, 3.38% crude fiber, and 2.81% fat.

Broiler chickens were immunized by active artificial act using vaccines in clean tap dechlorinated water after deprivation for 4 hours against infectious bronchitis on the 7th day with PESTIKAL B1 SPF H120 $\geq 10^{3.5}$ live attenuated viruses, infectious bursal disease on the 14th and 21st days with SER-VAC D78 Strain VMG91 $\geq 10^{3.0}$ live attenuated viruses, and Newcastle virus disease on the 16th and 26th days with PESTIKAL Lasota $\geq 10^{6.0}$ live lentogenic viruses.

Microclimatic conditions such as temperature, relative humidity, ventilation rate, and lighting program were kept under strict observation, as well as broilers for the development of any abnormalities, morbidity rates, and mortality rates (specific and non-specific) during the entire fattening cycle that was designed to last for 39 days.

**Water supply and challenge**

Chicks were supplemented in a daily regimen with water treated with Tilapia bone powder (1 g/l) for 4–6 hours starting from the 3rd day of the fattening cycle. Polluted water was provided to chicks as follows: the 1st group (G1) was challenged with magnesium chloride polluted water at a rate of 100 mg/l with total hardness levels up to 2,650 mg/l $CaCO_3$ and calcium hardness up to 1,960 mg/l $CaCO_3$, the second group was challenged with lead nitrate polluted water at a rate of 350 mg/l with lead concentrations up to 20 mg/l, the 3rd group (G3) was challenged with *E. coli* contaminated water at a rate of $2.4 \times 10^{12}$ CFU/ml, the 4th group was challenged with *S. Typhimurium* at a rate of $1.8 \times 10^8$ CFU/ml, and the 5th group was assigned as a negative control group (non-challenged and non-treated). Polluted water was provided to the broilers weekly on the 7th, 14th, 21st, 28th, and 35th days. On the challenge day, water deprivation for 4 hours was executed to ensure the weekly challenge act. Broilers were supplemented with water treated with Tilapia bone powder (1 g/l) for 4–6 hours after the challenge.

**Performance indices (PI)**

A random representative sample of about 45 birds per group was weighed weekly (LBW/g) using a digital scale (WONHENG® Computing Electronic Digital Counting Weight Balance Scale, 220 V, 30 kg, Zhejiang Wonheng Industry, and Trade Co., China). The random representative sample size was calculated using a simple random sampling design after Thrusfield and Christley (2018) with an expected error of 5%:

$$N = (Z_{1-\alpha/2})^2.p\,(1\text{-}p)/\,d^2$$

where (*n*) was the number of samples, $(Z_{1-\alpha/2})$ was the standard normal, (*p*) was the expected proportion in the population, and (*d*) was the absolute precision.

The PI were calculated after Soliman and Hassan (2017). These performance indices such as the weight gains (WG/g) were calculated based on the weekly final weight $(W_*)$ to the initial weekly weight $(W_0/W_*)$.

Feed (FI/g) and water (WI/ml) intakes were calculated based on proportioning the amount of food and water consumed to the number of live birds in a group. The feed conversion ratios (FCRs) were calculated by proportioning feed intake (FI/g) to weight gains (WG/g). PI was calculated by proportioning body weight (kg) to the FCRs.

**Sampling**

A total of 2,250 samples were collected during the study periods. The samples were 90 Tilapia-pollutants mixes (2 pollutants × triplets × 3 replicates × 5 sampling times), 480 Tilapia-bacterial/fungal mixes (96 well × 5 sampling times), 240 sera, 240 intestinal (duodenal/cecal) swabs, 1,200 tissue samples such as breast muscles, liver, spleen, heart, and bursa of Fabricius. Intestinal swabs (duodenal and/or cecal) were harvested on 9 ml tryptone soy broth (Thermo Scientific™ Oxoid™ Tryptone Soya Broth, CM0129, 500 g) and preserved in a dry ice box to be transferred to the laboratory for examination within 2–3 hours.

A total of 240 birds (48 per group) were sacrificed after the 39th day of the *in-vivo* experiment for blood sample collection. Blood samples into serum vacutainer tubes (BD Vacutainer® Serum tubes, 10.0 ml, 16 × 100 mm, Plastic, Additive: Clot Activator, Silicone Coated, Red Conventional Closure, and Paper Label) were held in a water bath (Thermo® water bath Precision series Standard, 20 l, 30℃ to 100℃, 392 mm, GP20) at 24℃ for 60 minutes and then centrifuged at 3,500 rpm for 10 minutes. The obtained clear sera were pipetted into 2.5 ml Eppendorf tubes with an automatic pipette (Thermo Scientific™ Finnpipette™ Adjustable Volume Single-Channel Micro Pipettor, 100 to 1,000 microliter Volume). The tubes were stored at −20℃ for analysis (Soliman *et al*., 2017).

The carcasses after blood sampling were de-feathered, eviscerated, weighed, and expressed in grams (CW/g). The breast muscles were sampled in a representative way and packed in polyethylene bags at −20℃ for the bacteriological examination. Edible organs (liver and heart) and immune organs (spleen and bursa of Fabricius) were harvested from the carcasses and weighed then kept frozen until histopathological examination. Evascirated carcasses after weighing were hygienically disposed of by burial with lime lining all over the burial bits.

**Biochemical profile**

Sera (*n* = 240) were thawed and examined for some biochemical and antioxidant parameters such as total protein (TP) (g/dl), alanine aminotransferase (ALT) (IU/l), urea (UREA) (mg/dl), creatinine (CREAT) (mg/dl), Glucose (GLUCO) (mg/dl), total cholesterol (TC) (mg/dl), and triglycerides (TG) (mg/dl), total antioxidant capacity (TAC) (mM/l), malondialdehyde (MDA) (nmol/ml), and superoxide dismutase (SOD) (U/ml) calorimetrically using ROCHE® COBAS Integra 400 Plus chemical analyzer. Cortisol hormone; CORT (mcg/dl) and total immunoglobulin G and M

concentrations (IgG and IgM, mg/dl) were measured by using ROCHE® ELECSYS 1010 Immunoassay Analyzer.

**Bacteriological examination**

Frozen tissue samples (breast muscles, n = 240) were thawed and placed in the stomacher (Seward® Lab blenders Stomacher®400 circulators, Wales, UK) and then pre-enriched into tryptone soy broth (Thermo Scientific™ Oxoid™ Tryptone Soya Broth, CM0129, 500 g). The pre-enriched tissue samples (n = 240) and intestinal swabs (n = 240) were subjected to tenfold serial dilutions up to $10^{-8}$ using physiological saline as recommended by American Public Health Association; APHA (2017).

Total bacterial, *Enterobacteriaceae*, and *Salmonella* counts of tissue samples and intestinal swabs were carried out using the drop plate technique after Kim and Lee (2016). Un-selective total bacterial counts onto standard plate count agar (Thermo Scientific™ Oxoid™ Plate Count Agar, CM0325, 500 g), Enterobacteriaceae counts onto eosin methylene blue agar (Modified Levine Eosin Methylene Blue Thermo Scientific™ Oxoid™, CM0069B, 500 g), and Salmonella counts onto CHROMagar™Salmonella (BD BBL™ CHROMagar™ Salmonella READY-TO-USE Plated Media) were incubated at 37°C for 24 hours. Colonies were counted on the screen of the Dark-field colony counter (R164109 Reichert-Jung Quebec Darkfield 3,325 Colony Counter) after Murray et al. (2015).

**Histopathological examination**

Tissue samples (*n* = 960) from the liver (*n* = 240), heart (*n* = 240), spleen (*n* = 240), and bursa of Fabricius (*n* = 240) that were harvested from broilers were thawed at room temperature. The samples were fixed in 10% buffered formalin. Specimens were cut into 5-mm thick, put into tissue cassettes, dehydrated by transferring through a series of alcohols with different concentrations, cleared in two changes of xylene, embedded in paraffin wax, cut into 4-μm thick sections, and stained with hematoxylin and eosin (HE) (Bancroff *et al.*, 1990). The histological sections of the examined organs were visualized using a Zeiss Axioplan microscope (Carl Zeiss MicroImaging, Thornwood, NY) under (×40) magnification under (×10) and (×20) magnification and photographed.

**Statistical analysis**

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS version 27.0, IBM Corp, NY, NY) software package (SPSS, 2020). Data were analyzed using multifactorial Analysis of Variance (two-tailed ANOVA) to determine the overall influence of the water pollutants/contaminants (chemical and microbial), broiler's age, and their interactions. The statistical model utilized in the analysis was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where $Y_{ijk}$ was the measurement of dependent variables; μ was the overall mean; $\alpha_i$ was the fixed effect of the water pollutants/contaminants (chemical and microbial), $\beta_j$ was the fixed effect of broiler's age, $(\alpha\beta)_{ij}$ was the interactions of the water pollutants by broiler's age, and $\varepsilon_{ijk}$ was the random error.

Total bacterial, *Enterobacteriaceae,* and *Salmonella* counts were transformed and expressed as logarithms (Log) using Microsoft Excel 2016. The results were expressed as highly significant at ($p < 0.01$), significant at ($p \leq 0.05$), and non-significant at ($p > 0.05$).

**Ethical approval**

The materials used, protocol, and study design were approved by the Scientific Research Ethics Committee on poultry researches, Faculty of Veterinary Medicine, Suez Canal University, Egypt with approval number (2022012). Broiler chickens were handled in human manners to reduce the suffering and the overwhelming challenges. The number of experimental birds was as minimized as possible and the 3Rs (replacement, reduction, and refinement) were followed to maintain birds' rights.

## Results

***In-vitro adsorption and antimicrobial activity***

Tilapia bone powder 1% reveals in Table 1 achieving a highly significant ($p < 0.01$) 100% reduction in the levels of lead (Pb²*) after 1 hour, total hardness levels after 0.5 hours, and calcium hardness levels after 0.5 hours. On the other hand, the antimicrobial activity (Table 1) reveals that Tilapia bone powder achieved 100% killing efficacy against *E. coli* O1527:H7, *S. Typhimurium*, *M. gallisepticum*, *A. niger*, *T. mentagrophytes*, and *C. albicans* after 0.5, 1, 1, 1, 1, and 1 hours, respectively.

***Water physicochemical and microbial analysis***

The analysis of Tilapia bone powder 1% treated water reveals highly significant (Table 2, $p < 0.01$) increases in the levels of dissolved oxygen (DO; mg/l), as well as highly significant ($p < 0.01$) declines in physico-chemical characteristics such as pH, electrical conductivity (EC; μS/cm), total dissolved solids (TDS; mg/l), total alkalinity (T. alkalinity; mg/l), total hardness (T. Hardness; mg/l $CaCO_3$), sulfates ($SO_4$; mg/l), and nitrates ($NO_3$; mg/l); electrolyte such as sodium (Na⁺; mg/l), potassium (K⁺; mg/l), calcium (Ca²⁺; mg/l), magnesium (Mg²⁺; mg/l), and chloride (Cl⁻; mg/l); heavy metals such as iron (Fe²⁺; mg/l), copper (Cu²⁺; mg/l), and zinc (Zn²⁺; mg/l); and microbial parameters such as total bacterial count (TBC; CFU/ml), total *Enterobacteriaceae* count (TEC; CFU/ml), and *Salmonella* count (TSC; CFU/ml) compared with tap water.

The levels of phosphate ($PO_4$; mg/l) and lead (Pb²⁺, mg/l) in Table 2 reveal no significant differences in Tilapia bone powder 1% treated water compared with tap water.

**Table 1.** *In-vitro* adsorption and antimicrobial efficacy (Reduction percentage mean ± SE) of tilapia powder 1% suspension.

| Contact time /hour | Chemical adsorption action mg/l | | |
|---|---|---|---|
| | Pb$^{2+}$ | Total hardness | Ca-hardness |
| 0.25 | 73.3$^c$ ± 0.66 | 93.7$^b$ ± 0.07 | 96.6$^b$ ± 0.23 |
| 0.5 | 94.7$^b$ ± 0.97 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| 1.0 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| 2.0 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| 4.0 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| p-value | **0.001** | **0.000** | **0.001** |

*Microbial survival CFU/ml*

| | Escherichia coli O157: H7 | Salmonella Typhimurium | Mycoplasma gallisepticum | Aspergillus niger | Trichophyton mentagrophytes | Candida Albicans |
|---|---|---|---|---|---|---|
| 0.25 | 93.2$^b$ ± 0.03 | 75.3$^c$ ± 0.12 | 76.3$^c$ ± 0.18 | 68.1$^c$ ± 0.09 | 71.5$^c$ ± 0.04 | 83.3$^c$ ± 0.01 |
| 0.5 | 100$^a$ ± 0.00 | 95.6$^b$ ± 0.06 | 97.4$^b$ ± 0.64 | 93.9$^b$ ± 0.05 | 95.0$^b$ ± 0.04 | 98.3$^b$ ± 0.04 |
| 1.0 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| 2.0 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| 4.0 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 | 100$^a$ ± 0.00 |
| p-value | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 |

*Initial levels of total hardness = 2,100 mg/l CaCO$_3$, calcium hardness = 1,600 mg/l CaCO$_3$, lead = 20 mg/l, E. coli O157: H7 = 2.4 × 10$^{12}$ CFU/ml, Salmonella Typhimurium = 1.8 × 10$^8$ CFU/ml, Mycoplasma gallisepticum = 1.5 × 10$^5$ CFU/ml, Aspergillus niger = 3.2 × 10$^9$ CFU/ml, Trichophyton mentagrophytes = 3.2 × 10$^9$ CFU/ml, and Candida albicans = 3.2 × 10$^9$ CFU/ml.*

**Table 2.** Water analysis (mean ± SE) in tap and tilapia bone-treated water.

| Elements | Tap water | Treated water | p-value | MPL |
|---|---|---|---|---|
| *Physicochemical analysis* | | | | |
| pH | 7.7$^a$ ± 0.07 | 7.3$^b$ ± 0.04 | **0.001** | 6.5–8.0 |
| EC (µS/cm) | 642.0$^a$ ± 0.00 | 148.3$^b$ ± 0.07 | **0.001** | 200 |
| TDS (mg/l) | 430.1$^a$ ± 0.71 | 99.3$^b$ ± 0.19 | **0.0002** | 500 |
| DO (mg/l) | 5.7$^b$ ± 0.01 | 10.6$^a$ ± 0.02 | **0.000** | 10 |
| Total alkalinity (mg/l) | 92.1$^a$ ± 0.04 | 34.4$^b$ ± 0.05 | **0.000** | 120 |
| Total hardness (mg/l CaCO3) | 68.8$^a$ ± 0.03 | 22.7$^b$ ± 0.05 | **0.000** | 500 |
| PO4 (mg/l) | 0.0$^a$ ± 0.00 | 0.0$^a$ ± 0.00 | -- | 0.1 |
| SO4 (mg/l) | 55.8$^a$ ± 0.04 | 4.3$^b$ ± 0.04 | **0.001** | 250 |
| NO3 (mg/l) | 9.1$^a$ ± 0.03 | 0.7$^b$ ± 0.04 | **0.001** | 45 |
| *Electrolytes analysis* | | | | |
| Na+ (mg/l) | 130.0$^a$ ± 0.33 | 95.5$^b$ ± 0.09 | **0.000** | 200 |
| K+ (mg/l) | 12.2$^a$ ± 0.33 | 2.8$^b$ ± 0.02 | **0.001** | 10 |
| Ca2+ (mg/l) | 55.6$^a$ ± 0.05 | 29.5$^b$ ± 0.03 | **0.002** | 75 |
| Mg2+ (mg/l) | 26.2$^a$ ± 0.04 | 9.0$^b$ ± 0.02 | **0.001** | 50 |
| Cl− (mg/l) | 126.4$^a$ ± 0.09 | 88.8$^b$ ± 0.06 | **0.001** | 200 |
| *Heavy metals analysis* | | | | |
| Fe2+ (mg/l) | 0.06$^a$ ± 0.01 | 0.00$^b$ ± 0.00 | **0.001** | 0.0 |
| Cu2+ (mg/l) | 0.06$^a$ ± 0.00 | 0.00$^b$ ± 0.00 | **0.001** | 1.0 |

*Continued*

| Elements | Tap water | Treated water | p-value | MPL |
|---|---|---|---|---|
| Zn2+ (mg/l) | 1.20ᵃ ± 0.02 | 0.0ᵇ ± 0.00 | **0.001** | 5.0 |
| Pb2+ (mg/l) | 0.0ᵃ ± 0.00 | 0.0ᵃ ± 0.00 | -- | 0.05 |
| Microbial assessment | | | | |
| TBC (CFU/ml) | 4.13ᵃ ± 0.01 | 1.96ᵇ ± 0.01 | **0.000** | Zero |
| TEC (CFU/ml) | 2.36ᵃ ± 0.074 | 0.18ᵇ ± 0.061 | **0.000** | Zero |
| TSC (CFU/ml) | 0.33ᵃ ± 0.01 | 0.00ᵇ ± 0.00 | **0.005** | Zero |

EC=Electrical conductivity as µS/cm, TDS=Total dissolved solids as mg/l, DO=Dissolved oxygen as mg/l, T. alkalinity=Total alkalinity as mg/l, T. hardness= Total hardness as mg/l CaCO₃, PO₄=Phosphate as mg/l, SO₄=Sulfate as mg/l, NO₃=Nitrate as mg/l, Na⁺=Sodium as mg/l, K⁺=Potassium as mg/l, Ca²⁺=Calcium as mg/l, Mg²⁺=Magnesium as mg/l, Cl⁻=Chloride as mg/l, Fe²⁺=Iron as mg/l, Cu²⁺=Cobber as mg/l, Zn²⁺=Zinc as mg/l, Pb²⁺=Lead as mg/l, TBC=Total bacterial logarithmic counts as CFU/ml, TEC=Total *Enterobacteriaceae* logarithmic counts as CFU/ml, TSC=Total *Salmonella* logarithmic counts as CFU/ml, SE=Standard error.

**Table 3.** Performance indices (mean ± SE) in different groups supplied with tilapia bone powder treated water (1 g/l).

| Groups | Age W | WG g | FCR % | PI ratio | FI g | WI ml | WI/FI ratio |
|---|---|---|---|---|---|---|---|
| Overall means concerning treatments | | | | | | | |
| G1 | | 396ᶜ ± 0.31 | 1.02ᵇ ± 0.03 | 8.96ᶜ ± 0.08 | 418ᵇ ± 0.40 | 776ᵇ ± 0.23 | 1.68ᵃ ± 0.05 |
| G2 | | 392ᶜ ± 0.31 | 1.07ᵇ ± 0.06 | 9.21ᶜ ± 0.09 | 413ᶜ ± 0.15 | 657ᶜ ± 0.92 | 1.53ᵇᶜ ± 0.03 |
| G3 | | 424ᵇ ± 0.34 | 0.93ᶜ ± 0.04 | 10.89ᵇ ± 0.08 | 398ᵈ ± 0.31 | 623ᵈ ± 0.51 | 1.56ᵇ ± 0.01 |
| G4 | | 465ᵃ ± 0.40 | 0.87ᶜ ± 0.03 | 12.97ᵃ ± 0.40 | 396ᵈ ± 0.70 | 587ᵉ ± 0.25 | 1.49ᶜ ± 0.01 |
| Gc | | 324ᵈ ± 0.25 | 1.54ᵃ ± 0.07 | 5.56ᵈ ± 0.06 | 481ᵃ ± 0.23 | 812ᵃ ± 0.87 | 1.65ᵃ ± 0.04 |
| p-value | | **0.002** | **0.000** | **0.000** | **0.002** | **0.001** | **0.005** |
| Overall means concerning the broiler's age | | | | | | | |
| First week | | 105ᵉ ± 0.28 | 0.98ᵇᶜ ± 0.05 | 1.73ᵉ ± 0.08 | 97ᵉ ± 0.88 | 142ᵉ ± 0.83 | 1.45ᶜ ± 0.01 |
| Second week | | 216ᵈ ± 0.20 | 0.99ᵇ ± 0.02 | 3.85ᵈ ± 0.11 | 213ᵈ ± 0.13 | 335ᵈ ± 0.16 | 1.57ᵇ ± 0.02 |
| Third week | | 458ᶜ ± 0.47 | 0.95ᵇᶜ ± 0.02 | 8.95ᶜ ± 0.02 | 434ᶜ ± 0.90 | 689ᶜ ± 0.36 | 1.58ᵇ ± 0.04 |
| Fourth week | | 717ᵃ ± 0.18 | 0.90ᶜ ± 0.03 | 18.16ᵃ ± 0.07 | 624ᵇ ± 0.07 | 1002ᵇ ± 0.82 | 1.60ᵇ ± 0.02 |
| Fifth week | | 505ᵇ ± 0.20 | 1.61ᵃ ± 0.09 | 14.90ᵇ ± 0.09 | 737ᵃ ± 0.87 | 1285ᵃ ± 0.58 | 1.72ᵃ ± 0.05 |
| p-value | | **0.000** | **0.001** | **0.000** | **0.001** | **0.000** | **0.003** |
| Interactions between treatments by broiler's age | | | | | | | |
| G1 | First week | 110ᵉ ± 0.65 | 0.84ᶜ ± 0.02 | 1.90ᵉ ± 0.06 | 93ᵉ ± 0.02 | 133ᵉ ± 0.60 | 1.42ᶜ ± 0.03 |
| | Second week | 200ᵈ ± 0.49 | 0.98ᵇ ± 0.01 | 3.65ᵈ ± 0.05 | 197ᵈ ± 0.94 | 301ᵈ ± 0.00 | 1.52ᵇ ± 0.02 |
| | Third week | 427ᶜ ± 0.98 | 0.94ᵇ ± 0.02 | 8.37ᶜ ± 0.19 | 403ᶜ ± 0.88 | 599ᶜ ± 0.17 | 1.49ᵇ ± 0.03 |
| | Fourth week | 675ᵃ ± 0.28 | 0.91ᵇᶜ ± 0.01 | 15.97ᵃ ± 0.12 | 618ᵇ ± 0.58 | 987ᵇ ± 0.56 | 1.59ᵇ ± 0.04 |
| | Fifth week | 568ᵇ ± 0.25 | 1.41ᵃ ± 0.08 | 14.93ᵇ ± 0.09 | 778ᵃ ± 0.87 | 1864ᵃ ± 0.84 | 2.39ᵃ ± 0.02 |
| G2 | First week | 113ᵉ ± 0.18 | 0.85ᶜ ± 0.02 | 1.89ᵉ ± 0.05 | 97ᵉ ± 0.65 | 134ᵉ ± 0.07 | 1.38ᶜ ± 0.02 |
| | Second week | 219ᵈ ± 0.22 | 0.98ᵇ ± 0.01 | 3.89ᵈ ± 0.04 | 215ᵈ ± 0.58 | 316ᵈ ± 0.45 | 1.46ᵇ ± 0.02 |
| | Third week | 459ᵇ ± 0.32 | 0.92ᵇᶜ ± 0.01 | 9.12ᶜ ± 0.09 | 423ᶜ ± 0.92 | 657ᶜ ± 0.79 | 1.55ᵇ ± 0.07 |
| | Fourth week | 724ᵃ ± 0.16 | 0.85ᶜ ± 0.02 | 18.3ᵃ ± 0.56 | 620ᵇ ± 0.61 | 1065ᵇ ± 0.62 | 1.71ᵃ ± 0.07 |
| | Fifth week | 445ᶜ ± 0.40 | 1.74ᵃ ± 0.19 | 12.8ᵇ ± 0.35 | 708ᵃ ± 0.94 | 1114ᵃ ± 0.50 | 1.57ᵇ ± 0.04 |

*Continued*

| Groups | Age W | WG g | FCR % | PI ratio | FI g | WI ml | WI/FI ratio |
|---|---|---|---|---|---|---|---|
| G3 | First week | $119^e \pm 0.23$ | $0.80^b \pm 0.02$ | $2.07^e \pm 0.09$ | $95^e \pm 0.12$ | $148^e \pm 0.85$ | $1.55^a \pm 0.02$ |
| | Second week | $229^d \pm 0.19$ | $0.86^b \pm 0.01$ | $4.52^d \pm 0.04$ | $199^d \pm 0.12$ | $318^d \pm 0.48$ | $1.60^a \pm 0.02$ |
| | Third week | $477^c \pm 0.43$ | $0.85^b \pm 0.02$ | $10.26^c \pm 0.23$ | $405^c \pm 0.31$ | $633^c \pm 0.90$ | $1.55^a \pm 0.01$ |
| | Fourth week | $773^a \pm 0.33$ | $0.82^b \pm 0.04$ | $20.52^a \pm 0.33$ | $625^b \pm 0.97$ | $983^b \pm 0.83$ | $1.57^a \pm 0.04$ |
| | Fifth week | $523^b \pm 0.42$ | $1.34^a \pm 0.09$ | $17.09^b \pm 0.53$ | $667^a \pm 0.10$ | $1034^a \pm 0.05$ | $1.55^a \pm 0.03$ |
| G4 | First week | $115^e \pm 0.17$ | $0.82^b \pm 0.02$ | $2.02^e \pm 0.06$ | $95^e \pm 0.15$ | $138^e \pm 0.02$ | $1.45^a \pm 0.04$ |
| | Second week | $232^d \pm 0.13$ | $0.86^b \pm 0.01$ | $4.62^d \pm 0.08$ | $201^d \pm 0.62$ | $316^d \pm 0.30$ | $1.57^a \pm 0.02$ |
| | Third week | $499^c \pm 0.50$ | $0.82^b \pm 0.01$ | $10.88^c \pm 0.17$ | $412^c \pm 0.23$ | $640^c \pm 0.05$ | $1.55^a \pm 0.02$ |
| | Fourth week | $863^a \pm 0.35$ | $0.72^c \pm 0.03$ | $24.78^a \pm 0.50$ | $618^b \pm 0.71$ | $906^b \pm 0.42$ | $1.46^a \pm 0.01$ |
| | Fifth week | $617^b \pm 0.49$ | $1.12^a \pm 0.09$ | $22.55^b \pm 0.02$ | $655^a \pm 0.71$ | $934^a \pm 0.09$ | $1.42^a \pm 0.02$ |
| Gc | First week | $68^e \pm 0.25$ | $1.59^b \pm 0.06$ | $0.75^e \pm 0.04$ | $108^e \pm 0.38$ | $158^e \pm 0.88$ | $1.46^b \pm 0.02$ |
| | Second week | $202^d \pm 0.26$ | $1.25^c \pm 0.02$ | $2.57^d \pm 0.06$ | $253^d \pm 0.71$ | $427^d \pm 0.94$ | $1.69^a \pm 0.05$ |
| | Third week | $428^b \pm 0.10$ | $1.23^c \pm 0.03$ | $6.12^c \pm 0.25$ | $526^c \pm 0.18$ | $918^c \pm 0.84$ | $1.74^a \pm 0.18$ |
| | Fourth week | $550^a \pm 0.26$ | $1.19^c \pm 0.07$ | $11.24^a \pm 0.78$ | $642^b \pm 0.42$ | $1073^b \pm 0.76$ | $1.66^a \pm 0.04$ |
| | Fifth week | $372^c \pm 0.25$ | $2.44^a \pm 0.04$ | $7.10^b \pm 0.05$ | $878^a \pm 0.65$ | $1482^a \pm 0.64$ | $1.68^a \pm 0.02$ |
| *p-value* | | **0.000** | **0.002** | **0.001** | **0.000** | **0.000** | **0.032** |

G1=Broilers challenged with magnesium chloride (100 mg/l), G2= Broilers challenged with lead nitrate (350 mg/l), G3=Broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU), G4=Broilers challenged with *Salmonella* Typhimurium ($1.8 \times 10^8$ CFU), G5=control-negative broilers.
WG=Weight gain, FCR=Feed conversion ratio, PI=Performance index, FI=Feed intake, WI=Water intake, WI/FI=Water to feed intake ratio, SE=Standard error.

### Growth traits
Broiler groups provided with drinking water treated with Tilapia bone powder 1% reveal in Table 3 highly significant ($p < 0.01$) increases in WG and PI, as well as highly significant ($p < 0.01$) improvements in FCR, FI, and WI in all challenged groups compared to the control. On a time scale, WG, FCR, PI, FI, and WI reveal highly significant ($p < 0.01$) increases/improvements in the 4th, 4th, 4th, 5th, and 5th weeks, respectively.

### Live, carcasses, and organs weights
Live body and carcasses weights in broiler groups provided with drinking water treated with Tilapia bone powder 1% reveal in Table 4 highly significant ($p < 0.01$) increases in G4 (challenged *S. Typhimurium*; $1.8 \times 10^8$ CFU), G3 (challenged *E. coli* O157:H7; $2.4 \times 10^{12}$ CFU), G1 (challenged with magnesium chloride; 100 mg/l), and G2 (challenged with lead nitrate; 350 mg/l) with no significant differences between the values in G1 and G2.
Liver weights reveal in Table 4 highly significant ($p < 0.01$) increases in G4 (challenged *S. Typhimurium*; $1.8 \times 10^8$ CFU) and G3 (challenged *E. coli* O157:H7; $2.4 \times 10^{12}$ CFU) compared to the other treated groups and the control with no significant differences between the values in the two groups. As shown in Table 4, heart, spleen, and bursa of Fabricius weights reveal highly significant ($p < 0.01$) increases in G4 (challenged *S. Typhimurium*; $1.8 \times 10^8$ CFU) compared to the other treated groups and the control.

### Biochemical profile
As shown in Table 5, broiler groups provided with drinking water treated with Tilapia bone powder 1% reveal highly significant ($p < 0.01$) improvements of TP, ALT, UREA, CREAT, GLUCO, TG, and TC levels in all treated broiler groups compared to the control group.

### Immunoglobulin concentrations
As shown in Table 6, immunoglobulins G concentrations reveal highly significant ($p < 0.01$) increases in G4 (challenged *S. Typhimurium*; $1.8 \times 10^8$ CFU) and G3 (challenged *E. coli* O157:H7; $2.4 \times 10^{12}$ CFU) compared to the other treated groups and the control with no significant differences between the values in the two groups. Meanwhile, IgM concentrations reveal in Table 6 highly significant ($p < 0.01$) increases in G4 (challenged *S. Typhimurium*; $1.8 \times 10^8$ CFU) compared to the other treated groups and the control.

**Table 4.** Live, carcass, and organ weights (mean ± SE) in different groups supplied with tilapia bone powder (1 g/l) treated water.

| Groups | LBW g | CW g | Edible organ weights g | | Immune organ weights g | |
|---|---|---|---|---|---|---|
| | | | Liver | Heart | Spleen | Bursa |
| *Overall means concerning treatments* | | | | | | |
| G1 | 2,033[c] ± 0.88 | 1,773[c] ± 0.13 | 47.8[b] ± 0.09 | 1.76[c] ± 0.01 | 11.1[c] ± 0.04 | 1.07[c] ± 0.01 |
| G2 | 2,017[c] ± 0.59 | 1,767[c] ± 017 | 45.8[c] ± 0.04 | 1.56[d] ± 0.02 | 9.17[d] ± 0.01 | 0.97[d] ± 0.00 |
| G3 | 2,170[b] ± 0.25 | 1,970[b] ± 0.13 | 50.3[a] ± 0.04 | 2.01[b] ± 0.01 | 14.1[b] ± 0.00 | 1.12[b] ± 0.00 |
| G4 | 2,380[a] ± 0.64 | 2,190[a] ± 0.76 | 51.5[a] ± 0.03 | 2.21[a] ± 0.02 | 15.4[a] ± 0.03 | 1.17[a] ± 0.01 |
| Gc | 1,673[d] ± 0.86 | 1,284[d] ± 0.11 | 36.0[d] ± 0.01 | 1.11[e] ± 0.03 | 7.8[e] ± 0.06 | 0.56[e] ± 0.03 |
| *p-value* | **0.000** | **0.001** | **0.000** | **0.000** | **0.000** | **0.000** |

G1=Broilers challenged with magnesium chloride (100 mg/l), G2= Broilers challenged with lead nitrate (350 mg/l), G3=Broilers challenged with *E. coli* O157:H7 (2.4 × 10¹² CFU), G4=Broilers challenged with *Salmonella* Typhimurium (1.8 × 10⁸ CFU), G5= control-negative broilers. LBW=Live body weight, CW=Carcass weight, SE=Standard error.

**Table 5.** Biochemical profile (mean ± SE) in different groups supplied with tilapia bone powder (1 g/l) treated water.

| Groups | TP g/dl | ALT IU/l | UREA mg/dl | CREAT mg/dl | GLUCO mg/dl | TG mg/dl | TC mg/dl |
|---|---|---|---|---|---|---|---|
| *Overall means concerning treatments* | | | | | | | |
| G1 | 5.6[e] ± 0.04 | 3.46[d] ± 0.03 | 19.5[d] ± 038 | 0.19[d] ± 0.01 | 67.2[d] ± 0.54 | 59.9[e] ± 0.16 | 93.7[c] ± 0.38 |
| G2 | 6.2[d] ± 0.03 | 3.62[c] ± 0.02 | 21.7[c] ± 0.35 | 0.31[c] ± 0.01 | 73.1[c] ± 0.49 | 63.8[d] ± 0.08 | 109.7[b] ± 0.41 |
| G3 | 6.6[c] ± 0.05 | 3.78[b] ± 0.04 | 22.4[c] ± 0.73 | 0.47[b] ± 0.00 | 74.3[bc] ± 0.48 | 67.6[b] ± 0.14 | 112.2[b] ± 0.34 |
| G4 | 6.7[b] ± 0.05 | 3.65[bc] ± 0.08 | 24.1[b] ± 0.55 | 0.43[b] ± 0.02 | 75.0[b] ± 0.78 | 66.7[c] ± 0.21 | 112.2[b] ± 0.21 |
| Gc | 7.5[a] ± 0.05 | 9.73[a] ± 0.07 | 29.5[a] ± 0.43 | 0.96[a] ± 0.01 | 104.7[a] ± 0.31 | 97.3[a] ± 0.14 | 162.7[a] ± 0.88 |
| *p-value* | **0.001** | **0.001** | **0.000** | **0.002** | **0.004** | **0.000** | **0.005** |

G1=Broilers challenged with magnesium chloride (100 mg/l), G2= Broilers challenged with lead nitrate (350 mg/l), G3=Broilers challenged with *E. coli* O157:H7 (2.4 × 10¹² CFU), G4=Broilers challenged with *Salmonella* Typhimurium (1.8 × 10⁸ CFU), G5= control-negative broilers. TP=Total protein, ALT=Alanine aminotransferase, UREA= Urea, CREAT=Creatinine, GLUCO=Glucose, TG=Triglycerides, TC=Total cholesterol, SE=Standard error.

**Table 6.** Immunoglobulin concentrations and stress markers (mean ± SE) in different groups supplied with tilapia bone powder (1 g/l) treated water.

| Groups | Immunoglobulin concentrations | | Stress marker | | | |
|---|---|---|---|---|---|---|
| | IgG mg/dl | IgM mg/dl | Cortisol mcg/dl | TAC mM/l | MDA nmol/ml | SOD U/ml |
| *Overall means concerning treatments* | | | | | | |
| G1 | 1,545[c] ± 0.68 | 456[c] ± 0.41 | 6.9[b] ± 0.24 | 2.35[a] ± 0.01 | 36.9[a] ± 0.20 | 309.5[a] ± 0.69 |
| G2 | 1,582[bc] ± 0.14 | 502[b] ± 0.81 | 6.1[c] ± 0.29 | 2.25[b] ± 0.01 | 36.5[a] ± 0.16 | 310.4[a] ± 0.82 |
| G3 | 1,650[a] ± 0.27 | 545[b] ± 0.55 | 6.0[cd] ± 0.25 | 2.21[b] ± 0.02 | 37.2[a] ± 0.16 | 308.4[a] ± 0.53 |
| G4 | 1,615[ab] ± 0.27 | 595[a] ± 0.16 | 5.3[d] ± 0.25 | 2.12[c] ± 0.03 | 34.4[b] ± 0.58 | 308.5[a] ± 0.26 |
| Gc | 1,256[d] ± 0.31 | 322[d] ± 0.65 | 15.3[a] ± 0.16 | 1.24[d] ± 0.00 | 23.4[c] ± 0.13 | 169.1[b] ± 0.24 |
| *p-value* | **0.001** | **0.002** | **0.004** | **0.001** | **0.004** | **0.005** |

G1=Broilers challenged with magnesium chloride (100 mg/l), G2= Broilers challenged with lead nitrate (350 mg/l), G3=Broilers challenged with E. coli O157:H7 (2.4 × 1012 CFU), G4=Broilers challenged with Salmonella Typhimurium (1.8 × 108 CFU), G5= control-negative broilers. IgG=Immunoglobulin G, IgM=Immunoglobulin M, CORT=Cortisol, TAC=Total antioxidant capacity, MDA=Malondialdehyde, SOD=Superoxide dismutase, SE=Standard error.

**Table 7.** Logarithmic bacterial counts (mean ± SE) in different groups supplied with tilapia bone powder (1 g/l) treated water.

| Treatments | TBCi CFU/ml | TBCb CFU/ml | TECi CFU/ml | TECb CFU/ml | TSC CFU/ml |
|---|---|---|---|---|---|
| *Overall means concerning treatments* | | | | | |
| *G1* | $4.06^b \pm 0.02$ | $2.06^a \pm 0.03$ | $1.81^b \pm 0.01$ | $0.68^{bc} \pm 0.01$ | $0.27^b \pm 0.04$ |
| *G2* | $3.91^c \pm 0.03$ | $1.88^b \pm 0.04$ | $1.74^{bc} \pm 0.02$ | $0.84^b \pm 0.09$ | $0.35^b \pm 0.02$ |
| *G3* | $3.80^d \pm 0.05$ | $1.89^b \pm 0.05$ | $1.68^c \pm 0.04$ | $0.62^{bc} \pm 0.01$ | $0.14^c \pm 0.00$ |
| *G4* | $3.69^e \pm 0.06$ | $1.69^c \pm 0.06$ | $1.54^d \pm 0.05$ | $0.57^c \pm 0.09$ | $0.07^c \pm 0.00$ |
| *Gc* | $4.18^a \pm 0.01$ | $2.15^a \pm 0.01$ | $1.98^a \pm 0.01$ | $1.20^a \pm 0.04$ | $0.48^a \pm 0.02$ |
| *p-value* | **0.000** | **0.002** | **0.000** | **0.004** | **0.001** |

G1=Broilers challenged with magnesium chloride (100 mg/l), G2= Broilers challenged with lead nitrate (350 mg/l), G3=Broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU), G4=Broilers challenged with *Salmonella* Typhimurium ($1.8 \times 10^8$ CFU), G5= control-negative broilers. TBCi=Total bacterial counts in intestinal swabs, TBCm= Total bacterial counts in breast muscles, TECi= Total Enterobacteriaceae counts in intestinal swabs, TECm= Total Enterobacteriaceae counts in breast muscles, TSC=Total Salmonella count in intestinal swabs, SE=Standard error.

### Stress markers

Cortisol hormone reveals in Table 6 highly significant ($p < 0.01$) declines in all treated broiler groups compared to the control group. Antioxidant enzymes such as TAC, MDA, and SOD levels reveal in Table 6 highly significant ($p < 0.01$) increases in all broilers provided with drinking water treated with Tilapia bone powder 1% compared to the control.

### Bacteriological assessments

Tilapia bone powder 1% treated water in Table 7 reveals highly significant ($p < 0.01$) declines in total bacterial counts of intestinal swabs (TBCi) and breast muscles (TBCb), total *Enterobacteriaceae* counts of intestinal swabs (TECi), and breast muscles (TECb), and TSC in all challenged broiler groups compared to the control group.
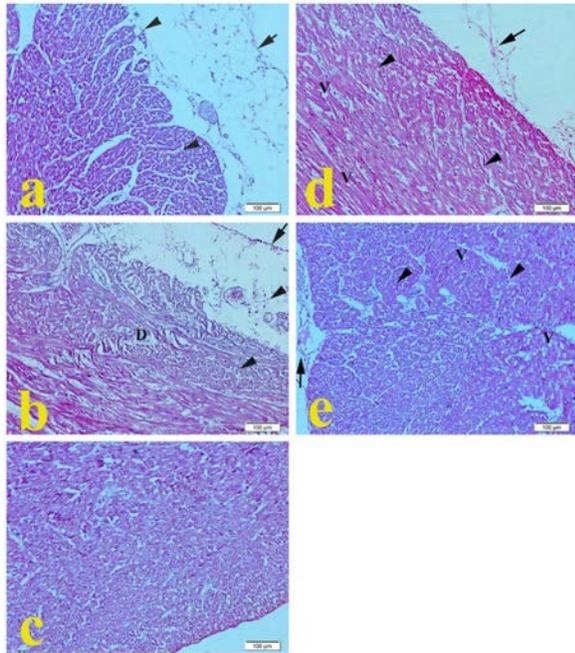
### Histopathological examination

Photomicrographs of the liver reveal severe congestion of the central vein, as well as hepatic cells, showed severe degeneration with severe vacuolation of cytoplasm and moderate leukocytic infiltration (Fig. 1a) in broilers challenged with magnesium chloride (100 mg/l) compared to control in Figure 1c. Figure 1b reveals congestion of blood vessels of the portal area with severe leukocytic infiltration. Hepatic cells showed degeneration with cytoplasmic vacuolation in broilers challenged with lead nitrate (350 mg/l). Mild congestion of central vein with hemosiderosis, hepatocytes showed mild vacuolar degeneration with mild leukocytic infiltration was recorded in Figure 1d in broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU) compared to control in Figure 1c. Figure 1e reveals congestion of blood vessels of the portal area and degeneration of the portal area with moderate focal leukocytic infiltration compared to control in Figure 1c. Hepatocytes showed moderate cytoplasmic vacuolation in broilers challenged with *S. Typhimurium* ($1.8 \times 10^8$ CFU) compared to the control (Fig. 1c).

Heart photomicrographs revealed in Figure 2a, fibrinous pericarditis and degeneration of cardiac muscle with mononuclear cell infiltration in broilers challenged with
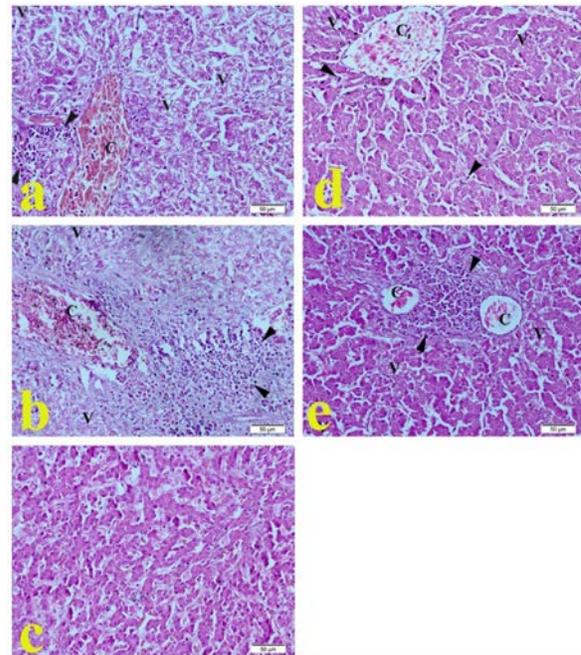


**Fig. 1.** Histopathological section of the liver by HE staining (**20×**): (a) Liver of broilers challenged with magnesium chloride (100 mg/l) showing congestion of central vein (C), mononuclear cell infiltration (arrowhead), and vacuolation of cytoplasm (V). (b) Liver of broilers challenged with lead nitrate (350 mg/l) showing congestion of central vein (C), vacuolation of cytoplasm (V), and severe mononuclear cell infiltration (arrowhead). (c) Liver of control-negative broiler chickens. (d) Liver of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU). € Liver of broilers challenged with *Salmonella Typhimurium* ($1.8 \times 10^8$). **Bar 50 μm.**

magnesium chloride (100 mg/l) compared to control in Figure 2c. Moderate pericarditis and mild myocardial degeneration with leukocytic infiltration were recorded in Figure 2b in broilers challenged with lead nitrate (350 mg/l) compared to the control in Figure 2c. Heart
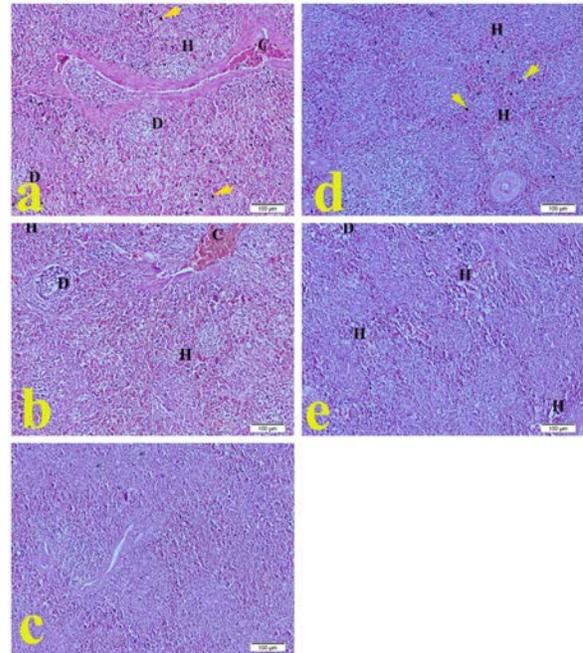
**Fig. 2.** Histopathological section of heart by HE staining (**20×**): (a) Heart of broilers challenged with magnesium chloride (100 mg/l) showing fibrinous pericarditis (arrow) and mononuclear cell infiltration (arrowhead). (b) Heart of broilers challenged with lead nitrate (350 mg/l). (c) Heart of control-negative broiler chickens. (d) Heart of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU). €Heart of broilers challenged with *Salmonella Typhimurium* ($1.8 \times 10^8$). **Bar 100 µm.**



**Fig. 3.** Histopathological section of the spleen by HE staining (**20×**): (a) Spleen of broilers challenged with magnesium chloride (100 mg/l) showing depletion of lymphocytes (D), congestion of splenic sinus (C), hemorrhage (H), and hemosiderosis (yellow arrow). (b) The spleen of broilers was challenged with lead nitrate (350 mg/l). (c) The spleen of control-negative broiler chickens. (d) The spleen of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU). €The spleen of challenged with *Salmonella Typhimurium* ($1.8 \times 10^8$). **Bar 100 µm.**

of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU) reveal in Figure 2d mild pericarditis, myocardial degeneration with mild hemorrhage, vacuolation of cytoplasm, and leukocytic infiltration compared to control in Figure 2c. Figure 2e of broilers challenged with *S. Typhimurium* ($1.8 \times 10^8$ CFU) reveals mild pericarditis, myocardium showed mild degeneration with congestion, mild hemorrhage, vacuolation of cytoplasm, and leukocytic infiltration compared to control in Figure 2c.

Photomicrographs of the spleen reveal in Figure 3a, mild lymphoid depletion, congestion of splenic sinus, and hemorrhage with hemosiderosis in broilers challenged with magnesium chloride (100 mg/l) compared to control in Figure 3c. Broilers challenged with lead nitrate (350 mg/l) reveal in Figure 3b, mild lymphoid depletion with severe congestion of splenic sinus and severe hemorrhage compared to control in Figure 3c. The spleen of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU) reveals in Figure 3d, mild hemorrhage with hemosiderosis, while in broilers challenged with *S. Typhimurium* ($1.8 \times 10^8$ CFU) reveals in Figure (3e), mild depletion with mild hemorrhage compared to control in Figure 3c.

Bursa of Fabricius reveals in Figure 4a, mild lymphocytic depletion of lymphoid follicles in broilers challenged with magnesium chloride (100 mg/l) compared to control in Figure 4c. Bursa of Fabricius of broilers challenged with lead nitrate (350 mg/l) reveals in Figure 4b normal follicular epithelium with moderate lymphoid depletion of follicles compared to control in Figure 4c. Bursa of Fabricius of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU) revealed in Figure 4d normal follicular epithelium and lymphoid follicles compared to control in Figure 4c. Normal follicular epithelium with moderate lymphoid depletion of follicles was recorded in Figure 4e in the Bursa of Fabricius of broilers challenged with *S. Typhimurium* ($1.8 \times 10^8$ CFU) compared to control in Figure 4c.

**Discussion**

Water represents an important requirement for all living organisms, as well as affects immune functions and homeostasis in broiler chickens (More-Bayona *et al.*, 2020). Water absence shortened the survival time among the broiler chickens (Abbas *et al.*, 2010). Broilers have to be supplied with a safe and plentiful amount of water of good quality for its influences on food intake and digestion, body temperature, nutritive transportation, and waste excretion (Manwar *et al.*, 2012). Water quality is an inter-relational factor in
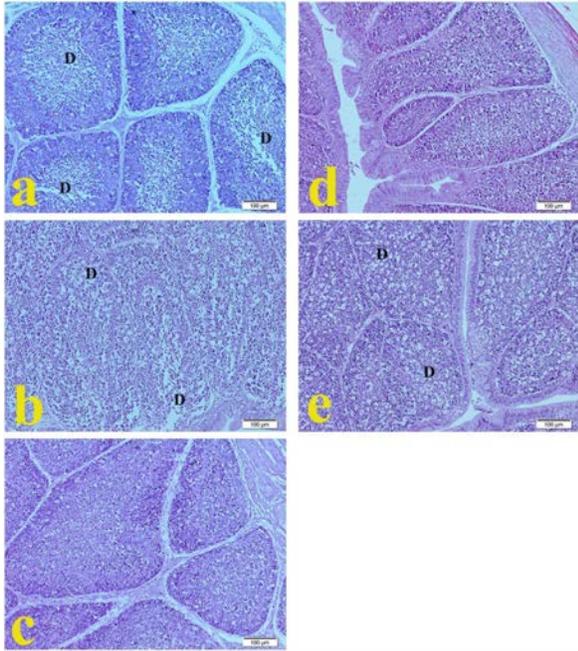
**Fig. 4.** Histopathological section of bursa of fabricius by HE staining **(20×)**: (a) bursa of fabricius of broilers challenged with magnesium chloride (100 mg/l) showing depletion of lymphocytes (D). (b) bursa of fabricius of broilers challenged with lead nitrate (350 mg/l). (c) bursa of fabricius of control-negative broiler chickens. (d) bursa of fabricius of broilers challenged with *E. coli* O157:H7 ($2.4 \times 10^{12}$ CFU). (e) bursa of fabricius of challenged with *Salmonella Typhimurium* ($1.8 \times 10^8$). **Bar 100 μm.**

the multi-factorial nature that influence broiler health including management procedures, type and design of the house, housing microclimatic conditions, and equipment (Abdullah, 2011). Recently many water contaminants had the opportunity to enter water bodies and streams contributing to water pollution (Goswami *et al.*, 2021). Water is a favorable medium for the growth and multiplication of many microorganisms and is mostly pathogenic with zoonotic significance. The comfort thermoneutral zone and low flow rates of water inside broiler housing usually encourage the growth and multiplication of micro-organisms, which is why many breeders tend to use some antibiotics in the water system to minimize microbial survival (Maharjan *et al.*, 2016; Maes *et al.*, 2019). The extensive use of antibiotics contributed to a resistance build-up in the micro-organisms causing diseases and potential eco-system imbalance (Becerra-Castro *et al.*, 2015).

Fish waste is known for its high content of water and enzymes making it very hazardous to the environment, which is why this waste has to be disposed of hygienically via burial or incineration (de Medeiros *et al.*, 2019). Fish waste can be better used for many applications such as fuel, medical, and water treatment. The medical application as reported by Júnior *et al.* (2020) and Ge *et al.* (2020) included burn injuries, reduce

pain, reduction of the dressing rate under anesthesia, and wound healing and all these uses were attributed to collagen and gelatin contents, fibroblast growth factor, and epidermal growth factor. The HAp derived from fish with all its active sites showed significant capabilities as filter media and efficient adsorbent (Liaw *et al.*, 2020). Tilapia bone is known for its natural content of some trace elements such as sodium (Na⁺), magnesium (Mg⁺²), zinc (Zn⁺²), and potassium (K⁺) giving the Tilapia fish bone advantageous importance for its use as a feed supplement and efficient adsorbent (Niakan *et al.*, 2015; Weeraphat *et al.*, 2016).

In this study, polluted water samples were treated using Tilapia bone powder to reduce pollutant levels which might contribute to harmful effects as they are non-biodegradable and can be accumulated in living tissues, causing various diseases and negative impacts on the environment. Tilapia bones can be used as a material for the removal or decrease of pollutants where Tilapia bone powder of 1% in this study reveals a highly significant 100% reduction in the lead after 1 hour, total and calcium hardness levels after 0.5 hours, as well as 100% killing efficacy against *E. coli* O1527:H7, *S. Typhimurium*, *M. gallisepticum*, *A. niger*, *T. mentagrophytes*, and *C. albicans* after 0.5, 1, 1, 1, 1, and 1 hours respectively. The results were compared with those of Omar *et al*. (2019) and Wang *et al*. (2017) who attributed the high adsorptive efficacy of Tilapia bones to the presence of HAp which processes hydroxyl (OH⁻) and phosphate ($PO_4^{3-}$) groups providing the capability to adsorb various heavy metal ions in water effectively. Alparslan *et al*. (2017), Marinah *et al*. (2017), and Othman and Zayadi (2016) also stated that HAp from fish bones and scales acts as a powerful adsorbent for heavy metals with special reference to lead from different sources as the presence of calcium ions (Ca²⁺) on HAp surface enhances the ion exchange between calcium and lead causing the high reduction of lead from the water. The action of HAp of Tilapia fish bone as an adsorbent as reported by Avram *et al*. (2017) is a multifactorial process including the initial lead concentrations, pH of the media, the microclimatic temperature, and the concentration of HAp ions. Ferri *et al*. (2019); Hao *et al*. (2017); Liu *et al*. (2017) showed that only 0.008 g of HAp is required to contribute to 99.5% adsorption of lead at an appropriate pH (2.2 to 5.0). The antimicrobial action of Tilapia bone was attributed to the shifting of the pH in the media making the conditions unfavorable for the growth and multiplication of micro-organisms. The current results revealed that Tilapia bone powder 1% treated water showed highly significant increases in the levels of dissolved oxygen and highly significant declines in physicochemical characteristics, electrolytes, heavy metals, and microbial parameters compared with tap water. Nasiebanda *et al*. (2020 and Nematidil and Sadeghi (2019) reported that fish bone can be considered an adsorbent of low cost for treating

wastewater and removal of some pollutants by up to 99%. Mitra *et al*. (2018) also showed that HAp biochar can be produced from Tilapia bone using carbonization at 550°C with high adsorptive power against heavy metals and dyes in water and wastewater. Olay *et al*. (2019) revealed the presence of a large amount of hydroxyl group in HAp derived from fish contributing to a significant increase of the active adsorbing sites for the heavy metals like lead, arsenic, copper, cadmium, and mercury with adsorption capability of 162.4 mg/g for copper and 128.7 mg/g for cadmium. Sricharoen *et al*. (2021) also reported a high adsorption capability of HAp derived from fish of up to 227.2 mg/g.

Broilers provided with drinking water treated with Tilapia bone powder 1% reveal highly significant increases in weight gains, PI, live body weights, carcasses, and organs weights, immunoglobulin concentrations, and antioxidant levels, as well as highly significant improvements in feed conversions, feed and water intakes, biochemical profile, cortisol hormone, and intestinal bacterial counts in all challenged groups compared to the control. The results were consistent with those of Kalita *et al*. (2021), Saidy *et al*. (2015), and Ibitoye *et al*. (2013) who reported that supplementing broilers with water treated using Tilapia bone contributed to significant increases in body weights and enhanced FCRs compared to the un-treated rain and well water. Jacobs *et al*. (2020) found that providing broiler chickens with sanitized and treated water using activated $ClO_2$ or some alternative biological and natural products could improve the body weights, carcasses quality, and the immune response in broiler chickens in clean or challenged microclimatic environment. Lee *et al*. (2022) reported the treating influence of Tilapia bone for the drinking and wastewater and producing water of high quality associated with immunostimulant and maintained hemostasis and they attributed these influences to the high content of Tilapia bone with HAp.

Thaib *et al*. (2021) evaluated triggerfish bone charcoal (1%, 2%, and 3%) influence on the performance and intestines of 240 Tilapia fingerlings and found that 3% charcoal contributed to enhanced length of intestinal villi up to 71.11 μm from the initial length and higher FCRs with the best feed efficiencies. Azhari *et al*. (2020) revealed that using fishbone as a feed supplement contributed to significant improvement in performance and blood profile (RBCs, WBCs, hemoglobin, platelets, and hematocrit) in Tilapia fish, as well as enhanced nutrient absorption and increase feed efficiency. Liao *et al*. (2020) recommended the use of peptides isolated from Tilapia bone as a supplement to enhance calcium absorption and in turn improve performance and production.

## Conclusion

The *in-vitro* activity of Tilapia bone powder 1% revealed achieving a 100% reduction in the levels of lead, total hardness, and calcium hardness after 1, 0.5, and 0.5 hours respectively, as well as achieved 100% killing efficacy against *E. coli* O1527:H7, *S. Typhimurium*, *M. gallisepticum*, *A. niger*, *T. mentagrophytes*, and *C. albicans* after 0.5, 1, 1, 1, 1, and 1 hours, respectively. Tilapia bone powder 1% enhanced the water's physicochemical characteristics by increasing dissolved oxygen levels and decreasing pH, electrical conductivity, total dissolved solids, total alkalinity, total hardness, sulfates, nitrates, electrolytes ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, and $Cl^-$), heavy metals ($Fe^{2+}$, $Cu^{2+}$, and $Zn^{2+}$), and microbial (total bacterial, *Enterobacteriaceae*, and *Salmonella* counts) levels.

The *in-vivo* study showed that Tilapia bone powder 1% revealed significant increases in weight gains, PI, live body weights, carcasses weights, organs weights, immunoglobulin concentrations, antioxidant parameters, as well as significant improvements in FCRs, feed and water intakes, biochemical parameters, and tissue architecture and cellular photomicrographs in all challenged groups. Tilapia bone powder 1% also revealed significant declines in cortisol hormone levels, total bacterial counts, *Enterobacteriaceae* counts, and *Salmonella* counts in all challenged and treated broiler groups.

## Authors' contributions

E.S.S. designed and executed the *in-vitro* and *in-vivo* studies' design, participated in the examinations (water analysis, biochemical profile, antioxidant analysis, and bacteriological examinations), provided a hand in the histopathological examination, and took a part in writing and reviewing the final edit of the manuscript. R.H.A. executed the PI recording and calculation, determined carcass and organ weights, participated in the biochemical and antioxidant analysis, and took a part in writing the manuscript. D.S.F. participated in the water examination, and biochemical and antioxidant analysis, and took a part in writing the manuscript.

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