

Effect of Different Intensities of Magnetized Water on Histological Characteristics and Growth Performance of the Sea Bream (*Sparus aurata*) Juveniles

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ABSTRACT

Applying magnetized water to marine aquaculture is still a new application, and studies are very scarce. Accordingly, our study was conducted on gilthead seabream, *Sparus aurata*, and juveniles to assess the impact of continuous exposure to magnetized treated water at various Tesla levels on water properties, as well as any subsequent consequences on juveniles' development, performance, and survival rate. A 60-day growth trial was carried out using magnetized treated water during the juvenile rearing period (from 60 to 120 days) with magnetized intensities corresponding to 0, 10, 40 and 60MT in the control, F₁, F₂ and F₃ treated groups, respectively. Each replicate treatment included 200 seabream juveniles. Determination of environmental factors, including water temperature, pH, salinity, and dissolved oxygen was carried out. The magnetization system was running around the clock. The findings showed that for 60 days, varying magnetic intensities had an impact on the survival rate of sea bream juveniles reared in magnetic water. At the end of the experiment, histopathological alterations were assessed between specimens of selected organs (intestine, liver, and kidney) collected from different fish juveniles treated groups. In this study, the magnetized water decreased bacterial load compared with the normal water (control) considering the antimicrobial efficacy of magnetized water. Overall, it was concluded that exposure of sea bream juveniles to magnetized water of intensity corresponding to 40MT represented the highest survival rate (51.67%), improved its growth performance and water properties, reduced microbial infected communities, as well as restored the normal architecture of intestinal and renal tissues in a recirculating healthy aquaculture system.

INTRODUCTION

Transparency, turbidity, water color, pH, hardness, carbon dioxide content, and unionized ammonia levels are all factors that influence water quality (Formicki *et al.*, 2021). Consequently, water treatment options such as exposing water to a magnetic field are used to improve water quality (Hassan & Rahman, 2016). In order to alter the structure of the water, magnetic water is created when it is exposed to a magnetic field (Brysiewicz & Formicki 2019; Abdelkhalek *et al.*, 2021). The biological characteristics of species that live in water and consume it will be affected by changes in the physical

and chemical features of magnetized water such as the rate of respiration, which will have an effect on their metabolic system. According to **Tyari *et al.* (2014)**, magnetized water increases the solubility of minerals, which improves the transfer of nutrients to all organs of the body. Overall, it has a positive impact on enhancing organism performance. Since magnetic therapy aids in the blood's transfer to the body's tissues, the organism becomes more alive and biologically active (**Habbas, 2005**).

Strong magnetic fields have been found in numerous studies to have altered the properties of cell membranes, cell metabolism, cell reproduction, and various other cellular functions like gene expression, protein biosynthesis, and enzyme activity (**Hassan *et al.*, 2018**). With respect to pH, total dissolved solids, conductivity, salinity, dissolved oxygen, temperature, minerals, organic matter, and total bacteria count, water quality dramatically improved when subjected to a magnetic field. Accordingly, its ability to penetrate cell walls would be enhanced, hastening the regular water diffusion required for the development of numerous organs (**Ebrahim & Azab, 2017**). According to numerous researchers such as **Khater and Ibraheim (2015)** and **Samiee and Samiee (2017)**, magnetic water may delay the onset of ageing and weariness by increasing the permeability of cell membranes.

Many living species including fish are affected by magnetic fields. Fish reactions are identified at many developmental stages, including embryonic, larval and adult development (**Brysiewicz & Formicki, 2019**). The Mediterranean regions produce a sizable amount of the highly valuable marine farmed fish known as the gilthead seabream (*Sparus aurata*) (**Tefalet *et al.*, 2023**). Due to the lack of literature about the effects of magnetic water on the aquaculture rearing of sea bream, its impact was reported on other farmed fish. **Tong *et al.* (2022)** recently investigated the possible use of static magnetic field (SMF) therapy in the preservation of sea bass that was exposed to SMF (5 MT) during cold storage; total viable counts, water holding capacity, pH, color, and textural qualities were examined. In the 45-day period of deteriorating conditions, **El-Sayed *et al.* (2022)** documented the impact of magnetized water on improving the larval aquaculture of the European sea bass.

In order to assess internal effects (nutrition) and exterior conditions (aqueous environment), histopathological analysis of fish tissues has been employed as a biomarker (**Bariiet *et al.*, 2018; Radwan *et al.*, 2023a**). The fact that histopathological biomarkers enable the assessment of certain target organs, such as the liver and kidneys, which are in charge of important activities, has led to their widespread application in environmental monitoring of fish (**Agamy 2012; El-Ghazaly *et al.*, 2017**). Additionally, the metabolic, physical health, and nutritional state of fish can be accurately determined by the histological analysis of their digestive systems (**Abdelkhalek *et al.*, 2021**). Only a few studies on different fish species have been done in aquaculture despite the fact that magnetic water has been successfully used in a variety of industries. According to certain authors' reports (**Nofouzi *et al.*, 2016; Hassan *et al.*, 2018**), the magnetic water treatment has favorable effects on fish development and water quality. Others revealed no impact of using the magnetic water on fish development or water quality (**Hassan *et al.*, 2019**).

As previously mentioned, few articles have been published recently using magnetic water treatments on sea bream survival and growth. In addition, the field of subjecting sea bream raised under different magnetic intensities is still a new approach and need

more research. Consequently, the current study's goal was to investigate the effects of utilizing a magnetic field with different intensities (10, 40, 60M Tesla) on some water quality parameters, growth performance, survival rate and microbial load of sea bream larvae during the juveniles rearing period. Furthermore, this study was carried out to examine the effect of these magnetized water intensities on the histopathological alterations that occurred in intestine, liver and kidney of sea bream juveniles drinking it.

MATERIALS AND METHODS

Experimental design

The hands-on activities were carried out at the marine hatchery of the National Institute of Oceanography and Fisheries (NIOF) in Alexandria, Egypt. In the control, F₁, F₂, and F₃ groups, respectively, a set of magnets with intensities of 0, 10, 40, and 60MT was used. One application of the magnetic flux was all that was needed to make the water magnetic. The goal of the experiment was to determine how different magnetized waters affected sea bream juvenile stage growth performance and condition parameters after 60 days in the hatchery. Twelve 100-liters round fiberglass containers with a capacity of 200 juvenile sea bream with an initial weight of 0.32 to 0.35g each were filled. The magnetic devices were used to magnetize the water for 60 days.

Each juvenile sea bream's total length was measured using an ocular micrometer, with a minimum precision of 0.5mm. The mono-pan balance was used to measure juvenile weight with an accuracy of 0.01mg. The following equations were used to calculate growth metrics, such as length gain (LG), weight gain (WG), and specific growth rate (SGR), as follows:

Weight gain (WG) (g/juvenile) = FW - IW

Where, IW initial mean weight of fish in g, and FW: final body weight of fish in g

Length gain (LG) (cm juvenile⁻¹)

Final mean length of juvenile (FL) in cm - Initial mean length of juvenile (IL) in mm

Specific growth rate (SGR) (%/ day) = 100 × (ln W_t - ln W₀)/ day

Where, ln: Natural logarithm

Average daily weight gain (ADWG) (g juvenile⁻¹ day⁻¹)

Final weight (FW) (g) - Initial weight (IW) (mg) / experimental period (days)

Survival percentage (S %) = {(No. of fish at end / No, of fish at the start)} × 100

Condition factor: (k-value) = (body weight. g) / (body length, L³) × 100

Proximate composition analysis

Upon completion of the trial, the biochemical composition of the examined sea bream juvenile from the different magnetic strengths (3 juveniles per treatment) was determined following **AOAC (2000)** standard method regarding crude protein, crude lipid, carbohydrate, crude ash and dry matter contents.

Magnetized water and condition factors

Water quality parameters were measured, included salinity (SAL) ppt, water temperature, pH and dissolved oxygen concentration (DO) mg L⁻¹(YSI ECO Sense® 9300 photometer, England).

Microbial analysis

The same tanks' ten fish/ aquaria were aseptically sampled alive using the three different magnetic strengths and placed in sterile bags. Fish mid-guts samples were treated with saline solution and a series of dilutions performed. After being incubated at 37°C for 24- 48 hours, one milliliter of each dilution of the fish, and water samples was inoculated on the selective media, and the viable aerobic bacterial counts were assessed using the pour plate method (APHA, 2005). Thiosulfate-citrate-bile salts-sucrose agar (TCBS) was used to isolate *Vibrio* species, while *Salmonella-Shigella* (SS) agar was used to distinguish between different strains of *Shigella* spp. and *Salmonella* spp. *Aeromonas hydrophila* is grown on base agar with ampicillin in the *Aeromonas* isolation medium. *Staphylococcus aureus* was grown using the selective and differentiating medium mannitol salt base agar (MSA).

Histological investigations

Six juveniles from each replica (two tanks per treatment) were randomly chosen out of the over 36 experimental sea bream specimens at the trial's end to undergo histological study, while the remaining specimens were maintained for further research. Fish juveniles' gut, liver, and kidney specimens were used for light microscopy (LM), while small pieces (5µm) were preserved in 10% neutral buffered formalin for 24 hours. Samples of the liver, kidney, and intestine were then dehydrated in a series of ethanol solutions, cleaned by methyl benzoate, and finally embedded in paraffin then stained with Hematoxylin and Eosin in accordance with standard histological procedures that is outlined by Radwan *et al.* (2023b).

Statistical analysis

SAS ANOVA was used to statistically analyze the data (SPSS Statistics 17.0). To ensure homoscedasticity; the assay data were subjected to the Bartlett test. There were no variations in homogeneity in the data. The data were subsequently subjected to a one-way classification variance analysis. When significant F values were noticed (Duncan, 1955), Duncan's multiple range test was performed to assess differences between treatment means at the $P \leq 0.05$ level.

RESULTS

1. Improving the survival and growth rates of the sea bream juveniles using different waves of magnetized water

1.1. Growth performance

Evaluation of using different magnetized water on the growth and survival growth rate of the sea bream juveniles are shown in Table (1). No significant differences ($P < 0.05$) were observed in IW among treatments. The measurements made on sea bream juveniles subjected to a 40ml Tesla magnetic field revealed the best growth performance metrics (FW, WG, ADG, SGR) with the following values: 1.04 ± 4.04 g/ fish, 0.70 ± 0.02 g/ fish, 0.011 ± 0.02 g/ fish/ day, 2.18 ± 0.02 %/ day, respectively. However, the lowest value was observed in juveniles' control, with the following results: FW 0.75 ± 4.04 g/ fish, WG

0.43 ± 0.04 g/ fish, ADG 0.009 ± 0.06 g/ fish/ day, and SGR 0.38 ± 0.01 %/ day, respectively.

1.2. Health condition

The results in (Table 1) indicated that condition factor (K) values ranged between 0.65 ± 0.04 and 0.87 ± 0.01 , with significant differences between juveniles treatments. FL (cm) measurements revealed the following values: 5.09 ± 1.09 for sea bream juveniles exposed to 40ml Tesla magnetic field, 3.42 ± 0.02 for fry exposed to 10.00ml Tesla magnetic field, 3.38 ± 0.04 for juveniles exposed to 60.00ml Tesla magnetic field, and 2.72 ± 0.04 for juveniles control. The highest condition factor (4.380.79) was observed in the data acquired from juvenile sea bream exposed to 60.00ml Tesla magnetic field, followed by juveniles group exposed to 40.00ml Tesla magnetic field (4.84 ± 0.69). Moreover, the condition factor (k value) for the control group of sea bream juveniles was 4.42 ± 0.53 , indicating a significant difference.

1.3. Survival%

In Table (1), the impact of magnetic fields on sea bream juveniles of various magnetic intensities is obvious. The information received demonstrated substantial ($P < 0.01$) variations across all treatments. In the control group, it was recorded that sea bream survival rate was equal to 41.00 ± 1.14 %. Besides, the highest survival was influenced by the magnetic intensity of 40.00MT which was 51.67 ± 1.33 %. In addition, the percentage of surviving under magnetic intensity of 10.00MT was 44.72 ± 1.24 %, while the lowest percentage of surviving 38.32 ± 1.28 % was recorded at the magnetic intensity of 60.00MT.

2. Effect of the magnetized water on sea bream juveniles' body composition%

The juvenile sea bream's body composition analysis is displayed in Table (2) (as DM basis) on crude protein, crude lipid, and ash %. Analysis of crude protein % revealed significantly ($P < 0.01$) higher levels for larvae exposed to F₂ (40MT) with a value of 61.11 ± 0.16 %, followed by larvae exposed to F₁ (10MT) and F₃ (60MT) with values of 60.14 ± 0.56 % and 58.22 ± 0.10 , respectively, compared to the lowest values noticed in the typical water control (56.12 ± 0.06 %). The highest crude lipid % value was recorded for juveniles in normal water (19.2 ± 0.06 %), followed by juveniles exposed to F₂ (40MT) and F₃ (60.00MT) compared to the lowest value for the juveniles exposed to F₁ (10 MT), with value of 18.02 ± 0.07 . Among different treatments, there was no discernible variation in the percentage of carbohydrates and the percentage of ash.

Table 1. Effect of the magnetized water on the growth performance and condition factor of sea bream juveniles from 60 : 120 days

Treatment	Intensity of magnetized water			
	0 MT	10 MT	40 MT	60 MT
IW (g/fish LK)	0.32±0.46	0.35±0.54	0.34±0.44	0.33±0.82
FW (g/fish)	0.75±4.04 ^d	0.95±2.02 ^b	1.04±4.04 ^a	0.88±3.04 ^c
WG (g/fish)	0.43±0.04 ^d	0.60±0.03 ^b	0.70±0.02 ^a	0.55±0.04 ^c
ADG (g/fish /day)	0.007±0.00 ^c	0.01±0.04 ^b	0.011±0.02 ^a	0.009±0.06 ^b
SGR (%/day)	0.38±0.01 ^d	2.05±0.01 ^b	2.18±0.02 ^a	1.42±0.01 ^c
IL (cm/fish)	1.26±0.01	1.33±0.01	1.43±0.02	1.30±0.01
FL (cm/fish)	4.42±0.53 ^c	4.84±0.69 ^b	5.09±1.09 ^a	4.38±0.79 ^d
LG (cm/fish)	3.16±0.03 ^c	3.51±0.09 ^b	3.66±0.07 ^a	3.08±0.06 ^d
k value	0.87±0.01 ^c	0.84±0.05 ^b	0.79±0.05 ^b	0.65±0.04 ^a
Survival (%)	41.00±1.14 ^c	44.72±1.24 ^b	51.67±1.33 ^a	38.32±1.28 ^d

IW: Initial mean weight, FW: Final body weight, WG: Weight gain, ADWG: Average daily weight gain, SGR: Specific growth rate, IL: Initial mean length, FL: Final mean length, LG: Length gain. Different superscript letters are significantly different ($P \leq 0.05$) in the same column.

Table 2. Effect of the magnetized water on body composition % of sea bream juveniles (DM basis) during juveniles rearing period from 60 : 120 days

Treatment	Intensity of magnetized water			
	0 MT	10 MT	40 MT	60 MT
Crude protein (%)	56.12±0.06 ^d	60.14±0.56 ^b	61.11±0.16 ^a	58.22±0.10 ^c
Crude lipid (%)	19.2±0.06 ^a	18.02±0.07 ^d	18.58±0.06 ^b	18.45±0.09 ^c
Carbohydrate (%)	8.96±0.02 ^a	6.16±0.02 ^c	6.12±0.02 ^d	7.32±0.02 ^b
Crude ash (%)	2.56±0.04 ^a	2.32±0.03 ^c	2.23±0.01 ^e	2.52±0.03 ^b
Dry Matter (%)	24.86±0.12 ^a	24.15±0.05 ^d	24.64±0.13 ^c	24.85±0.60 ^b

Different superscript letters are significantly different ($P \leq 0.05$) in the same column.

3. Physicochemical Parameters of water in fish tanks

The water temperature was between 18.72 and 19.01°C during the experiment, while the salinity was between 39.34 and 39.44ppt. Furthermore, it was noted that the pH and the dissolved oxygen showed a slight increase in their levels among the different experimental magnetic intensities groups ranging from 7.60– 7.75 and 8.15– 8.68mg/ L, respectively, as shown in Table (3).

Table 3. Effect of exposure of magnetic field on some physicochemical properties of water during sea bream juveniles rearing period from 60 : 120 days

Treatment	Intensity of magnetized water			
	0 MT	10 MT	40 MT	60 MT
Salinity (ppt)	39.44±0.05 ^e	39.43±0.04 ^a	39.34±0.02 ^d	39.39±0.07 ^c
Water Temperature (°C)	19.01±0.02 ^a	18.85±0.02 ^b	18.72±0.00 ^d	19.00±0.02 ^a
pH	7.60±0.02 ^a	7.68±0.02 ^e	7.69±0.02 ^d	7.75±0.00 ^b
D.O ² (ppm)	8.15±0.03 ^e	8.42±0.04 ^c	8.63±0.04 ^a	8.68±0.03 ^d

Different superscript letters are significantly different ($P \leq 0.05$) in the same column.

4. Microbial analysis for both sea bream juvenile's body and magnetized water

Fig. (1A) shows the microbiological examination of sea bream juveniles being reared in magnetized water. The findings revealed that the highest *Aeromonas* sp. count was detected in the normal water control with value of $1.23 \pm 1.45 \times 10^2$ CFU/ ml, followed by the juveniles exposed to F₃ (60ml Tesla) with values of $0.24 \pm 1.20 \times 10^2$ CFU/ ml, and no count was observed in the juveniles exposed to F₁ and F₂. The highest *Shigella* sp. value was detected in the control ($9.30 \pm 25.17 \times 10^2$ CFU/ ml), however complete bacterial removal was observed in the juveniles exposed to the rest of treatments. The highest *Staphylococcus* sp. count was in the normal water ($15.71 \pm 123.88 \times 10^2$ CFU/ ml), followed by the juveniles exposed to F₃ (60ml Tesla) with values of $4.22 \pm 1.45 \times 10^2$ CFU/ ml. Then, F₁ (10ml Tesla) was recorded with a value of $26.74 \pm 3.48 \times 10^2$ CFU/ ml, and the lowest count was observed in the juveniles exposed to F₂ (40ml Tesla) with a value of $7.51 \pm 1.00 \times 10^2$ CFU/ ml. The highest *Vibrio* sp. count in control water was assessed with a value of $40.17 \pm 16.67 \times 10^2$ CFU/ ml, followed by the *Vibrio* count of juveniles exposed to F₃ and F₁ with values of 3.74 ± 2.65 and $15.74 \pm 2.91 \times 10^2$ CFU/ ml, respectively, while no growth was shown in F₂. Sea bream juveniles exposed to regular water experienced a significant increase in the total bacterial count with a value of $860.26 \pm 14.45 \times 10^2$ CFU/ ml, followed by F₁ (10ml Tesla), F₃ (60ml Tesla), and F₂ (40ml Tesla) with values of 510.79 ± 668.33 , 26.47 ± 27.01 , and $26.26 \pm 2.89 \times 10^2$ CFU/ ml, respectively.

The microbiological analysis for the magnetized water of sea bream juveniles is illustrated in Fig (1B). The results showed that, no data were recorded for both *Shigella* and *Aeromonas* sp. within all treatments. The highest counts for both *Staphylococcus* sp. and *Vibrio* sp. were detected in the normal water with a value of $5.00 \pm 1.15 \times 10^2$ CFU/ ml, while no growth was observed in F₁, F₂, and F₃ treatments. The total bacterial count in normal water was $33.72 \pm 1.45 \times 10^2$ CFU/ml followed by that juvenile exposed to F₁ (10ml Tesla) and F₃ (60ml Tesla) with values of 19.73 ± 1.53 and $13.46 \pm 3.06 \times 10^2$ CFU/ ml, respectively, compared the lowest value observed in F₂ (40ml Tesla), having a value of $5.74 \pm 2.08 \times 10^2$ CFU/ ml.

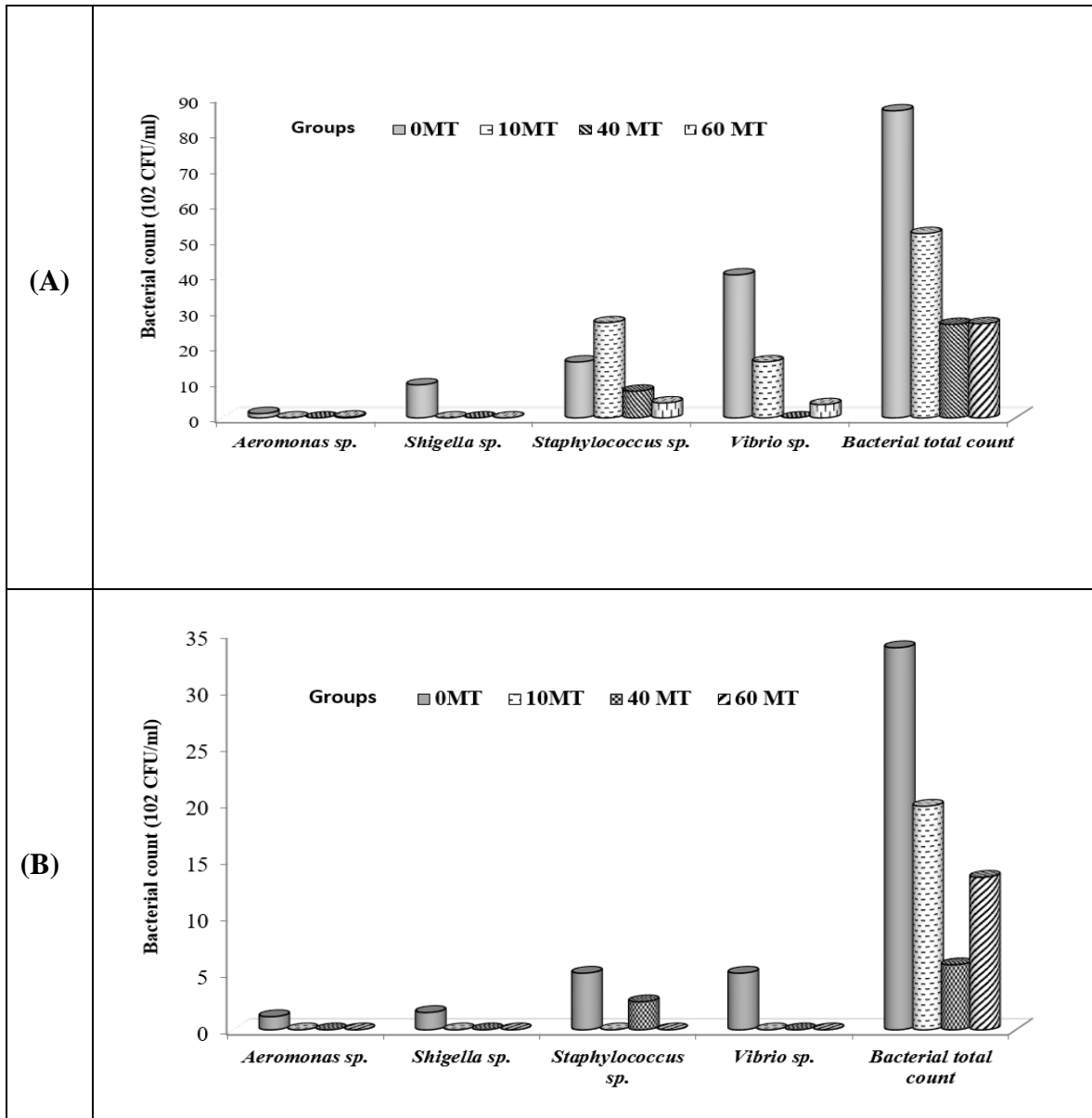


Fig. 1. Microbiological analysis for sea bream showing: (A) Juvenile's body rearing, and (B) The magnetized water

5.1. Macroscopic study

It is worth to mention that, the macroscopic observation of sea bream juveniles performed during sampling of this study did not reveal any macroscopic abnormality or clinical sign.

5.2. Microscopic studies

5.2.1 Histological studies of intestine

Intestine of control group

The basic organization of the intestinal wall in the control group was made up of the mucosa, lamina propria-submucosa, tunica muscularis, and outermost serosa. Intestinal villi, or longitudinal folds, were seen on the mucosal surface. An inner circular and an outer longitudinal layer of smooth muscle made up the tunica muscularis (Fig. 2A).

Intestine of treated groups

For sea bream juveniles exposed to 10MT in F₁ group: Intestine of sea bream juveniles showed a beginning of hyperplasia of goblet cells, shrinkage of lamina propria (LP) and rupture of the tip of villi. Hypertrophy of submucosa (H) layer was also noted (Fig. 2B). However, intestine of seabream juveniles of F₂ group exposed to 40MT demonstrated a typical structure and normal intestinal wall structure including a longitudinal villi with goblet cells that are activated (Fig. 2C). Moreover, the severity and frequency of these histopathological changes were observed in the intestine of sea bream juveniles of F₃ group exposed to 60MT including shrinkage and disintegration of the intestinal tissue, missing and fusion or flattening of villi with hyperplasia in some regions and disintegration of mucosa layer in other areas. Furthermore, some intestinal specimens showed disintegration and in some cases massive atrophy of lamina propria and presence of cellular debris (Fig. 2D).

5.2.2 Histological studies of Liver

Liver of control group

Histological sections of hepatic tissue from control sea bream juveniles are illustrated in Fig. (3A). The liver of the control fish showed no histological alterations, and the hepatic parenchyma displays a consistent distribution of hepatocytes grouped around the circulatory system (sinusoids).

Liver of treated groups

Concerning seabream juveniles exposed to 10MT in F₁ group, the histological sections of liver belonging to this group showed vacuolar degeneration and vacuoles in most of hepatocytes, infiltration of liver parenchyma, focal necrosis and hepatocytes degeneration (Fig. 3B). At 40MT the liver sections of this F₂ group represented fat vacuoles fusion, fatty degeneration, hyperaemia represented by congested portal vein containing red blood cells, narrowing in blood sinusoids, hydropic degenerated hepatocytes possessing nuclei with clumped chromatin (Fig. 3C). In addition, the hepatic nuclei of hepatocytes appeared either swollen or pyknotic. It is important to note that, the histopathological alterations were more obvious in the liver sections of sea bream juveniles of F₃ group exposed to 60MT, in which its liver parenchyma architecture was distorted (Fig. 3D). These changes were seen as hemorrhage, dilatation, and thrombus development in the central vein, with the majority of hepatocytes exhibiting the most severe vacuolar degeneration. Furthermore, the appearance of blood streaks amongst

hepatocytes of this group as a marked change due to necrosis of vascular impairment is evident. The presence of melanomacrophage centers was also noted.

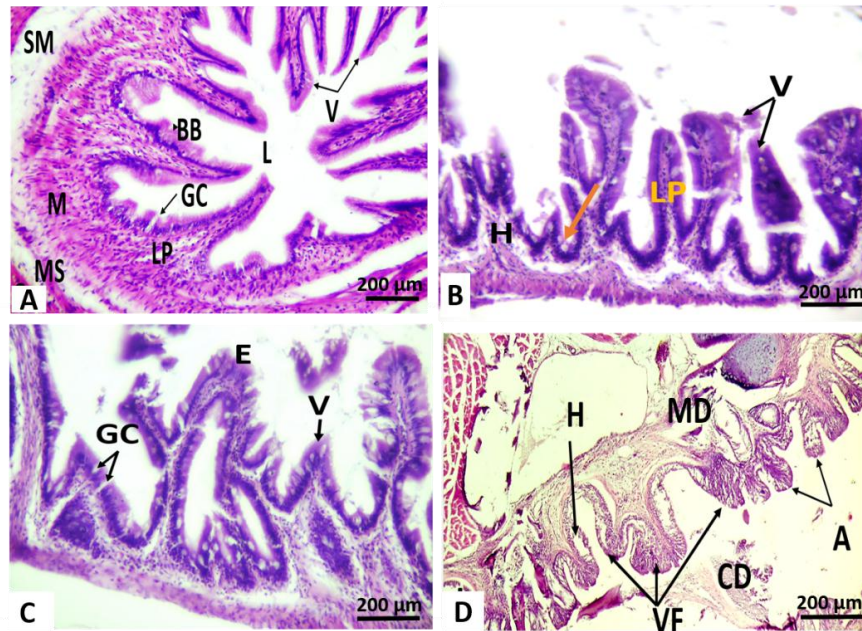


Fig. 2. Photo micrographs of TS in intestine of sea bream juvenile showing: **A)** Control group with typical structure of (M) mucosa, (LP) lamina propria, (SM) submucosa, (MS) tunica muscularis layers, (GC) goblet cells, (V) well-formed villi, (BB) brush border cells, and (L) lumen. **B)** F₁ group exposed to 10MT demonstrating hyperplasia of goblet cells (orange arrow), (LP) shrinkage of lamina propria, (V) rupture of villi tip, (H) hypertrophy of submucosa layer. **C)** F₂ group exposed to 40MT showing normal organization of intestinal wall, (E) longitudinal villi with simple columnar epithelium. **D)** F₃ group exposed to 60MT representing (VF) fusion and flattening of villi, (H) hyperplasia of goblet cells, (MD) disintegration of mucosa layer, (A) atrophy of lamina propria, and (CD) cellular debris. (H&E stain was used)

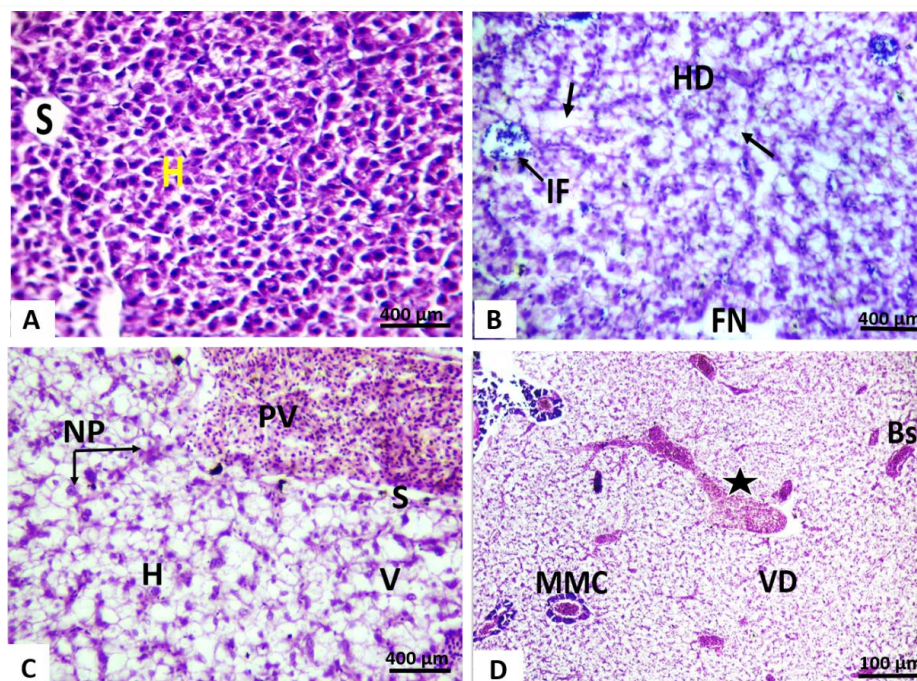


Fig. 3. Photomicrographs of liver of sea bream juvenile representing: **A)** Control liver showing normal (H) hepatocytes and (S) sinusoids. **B)** F₁ group exposed to 10MT showing vacuolar degeneration and fatty changes (black arrows), (IF) infiltration of liver parenchyma, (FN) focal necrosis, and (HD) hepatocytes degeneration. **C)** F₂ group exposed to 40MT demonstrating (V) fat vacuoles fusion, marked dilation in (PV) portal vein, (S) narrowing in blood sinusoids, (H) hydropic degenerated hepatocytes, and (NP) pyknotic nuclei. **D)** F₃ group exposed to 60MT illustrating dilation and thrombosis in central vein (star), (VD) vacuolar degeneration, (BS) blood streaks, and (MMC) melanomacrophage centers. (H&E stain was used).

5.2.3 Histological studies of kidney

Kidney of control group

The control fish's kidney showed no histopathological alterations. Similar to other teleosts, the kidney's fundamental unit is made up of a renal corpuscle, Bowman's capsule, a glomerulus, and numerous renal tubule segments. Well-formed distal and proximal renal tubules made of epithelial cells and encircled by interstitial lymphoid-hematopoietic tissue serve as a representation of these tubules (Fig. 4A).

Kidney of treated groups

Seabream juveniles of F₁ group exposed to 10MT showed shrinkage of proximal and distal renal tubules, thinning of tubular epithelium, lifting of tubular lining epithelium from the basement membrane and appearance of fibrinoid deposition around tubules (Fig. 4B). Concerning sea bream juveniles of F₂ group exposed to 40MT, micrographs of its kidneys illustrated restoration of epithelial cells lining of the renal tubules and restoration of the interstitial hematopoietic tissue (Fig. 4C). For seabream juveniles of F₃ group exposed to 60MT, the kidneys micrographs of this group represented structural and

architectural alterations with hyperaemia. Infiltration, necrosis and reduction of the hematopoietic tissue were observed. Degeneration of renal tubules and appearance of fibrinoid deposition that surrounds the tubules were also noted. Moreover, other kidney tubules were swollen, ruptured and missing in many places (Fig. 4D).

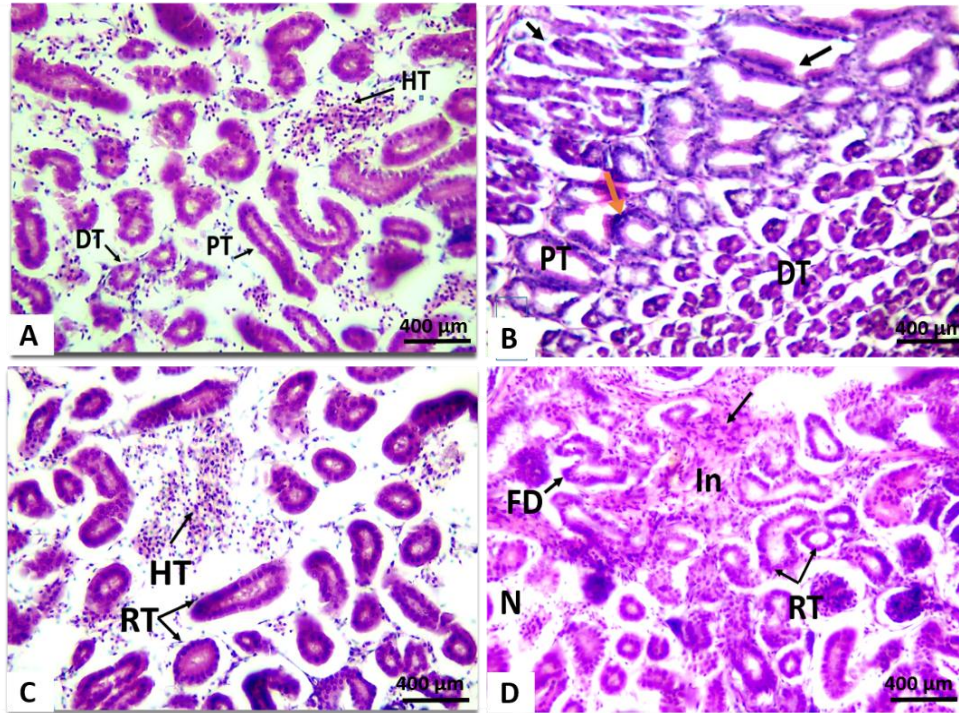


Fig. 4. Photomicrographs of kidney of sea bream juvenile showing: **A)** Normal kidney in control group with well-formed (PT) proximal renal tubules, (DT) distal renal tubules, and (HT) hematopoietic interstitial tissue. **B)** F₁ group exposed to 10MT representing thinning and lifting of tubular lining epithelium (black arrows), appearance of fibrinoid deposition around tubules (orange arrow). **C)** F₂ group exposed to 40MT illustrating restoration of epithelial cells lining (RT) renal tubules and (HT) hematopoietic tissue. **D)** F₃ group exposed to 60MT demonstrating architectural alterations with (In) infiltration and (N) necrosis of the hematopoietic tissue, (RT) degeneration of renal tubules, appearance of (FD) fibrinoid deposition, kidney with hyperaemia (black arrows). (H&E stain was used)

DISCUSSION

To the best of the authors' knowledge, this study is the first to look into how employing various intensities of magnetized water affect the upbringing of sea bream juveniles (*Sparus aurata*). Hence, we discussed our results with other relevant studies on other marine fish due to the lack of literature about the effects of magnetized water intensities during the aquaculture rearing of sea bream.

1. Survival rate of seabream juveniles

The maximum survival rate of sea bream juveniles in the second treatment was 51.67%, which is considered to be higher than the survival rate of the larvae of the

European sheat fish (*Silurus glanis*) treated with the magnetized water in a RAS, reaching 80.9% (Krziemiewski *et al.*, 2004). However, Hassan *et al.* (2019) reported that, the survival rate of Jade Perch (*Scortum barcoo*) juveniles reared in a recirculating aquaculture system under the exposure of different magnetic field intensities was 88. The longer exposure time, higher field activity, and varying species sensitivity in this study may have contributed to the higher survival rate (Irhayyim *et al.*, 2020). On the other hand, Hassan *et al.* (2018) demonstrated that the survival rate was unaffected by the level of magnetized water by studying the effects of continuous exposure to magnetized water on the red hybrid tilapia (*Oreochromis* sp.) over the course of 70 days using various water intensities of Tesla. Additionally, Hassan *et al.* (2019) reported that juvenile Jade Perch (*Scortum barcoo*) quadruplicate treatments exposed to continuous magnetized water at 0.00, 0.10, 0.15, and 0.20 Tesla in a recirculating system had no effect on survival rates. Additionally, according to Fey *et al.* (2019), neither static nor time-varying MFs appear to have an impact on larval survival.

2. Effect of magnetic field exposure on the characteristics of water

According to Hochachka and Lutz (2001), the magnetic intensity may be the cause of physicochemical parameter changes in water and the crucial parameters needed for all aerobic metabolism processes in all living things. This is especially true for the respiratory process, which uses protein, lipid, and carbohydrate sources to produce energy. Dissolved oxygen plays a key function in the respiratory process that produces energy; it may also have an impact on how fish use protein or energy to grow (Ayoola & Kuton, 2009). Jabir (2019) used magnetized treated water of 0.1T in the first treatment and 1.5T in the second treatment to investigate the impact of magnetized water on the growth of the common carp *Cyprinus carpio*. Using increasing amounts of magnetized water strength, Hassan *et al.* (2018) conducted a 70-day study to evaluate the effects of continuous magnetized water exposure on the red hybrid tilapia (*Oreochromis* sp.). They noticed that as magnetic intensity increased from 0.00- 0.20T, water characteristics like pH showed a small improvement but dissolved oxygen and salt concentration remained within the normal range. Aligning with the study of Ahmed and Abd El-Hamed (2020), magnetic water had higher DO and pH levels than control water. According to a research by Hassan *et al.* (2019), magnetized water had a slight improvement in water quality measures like DO, which was also simpler to maintain than control water. Mahmoud *et al.* (2019) reported similar findings in earlier investigations. According to Yacout *et al.* (2015), the decrease in organic matter in magnetic water is what led to the increase in DO. Due to salt dissociation brought on by a magnetic field, high pH may be associated with a rise in the amount of free carbonate in water (Alabdraba *et al.*, 2013). The results from Irhayyim *et al.* (2020), who investigated the impact of magnetized water on common carp (*Cyprinus carpio* L.) development and water quality parameters in six separate integrated recirculating aquaculture systems, disagree with the ones from the present study. They stated that there was no variation in temperature, pH, or DO means between the treatments that was statistically significant ($P > 0.05$). It was discovered that the salinity of the water in the current study falls within the same range for all treatments as the control group. Hassan and Rahman (2016), in contrast, demonstrated a general pattern of rising water salinity with magnetization times. According to Oyugi *et al.* (2012), the water temperature in the fish tanks was generally around 24°C, which is

between the ideal temperatures for common carp feeding and growth (24 to 28°C). This finding is consistent with the findings of the current study.

3. Microbial assays

In this study, the magnetized water decreased bacterial load compared with the normal water (control). All pathogenic bacteria species including *Aeromonas* sp., *Shigella* sp., *Staphylococcus* sp., and *Vibrio* sp. showed lower or no count in almost treatment considering antimicrobial efficacy of magnetized water. *Staphylococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli* were subjected to a magnetic field exposure experiment utilizing ferrite magnets to study the biological effects of magnetic fields. The results demonstrated that when *S. mutans* and *S. aureus* were cultivated under anaerobic conditions, the ferrite magnet induced a strength-dependent decrease in the growth rate and growth of the maximum number of bacteria (Kohno *et al.*, 2000). Another study by Goyal *et al.* (2017) supports the same theory, showing that since magnetized water is alkaline and *S. mutans* is an anaerobic bacteria, its alkaline nature prevents the anaerobic bacteria from growing, hence reducing the count.

4. Histological findings

The present histological findings of the control group revealed that the characteristics of the intestinal layers previously reported by Abdel Mohsen *et al.* (2018) and Wassef *et al.* (2020) are consistent with the intestinal structure of the gilthead sea bream (*Sparus aurata*). The mucus-producing goblet cells, the cellularity of the lamina propria, the amount of connective tissue, the degree of mucosal folding, and the infiltration of the epithelium or lamina propria by inflammatory cells are frequently altered in the histopathological alterations of the intestine (Hernández *et al.*, 2012; Couto *et al.*, 2015). Histopathological changes in the gut described in the current study demonstrated unequivocally that magnetic water has harmful effects on the various layers of the intestine. These changes were more severe at higher tesla intensities and included degenerative mucosal epithelial changes (hypertrophy, vacuolation, cellular debris, and necrosis of submucosa), loss of structural integrity of mucosal folds, and flattening, fusion, and rupture of villi tips. According to Abdelkhalek *et al.* (2021), intestinal histomorphometric analyses of the Nile tilapia (*Oreochromis niloticus*) significantly improved ($P < 0.01$) in magnetic water groups treated with magnetic Tesla at 0.2 compared to the water of the control group. The present results regarding the improvement of the intestine in F₂ group with normal organization of intestinal wall and longitudinal villi are in agreement with their findings. At the end of the trial after eight weeks, they found that the treated groups had a larger abundance of intraepithelial lymphocytes and goblet cells, as well as the biggest growth in villi and epithelial lengths. This improvement in growth performance and fish health status is accompanied by an improvement in the intestinal absorption of nutrients (Pirat *et al.*, 2011). Water becomes even more essential as a result, improving digestive system performance and removing many chemical pollutants and germs (Mushattat *et al.*, 2009). At the same time, it improves blood flow to various body organs and cell types (Pang 2005), activating the gut immune system's defense mechanisms. Due to its function, location, and blood supply, fish liver histology may be used as a model to explore the connections between environmental factors and hepatic architecture and functions (Maharajan *et al.*, 2016). In line with Hassan *et al.* (2019), the influence of continuous magnetic field exposure on the liver histopathology of Jade Perch *Scortum barcoo* in the control and 0.10T groups

exhibits a normal architecture with a typical parenchymatous appearance and no pathological abnormalities. The current histological results of the control liver support these findings.

The current study found that higher magnetic water intensity (F_3) exhibits greater modifications than lower magnetic water intensities (F_1 and F_2). Major degenerative changes in the current histological results included necrosis, vacuolar, and nuclear degeneration. The gap between neighboring hepatocytes seen in the livers of treated juveniles is likely connected to cell necrosis since "necrosis" is given the highest importance factor since it is thought to be a direct effect associated to the exposure to magnetic water. This separation also points to structural proteins in the hepatocyte membrane, which are typically responsible for keeping the hepatic parenchyma a compact, homogeneous tissue (**Agamy, 2012**). The liver parenchyma architecture was disrupted in the fish treated with the F_3 group, resulting in more severe hepatocyte separation. The nuclear pyknosis and hepatocyte necrosis found in the current hepatic preparations were typically evident in the F_1 and F_3 treatment groups. In a 150-day study, **Hassan et al. (2019)** exposed juvenile Jade Perch in quadruplicate treatments to different magnetized waters continuously. They noted clogged blood vessels, an inflammatory response based on white blood cell infiltrations, and lipofuscin-like material in the livers of fish exposed to a 0.15T treatment. According to **Agius and Roberts (2003)**, this happened as a result of an accumulation of oxidized lipids brought on by the liver's reduced function. According to **Kamarudinet al. (2018)**, DNA damage and interference with cellular communication were proposed as potential causes of necrosis and hyperplasia, respectively. The integrity of the cellular membranes being compromised is another potential scenario (**Hassan et al., 2019**). Furthermore, the current alterations discovered by **Maharajan et al. (2016)** are supported by those seen in the histological sections of the treated liver in the three experimental groups, particularly in the F_3 group. These changes included atrophy, disappearance of the hepatocytes' cell wall, and arrangement of the hepatic cords.

In line with **Thophon et al. (2003)**, the teleostean kidney that receives the majority of postbranchial blood showed changes that were found in the kidneys of the sea bream juveniles from the F_1 group. These changes were shown by the moderate loss of interstitial hemopoietic tissue, tubular contraction, and degeneration of renal tubular epithelial cells. The kidneys in the F_3 group that were subjected to higher tesla intensity (60MT) had more reduction of the interstitial hematopoietic tissue, as well as a significant necrosis and atrophy of tubular degeneration. Tubular resorption may be hampered as a result of the tubules' reduced lumen (**Bhatnagar et al., 2007**). The current findings showed that magnetic exposure increased the frequency and severity of histological abnormalities in the cortical region of the kidney compared to the control. According to **Khater and Ibraheim (2016)**, this may be explained by the effect of magnetic fields on the hydration of ions in exposed water, which alters the way molecules are arranged around charged ions in cells, leading to disruption and deformation of tissue cells.

CONCLUSION

This research would help scientists better understand how magnetism affects water characteristics and the biology of cultured organisms like sea bream. Additionally, as improved growth and survival rates would increase and fish seed supply would decrease, the study's findings have favorable effects on aquaculture. The results showed that growth performance, survival rate, and water quality parameters of the magnetic water technique all significantly improved with prolonged exposure to magnetized water in the second treatment using moderate magnetized water intensity equivalent to 40m Tesla. Microbial burden was decreased. In order to improve the growth, biology, and cost-effectiveness of sea bream production within 60 days of exposure, the use of 40MT is advised. The modest tested T level equivalent to 40MT revealed mild hepatic histopathological abnormalities with a significant restoration of intestinal and renal tissues compared to the control group.

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