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Synergistic interactions of zeolite, stocking density, and water exchange: A holistic approach to optimizing aquaculture performance of juvenile European seabass (*Dicentrarchus labrax*)

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ABSTRACT

Effective water management is an essential component of sustainable fish farming practices, particularly in the context of global water scarcity. The accumulation of ammonium ions ($NH4+$) from fish metabolism necessitates frequent water changes, posing a challenge to the sustainability of fish farming operations. Zeolite materials have emerged as a promising solution, offering enhanced ammonium cation removal compared to conventional nitrifying bacteria. This innovative approach alleviates the pressure on water resources and promotes environmental sustainability in fish farming. Over 75 days, this study examined how zeolite, stocking density, and water exchange affected European seabass water quality and growth. 250 kg of seabass, 25.61 ± 2.39 g/fish. Three factors: zeolite levels (Z: 0, 10, and 15 ppt), density (D: 1, 2.5, and 5 kg/m^3), and water exchange (W: 10, 25, and 50 %), using 81 hapas with 0.5 m^3 vol each (triplicates for each treatment) fixed in concrete ponds, fish were randomly distributed among 81 experimental hapas in 27 ponds. This research illuminates the potential benefits of various therapies. Zeolite in seabass culture improved water quality. It reduced ammonia derivatives, improving water quality. Adjusting seabass stocking density to low or high improved water quality measures. This change kept dissolved oxygen levels within the target range, providing a good home for farmed fish. Zeolite supplements, reduced stocking density, and optimum water exchange improved European seabass growth, along with water quality improvements. These interventions improved feed consumption and growth rates. These approaches promoted fish growth and reduced stress by limiting the negative effects of high stocking density. The study also examined immune-related, hematobiochemical, and plasma biochemical characteristics after the interventions. Zeolite supplementation, to low stocking density, and water exchange improved these characteristics. They strengthened the European seabass' immune system and preserved healthy plasma and hematobiochemical parameters. In conclusion, optimizing European seabass culture with zeolite (15 ppt), stocking density (1 kg/m^3), and water exchange (50 %) enhances water quality, growth performance, and physiological parameters. This contributes to improved aquaculture

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sustainability and efficiency. Further research is required to fully elucidate the molecular mechanisms of those factors that affect fish health and aquaculture practices.

Introduction

For future food security and to alleviate pressure on wild-catch fisheries, it is essential to ensure the sustainable expansion of the global fish aquaculture industry [1,2]. While optimal water exchange rates and fish densities are important for aquaculture, reducing water exchange levels and increasing the number of fish is becoming a priority [3]. To address this, the aquaculture sector is turning to alternative natural resources to create efficient recirculating systems that maintain prolonged water quality [4]. However, using natural resources in aquaculture can result in excess costs and increased production [5,6].

The density of fish emerges as a significant limiting factor, playing a pivotal role in negatively affecting fish performance and welfare, thereby influencing the operational costs of fish production [7]. In the aquaculture of seabass, varying densities are commonly employed based on the rearing system and size [8]. Elevated stocking densities generally lead to increased energy demand, modifications in digestive enzyme activities [9], and alterations in body composition [10]. Previous research has demonstrated the combined impact of high density and compromised growth [11]. Another study on fish welfare has distinguished between the specific effects of biomass increase and those resulting from compromised water quality [12].

Dissolved oxygen (DO) and ammonia (NH_3) concentrations frequently limit the number of fishes that may be stocked in aquariums and aquaculture systems [13]. Ammonia is produced during fish protein metabolism [14] and the bacterial degradation of nitrogen-containing organic materials [15]. Ammonia is always released into the water as a result of the degradation of proteins. It is the most important limiting water quality parameter in aquarium and aquaculture systems, with lethal concentrations ranging from 0.2 to 0.5 mg/L [16]. Ammonia removal is a much more pressing issue in intensive aquaculture systems and aquariums [17]. Fish and other aquatic species can become poisonous as a result of ammonia toxicity because it can reduce the amount of dissolved oxygen in the water [18]. Ammonia (NH_3) must be eliminated, and fish culture systems' water quality must be improved. Additionally, water hardness has an impact on the survival and hatching rates of angelfish larvae [19].

One highly effective method for removing ammonia from contaminated water involves ion exchange using natural zeolite [20]. Zeolites are a type of mineral with numerous applications in water and tertiary wastewater treatment. Because of their inherent qualities, such as high ion exchange capacity, large specific surface area, high thermal stability, and lattice stability, many types of zeolites are utilized in filtration, ion exchange, adsorption, photocatalytic degradation, and membrane separation technologies [21]. Zeolites are composed of tetrahedral [SiO_4] 4- and [AlO_4] 5-groups connected by oxygen bridges. When aluminum (Al) replaces silicon (Si) in the zeolite framework, a negative charge is created on the oxygen atom above. Cations, primarily alkali metals (Li^+, Na^+, K^+) and alkaline earth metals (Ca^{2+}, Mg^{2+}), neutralize oxygen's negative charge [22]. Due to their chemical structure, zeolites have a remarkable capacity to assimilate cations. They favour cations with a larger radius and a single charge, such as Cs^+ and NH_4^+. Consequently, zeolites make outstanding ion exchange materials for water treatment and purification [23]. The porous structures of zeolites enable ion exchange to occur not only at the surface but also deep within the zeolite structure, which significantly improves their effectiveness. After saturation, zeolite materials can be regenerated by soaking in NaCl solutions [24].

Previous research has demonstrated that native Iranian clinoptilolite zeolite can lower cadmium levels in water [25]. Several natural zeolites, including clinoptilolite, have also proven successful at removing ammonia from wastewater [26,27]. Clinoptilolite has been shown to be able to lower ammonia levels during the transportation of ornamental fish by Bower and Turner [28]. Obradov *et al.* proposed that using natural zeolite as an environmental corrector in rainbow trout ponds improved fish growth and nutritional parameters [29], whereas Danabas and Altun found no differences in water parameters and rainbow trout growth after adding different levels of zeolite to the experimental ponds [30].

European seabass (*Dicentrarchus labrax*) is a crucial fish species produced in the Mediterranean region [31]. The use of zeolite alone or in combination with other factors has been studied to assess its effects on water quality, growth performance, feed utilization, and biochemical parameters of European seabass [32,33]. The industry utilizes high amounts of zeolite in the diets and/or water for this species. To the best of our knowledge, studies that clarify the interaction between density, water quality, and water exchange rate in aquatic animals is rarely found. Therefore, this study was conducted to detect the interactive effects of zeolite, stocking density, and water exchange on growth performance, feed utilization, water quality, and biochemical parameters of juvenile European seabass (*Dicentrarchus labrax*). The current research aligns with the aspirations of Africa's Union's Agenda 2063, particularly in the areas of promoting healthy and well-nourished citizens, fostering modern agriculture for enhanced productivity and production, and harnessing the potential of the blue/ocean economy. By advocating for an eco-friendly approach to aquaculture that increases fish production, this study contributes to the realization of these ambitious goals [2,34]. The study's comprehensive analysis provides invaluable insights into the complex interplay between zeolite, stocking density, and water exchange, establishing a solid foundation for further research and innovation in seabass aquaculture.

Materials and methods

Ethics statement: The College of Agriculture Committee for Animal Care at Alexandria University Egypt approved and evaluated the experimental animals (Ref. AU: 19/23/05/23/3/32). The experiment was conducted with the aim of minimizing animal suffering.

Preparation of experimental tanks

27 cuboid concrete ponds (24 m^3 each) of the same size were used in this experiment. A pond with dimensions (3 $m \times 8\,m \times 1$ m, length × width × height, respectively) was prepared for rearing animals. Moreover, 100 mg/kg of chlorine was used to sterilize all the ponds by washing and drying them before the experiment. Also, all dirty ponds were washed with fresh water three times with fresh water and seawater and lastly cleaned with fresh water. Once the concrete had cured, the pond was filled with water and let stand for at least a week to allow any chemicals to leach out and the pH to stabilize. During this time, the water quality was monitored and adjusted as needed to ensure it was suitable for the intended aquatic organisms. Finally, any necessary equipment was installed such as aerators, filters, or heaters, and tested to ensure they were functioning properly.

Source of natural zeolite as ammonia removal product

The natural clinoptilolite (zeolite) was purchased from Alix Zeolite, Yemen (http://alixzeolite.com/en/) and used as an adsorbent for ammonia. The chemical composition of Clinoptilolite is as follows (SiO_2, 62.22 %), (Fe_2O_3, 4.033 %), (BaO, 0.085 %), (Al_2O_3, 11.096 %), (K_2O, 3.266 %), (P_2O_5, 0.033 %), (Na_2O, 0.78 %), (TiO_2, 0.339 %), (ZnO, 0.025 %), (MgO, 0.599 %), (ZrO, 20.112 %), (SrO, 0.047 %), (CaO, 3.583 %), (Cl, 0.025 %), and (MnO, 0 %). The zeolite was placed in experimental ponds at three concentration levels (0, 10, and 15 ppt) to maintain a zeolite concentration of 0, 10, and 15 ppt, respectively. The zeolite was removed and washed with fresh tap water every week to prevent clogging.

Fish selection and acclimatization

Around 250 kg of juvenile European seabass, with an average initial weight ± SE of 25.61±2.39 (g/fish), were sourced from the Marine Fish Rearing Unit, General Authority for Fish Resources and Development (GAFRD) in Alexandria, Egypt. These fish were then kept as part of the fish stock in 15 large-scale open systems, each with a volume of 50 m^3, at the El-Max Research Station of the National Institute of Oceanography and Fisheries (NIOF) in Alexandria, Egypt. During the acclimatization period, the experimental animals were provided with a basal diet for a duration of seven days.

Experimental diets and feeding

The fish were provided with a dry pelleted commercial feed that was formulated and prepared at the El-Max Research Station feed mill. The feed had the following composition: moisture (9.23 %), dry matter (DM=90.77 %), crude protein (54.95 % DM basis), crude fat (13.95 % DM basis), crude fiber (1.25 % DM basis), ash (12.20 % DM basis), and Nitrogen-free extract (NFE, 17.65 % DM basis). Samples of the feed were kept to analyze its proximate composition, including the moisture, protein, lipid, and ash content, according to the methodology described in AOAC [35]. The feeds were kept at -20 °C so as to avoid rancidity and corrosion in well-labeled plastic black bags. The experimental animals (European seabass) were hand-fed to apparent satiation three times daily, six days per week. The remaining feeds were constantly removed on a daily basis by siphoning. Also, the leftover feeds were assessed to know the feeding rate.

Fish experimental design and rearing

In this experiment, a 3 × 3 × 3 factorial design was utilized to investigate three factors: zeolite levels (Z), density (D), and water exchange (W), using 81 hapas (Hapa is a cage-like, rectangular or square net impoundment placed in a pond for holding fish) with 0.5 m^3 vol each (three hapas per pond as three replicates for each treatment), fixed in the concrete ponds, fish were randomly distributed among 81 separate experimental hapas fixed in 27 ponds. Hapas were provided with 3 different levels of zeolite (Z) (0, 10, and 15 ppt) at three stabilized rearing densities: 1, 2.5, and 5 kg/m^3 and three water exchange rates (W): 10, 25, and 50 % from the total volume of ponds water, as shown in Table 1.

Throughout the experiment, the water quality parameters were monitored and managed to ensure that they remained within the recommended safe levels for European seabass. The experiment utilized saline well water sourced from a deep well with a salinity range of 28–31 %. The temperature requirements for the experimental European seabass were based on the optimal temperature range for performance reported in the literature, which is between 18 and 22 °C. Therefore, the fish were maintained within this temperature range throughout the experiment using a temperature control system. Water temperature was monitored daily using a thermometer, and any deviations from the target temperature range were corrected immediately. Furthermore, we utilizing the natural light

Table 1
Experimental design: factorial design with three factors.

Z (ppt) W%	D (kg/m^3)								
	10			25			50		
0	1	2.5	5	1	2.5	5	1	2.5	5
10ppt	1	2.5	5	1	2.5	5	1	2.5	5
15ppt	1	2.5	5	1	2.5	5	1	2.5	5

Where, Z = Zeolite, D = Density, W = Water exchange.

throughout the experimental period and constantly checked and removed any dead European seabass daily.

Sample collection and analytical procedures

After 75 days and prior to biometric sampling, the fish were fasted for 24 h and anesthetized in their tank using 2-phenoxyethanol (220 ppm/m^3) [36]. Ten fish per hapa were randomly selected, and their weight and length were measured. Blood samples were taken from the caudal vein using sterilized syringes and needles. The samples were transferred into sterile vacutainer tubes containing heparin sodium as an anticoagulant. Plasma was separated from the blood samples by centrifugation at 4000 rpm for 10 min and stored at -80 °C until analysis.

Measurements of growth parameters, survival rate, feed utilization, and condition factor

The following calculations were used to determine the parameters:

Weight gain (WG, g) = ($W_f - W_i$), where W_f is the final wet weight, and W_i is the initial wet weight.

Average daily gain (ADG; g/d) = ($W_f - W_i$) / d.

Specific growth rate (SGR,%/d) = [(ln W_f – ln W_i)/t] × 100, where t is the number of days.

Survival rate (SR,%) = 100 × the final number of fish/the initial number of fish.

Feed conversion ratio (FCR) = total feed fed (g) / WG (g).

Protein protective value (PPV,%) = (Weight gain (g) ÷ Protein intake (g)) x 100.

Condition factor (KF) = 100 × (body weight (g) / total length^3 (cm)).

Hematological factors measurements

Hematological factors analyses were performed to evaluate the blood parameters of the European seabass. Blood samples were collected from the caudal vein of each fish and analyzed for hematological factors such as red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), and hemoglobin (Hb) concentrations, as well lymphocytes, monocytes, and neutrophils. All the previous parameters related to hematological factors were measured using an automated hematology analyzer according to the manufacturer's instructions [37,38].

Plasma biochemical and immune-related indexes assays

The concentrations of various biochemical parameters were evaluated using different methods. Total protein (TP), Albumin (ALB), Globulin (GLOB), and Total cholesterol (CL), the constituents were evaluated with a spectrophotometer. While strictly following the manufacturer's directives, using commercial assay kits (NS BIOTEC) obtained from Camp Chezar, Alexandria, Egypt. The lysozyme (LYZ) activity was evaluated by turbidimetry [39]. The concentrations of growth-related hormones, specifically growth hormone (GH), were analyzed using Enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocol as described by Songlin et al. [40]. The activities of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were detected using the method reported by Gella et al. [41]. Cortisol levels throughout the body were measured using a competitive RIA that was modified from Young's method [42]. The method described by Chaney and Marbach in 1962 was used to determine the amounts of urea and uric acid [43]. The assays utilized commercial kits sourced from DIALAB® Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H., Neudorf, Austria.

Water physicochemical parameters

The water physicochemical parameters including dissolved oxygen (DO), pH, ammonium (NH_4), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), and alkaline reserve (ARRS) were measured during the experiment. The DO, pH, and temperature were measured using a handheld water quality meter (Hach Co., Loveland, CO, USA) before each sampling. The concentrations of NH_4, NH_3, NO_2, and NO_3 were analyzed according to the standard methods outlined by American Public Health Association [44]. The ARRS was measured by titration with hydrochloric acid (HCl) and sodium hydroxide (NaOH) using bromocresol green/methyl red as an indicator [45].

Data collection and statistical analysis

The acquired data underwent rigorous statistical analysis to unveil the nuanced effects of zeolite, density, and water exchange on various parameters in juvenile European seabass. A multivariate analysis of variance (MANOVA) was conducted to comprehensively assess the collective impact of these three factors on multiple measured variables, including growth, feed utilization, water quality, and biochemical tests. The MANOVA allowed for the simultaneous evaluation of these dependent variables, exploring not only the individual effects of each factor but also their potential interactions. By utilizing SPSS 23 statistical software and setting a significance level at ($p < 0.05$), the study rigorously determined the statistical significance of observed differences, ensuring that the identified effects were likely attributed to the experimental variables rather than random chance.

Results

Growth performance, survival rate, feed utilization, and condition factor

Tables 2A **and** 2B display the growth performance, survival rate, feed utilization, and condition factor of European seabass

D. labrax fed with varying levels of zeolite, density, and water exchange for 75 days. At the conclusion of a 75-day feeding experiment, the highest mean of the core effect for final body weight (FBW), daily weight gain (WG), average daily weight gain (ADG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), Protein protective value (PPV) and Condition factor (KF) was found at 0, 10, and 15 g/kg of zeolite, 1, 2.5, and 5 Kg/m^3 of density, and 10, 25, and 50 water exchange. According the result of Analysis of variance (ANOVA) revealed that FBW, WG, ADG, SGR, SR, FCR, and PPV were significantly affected by different dietary levels by zeolite level, density, and water exchange ($P < 0.05$); but the results demonstrate that density had no significant effect on that KF. While the interrelationship between zeolite level and density (Z x D), revealed a significant effect on FBW, WG, ADG, SGR, SR, FCR, PPV, and KF, ($P < 0.05$). Similarly, the interrelationship between zeolite level and water exchange (Z x W) had a significant on all the above parameters ($P < 0.05$), with the exception of KF which revealed no significant effect in the association between zeolite level and water exchange. The interaction between density and water exchange (D x W) had a significant effect on survival rate and KF ($P < 0.05$). However, the interaction between zeolite level, density, and water exchange (Z x D x W) had a significant effect on FBW, SR, and FCR ($P < 0.05$). Although the interaction between zeolite level, density, and water exchange (Z x D x W) had no significant effect on the WG, ADG, SGR, PPV, and KF of *D. labrax* fed with different levels of zeolite, density and water exchange.

Hematological factors

The effects of zeolite, density, and water exchange on hematological parameters of *D. labrax* such as red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), white blood cells (WBC), lymphocytes, monocytes, neutrophils for 75 days are presented in (Tables 3A **and** 3B). The 15ppt zeolite showed a significant increase in RBCs count, HB, WBC, lymphocytes, monocytes, and neutrophils compared to control ($P < 0.05$). In contrast, 5 (Kg/m^3) density revealed a significant decrease ($P < 0.0001$) in BCs count, HB, WBC, lymphocytes, monocytes, compared to 1(Kg/m^3) density at 75 days with the exception. However, in *D. labrax,* 50 % water exchange significantly increased ($P < 0.05$) RBCs count, HB, WBC, lymphocytes, monocytes, and neutrophils compared to 10 and 25 % water exchange. The interaction between zeolite and density (Z x D) had a significant effect on RBCs count, HB, WBC, lymphocytes, monocytes, and neutrophils. The interaction effect of zeolite and water exchange (Z x W) had a significant effect on HB content, lymphocytes, and neutrophils, but did not have any significant effect on other hematological factors such as RBCs content, HCT, WBC, and monocytes. Also, the interaction between density and water exchange in (D x W) *D. labrax* affected only the percentage of lymphocytes, and neutrophils significantly ($P < 0.05$), but did not have any significant effect on other hematological factors. Furthermore, the interaction between levels of zeolite, density, and water exchange percentage (Z x D x W) was found to have a

Table 2-A
Growth performance, survival rate, feed utilization, and condition factor of European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days (mean [$n = 3$]).

Factors			Parameters							
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	FBW (g)	WG (g)	ADG (g)	SGR (%)	SR (%)	FCR	PPV%	KF
Contributed (Pooled) treatment means										
0	1	10	67.52 ^{k}	42.62 ^{jk}	0.57 ^{ij}	1.33 $^{f-h}$	67.67 ^{ij}	2.06 ^{ef}	35.83 ^{jk}	0.80 ^{fg}
		25	80.42 ^{j}	55.25 ^{i}	0.74 ^{h}	1.55 ^{ef}	73.00 ^{h}	1.79 ^{g}	55.99 $^{e-i}$	0.83 $^{e-g}$
		50	94.31 ^{i}	68.00 ^{h}	0.91 ^{g}	1.70 ^{de}	83.33 ^{ef}	1.68 ^{gh}	67.29 $^{b-e}$	0.91 $^{d-f}$
	2.5	10	57.83 ^{l}	31.50 ^{m}	0.42 ^{l}	1.05 ^{ij}	62.67 ^{k}	2.45 ^{ab}	21.17 ^{l}	0.76 ^{fg}
		25	63.96 ^{kl}	39.38 $^{k-m}$	0.53 $^{j-l}$	1.27 $^{g-i}$	79.00 ^{fg}	2.32 ^{bc}	34.89 ^{jk}	0.84 $^{e-g}$
		50	76.75 ^{j}	50.38 ^{ij}	0.68 ^{hi}	1.43 ^{fg}	75.67 ^{gh}	2.18 $^{c-e}$	43.36 ^{ij}	0.85 $^{e-g}$
	5	10	47.31 ^{m}	22.05 ^{n}	0.29 ^{m}	0.84 ^{j}	57.00 ^{l}	2.57 ^{a}	12.29 ^{l}	0.71 ^{g}
		25	59.10 ^{l}	33.50 ^{lm}	0.45 ^{kl}	1.12 ^{hi}	63.33 ^{jk}	2.41 ^{ab}	23.18 ^{kl}	0.76 ^{fg}
		50	67.20 ^{k}	41.75 $^{j-l}$	0.56 ^{jk}	1.29 ^{gh}	71.33 ^{hi}	2.23 ^{cd}	35.34 ^{jk}	0.80 ^{fg}
10	1	10	101.34 ^{gh}	75.83 ^{gh}	1.01 ^{fg}	1.84 $^{b-d}$	91.00 $^{a-c}$	1.59 ^{hi}	56.34 $^{d-h}$	0.93 $^{d-f}$
		25	111.91 $^{d-f}$	86.45 ^{ef}	1.15 ^{de}	1.98 $^{a-c}$	92.67 ^{ab}	1.50 $^{i-k}$	63.91 $^{b-g}$	1.01 $^{c-e}$
		50	126.51 ^{ab}	100.80 ^{a}	1.34 ^{a}	2.13 ^{a}	94.97 ^{a}	1.35 $^{k-n}$	68.95 $^{a-d}$	1.28 ^{a}
	2.5	10	97.53 ^{hi}	71.87 ^{h}	0.96 ^{g}	1.78 $^{c-e}$	86.67 $^{c-e}$	1.68 ^{gh}	54.21 $^{f-i}$	1.07 $^{b-d}$
		25	108.42 ^{ef}	82.83 ^{fg}	1.10 ^{ef}	1.93 $^{a-d}$	91.00 $^{a-c}$	1.49 $^{i-l}$	66.94 $^{b-e}$	1.16 $^{a-c}$
		50	124.51 ^{ab}	99.25 $^{a-c}$	1.32 ^{ab}	2.13 ^{a}	92.67 ^{ab}	1.55 $^{h-j}$	67.76 $^{a-e}$	1.18 $^{a-c}$
	5	10	97.63 ^{hi}	72.10 ^{h}	0.96 ^{g}	1.79 ^{cd}	85.67 ^{de}	2.10 $^{d-f}$	44.79 $^{h-j}$	1.06 $^{b-d}$
		25	107.26 ^{fg}	82.32 ^{fg}	1.09 ^{ef}	1.95 $^{a-c}$	89.00 $^{b-d}$	1.96 ^{f}	51.38 $^{g-i}$	1.15 $^{a-c}$
		50	121.12 ^{bc}	95.65 $^{a-d}$	1.28 $^{a-c}$	2.08 ^{a}	88.67 $^{b-d}$	1.79 ^{g}	57.55 $^{d-g}$	1.17 $^{a-c}$
15	1	10	125.48 ^{ab}	99.97 $^{a-c}$	1.33 ^{ab}	2.13 ^{a}	95.67 ^{a}	1.49 $^{i-m}$	64.44 $^{b-f}$	1.18 $^{a-c}$
		25	125.59 ^{ab}	100.7 ^{ab}	1.34 ^{a}	2.16 ^{a}	95.00 ^{a}	1.36 $^{k-n}$	71.94 $^{a-c}$	1.24 ^{ab}
		50	128.22 ^{a}	102.72 ^{a}	1.37 ^{a}	2.16 ^{a}	94.33 ^{a}	1.33 ^{mn}	80.30 ^{a}	1.32 ^{a}
	2.5	10	116.95 ^{cd}	91.48 $^{c-e}$	1.22 $^{b-d}$	2.03 ^{ab}	95.00 ^{a}	1.51 $^{i-k}$	61.99 $^{c-g}$	1.15 $^{a-c}$
		25	121.25 $^{a-c}$	95.40 $^{a-d}$	1.27 $^{a-c}$	2.06 ^{ab}	94.67 ^{a}	1.34 $^{l-n}$	73.33 $^{a-c}$	1.20 $^{a-c}$
		50	124.96 ^{ab}	99.99 $^{a-c}$	1.33 ^{ab}	2.15 ^{a}	95.67 ^{a}	1.33 ^{n}	76.38 ^{ab}	1.28 ^{a}
	5	10	114.47 $^{c-e}$	89.08 $^{d-f}$	1.19 $^{c-e}$	2.01 $^{a-c}$	91.67 ^{ab}	1.40 $^{j-n}$	63.78 $^{b-g}$	1.14 $^{a-c}$
		25	116.73 ^{dc}	92.09 $^{b-e}$	1.23 $^{b-d}$	2.07 ^{ab}	92.9 ^{ab}	1.31 ^{n}	73.08 $^{a-c}$	1.23 ^{ab}
		50	124.02 ^{ab}	98.84 $^{a-c}$	1.32 ^{ab}	2.13 ^{a}	92.00 ^{ab}	1.33 $^{l-n}$	70.41 $^{a-c}$	1.27 ^{a}

FBW, Final body weight; WG, Weight gain; ADG, Average daily gain; SGR, Specific growth rate; SR, Survival rate; FCR, Feed conversion ratio; PPV, Protein protective value; KF, Condition factor.

Table 2-B
Means of the main impact and ANOVA for growth performance, survival rate, feed utilization and condition factor of European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days.

Factors			Parameters							
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	FBW (g)	WG (g)	ADG (g)	SGR (%)	SR (%)	FCR	PPV%	KF
Means of main impact										
0			68.27 ^{C}	42.71 ^{C}	0.57 ^{C}	1.29 ^{C}	70.33 ^{C}	2.19 ^{A}	36.59 ^{C}	0.81 ^{C}
10			110.69 ^{B}	85.23 ^{B}	1.14 ^{B}	1.96 ^{B}	90.22 ^{B}	1.67 ^{B}	59.09 ^{B}	1.11 ^{B}
15			121.96 ^{A}	96.70 ^{A}	1.29 ^{A}	2.09 ^{A}	94.00 ^{A}	1.38 ^{C}	70.63 ^{A}	1.22 ^{A}
	1		106.81 ^{A}	81.37 ^{A}	1.08 ^{A}	1.89 ^{A}	87.48 ^{A}	1.57 ^{C}	62.78 ^{A}	1.06 ^{A}
	2.5		99.13 ^{B}	73.56 ^{B}	0.98 ^{B}	1.76 ^{B}	85.89 ^{B}	1.76 ^{B}	55.56 ^{B}	1.06 ^{A}
	5		94.98 ^{C}	69.71 ^{C}	0.93 ^{C}	1.70 ^{C}	81.19 ^{C}	1.90 ^{A}	47.98 ^{C}	1.03 ^{A}
		10	91.78 ^{C}	66.28 ^{C}	0.88 ^{C}	1.64 ^{C}	81.44 ^{C}	1.87 ^{A}	46.09 ^{C}	0.98 ^{C}
		25	99.41 ^{B}	74.21 ^{B}	0.99 ^{B}	1.79 ^{B}	85.52 ^{B}	1.72 ^{B}	57.18 ^{B}	1.05 ^{B}
		50	109.73 ^{A}	84.15 ^{A}	1.12 ^{A}	1.91 ^{A}	87.59 ^{A}	1.64 ^{C}	63.04 ^{A}	1.12 ^{A}
Analysis of variance (ANOVA, *p-values*)										
Z			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
D			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	ns
W			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Z x D			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.006
Z x W			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	ns
D x W			ns	ns	ns	ns	0.0002	ns	ns	0.03
Z x D x W			0.003	ns	ns	ns	0.0001	0.003	ns	ns

FBW, Final body weight; WG, Weight gain; ADG, Average daily gain; SGR, Specific growth rate; SR, Survival rate; FCR, Feed conversion ratio; PPV, Protein protective value; KF, Condition factor; Z, Zeolite; D, Density; W, Water exchange; ns = $p > 0.05$.

significant ($P < 0.05$) effect on the RBCs content, WBC, lymphocytes, monocytes, and neutrophils percentages of *D. labrax* but have no significant on HB and HCT percentage.

Plasma biochemical and immune-related indexes

The plasma biochemical and immune-related parameters in *D. labrax* treated with different levels of zeolite, density, and water exchange for 75 days were presented in Tables 4A and 4B. Plasma total protein (TP), globulin (GLOB), lysozyme (LYZ), and GH were significantly increased with the increase in zeolite levels at 10 and 15 ppt compared to the control group in *D. labrax*. In contrast cholesterol, cortisol, AST, ALT, and uric acid decreased significantly in *D. labrax* treated with different levels of zeolite compared to control group. Meanwhile, the level of urea and albumin (AL) exhibit no significant difference between control and treatment groups. The cholesterol, cortisol, AST, ALT urea, and uric acid were significantly increased in *D. labrax* raised at the highest density. However, the level of TP, GLOB, LYZ, and GH significantly decrease when raised at high density, while the level of albumin has not been affected when raised at the highest density. The water exchange significantly increases the levels of TP, ALB, GLOB, LYZ and GH but on the other hand, the levels of cholesterol, cortisol, AST, ALT, urea, and uric acid significantly decrease in *D. labrax*. The interaction between zeolite and density (Z x D) had a significant effect on plasma biochemical and immune-related indexes in European seabass with the exception of albumin. In contrast, the interaction between zeolite and water exchange (Z x W) significantly affected all the plasma biochemical parameters with the exception of ALB and GH which display no significant difference. However, the interaction between density and water exchange (D x W) only TP, ALB, and LYZ show no significant difference while the rest of the parameters were significantly affected by the interaction. Lastly, the interaction between zeolite, density, and water exchange (Z x D x W) significantly affected all the plasma biochemical parameters that have been tested with the exception of total protein and lysozymes in European seabass for 75 days.

Physicochemical parameters of water

Tables 5A and 5B present the effects of zeolite, stocking density, and water interventions on the physiochemical parameters of *D. labrax*. The addition of zeolite substantially increased the levels of DO, pH, NO3, and ARRs% in water treated with zeolite. Meanwhile, water treatment with the addition of zeolite significantly decreased the level of NH_4, NH_3, and NO_2 in *D. labrax* for 75 days. The stocking density significantly decreases the DO, pH, NO_3, and ARRs% with increasing the stocking density. Correspondingly, the level of NH_4, NH_3, and NO_2 significantly increased with increasing of the stocking density. Furthermore, the water exchange significantly increased the levels of DO, pH, NO_3, and ARRs%, on the other hand, decreased the level of NH_4, NH_3, and NO_2 significantly in raised European seabass at 75 days. However, the interaction between zeolite and density (Z x D) significantly affected all the physical parameters of the water with exception of NH_3 which exhibited no significant difference. The interaction between zeolite and water exchange (Z x W) significantly affected all the water physiochemical parameters, while the interaction between the density and water exchange (D x W) demonstrated no significant differences in the level of DO, NH_3, NO_2, and NO_3. Lastly, the interaction between zeolite, stocking density, and water exchange (Z x D x W) significantly affected all the water physiochemical parameters with exclusion of NH_3 level.

Table 3-A
Hematological factors of European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days (mean [$n = 3$]).

Factors			Parameters						
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	RBC ($\times 10^6$/mm^3)	HB (g/dl)	HCT (%)	WBC (10^3/mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Contributed (Pooled) treatment means									
0	1	10	1.67 ^{fg}	7.22 $^{i-k}$	20.64 $^{h-k}$	27.61 $^{f-h}$	66.54 $^{b-d}$	2.41 $^{e-g}$	19.60 ^{fg}
		25	1.85 ^{ef}	8.05 $^{e-i}$	24.15 $^{e-i}$	31.28 $^{b-f}$	69.65 ^{ab}	2.51 $^{b-e}$	21.18 $^{c-e}$
		50	1.89 $^{d-f}$	8.99 $^{a-e}$	27.72 $^{b-f}$	31.73 $^{a-f}$	69.78 ^{ab}	2.50 $^{c-e}$	21.27 $^{b-e}$
	2.5	10	1.52 ^{gh}	6.85 ^{jk}	15.51 ^{jk}	24.51 $^{h-j}$	63.09 ^{d}	2.29 ^{fg}	18.03 ^{h}
		25	1.83 ^{ef}	7.58 $^{g-j}$	22.76 $^{g-j}$	30.70 $^{b-f}$	69.99 ^{ab}	2.52 $^{b-e}$	21.18 $^{c-e}$
		50	1.86 ^{ef}	8.52 $^{a-g}$	25.55 $^{d-g}$	31.85 $^{a-f}$	69.29 ^{ab}	2.49 $^{c-e}$	21.18 $^{c-e}$
	5	10	1.19 ^{i}	5.48 ^{l}	15.89 ^{k}	2032 ^{j}	45.89 ^{f}	2.06 ^{i}	15.89 ^{i}
		25	1.30 ^{hi}	6.28 ^{kl}	16.24 ^{k}	21.67 ^{ij}	54.33 ^{e}	2.07 ^{hi}	16.18 ^{i}
		50	1.51 ^{gh}	7.26 $^{h-k}$	19.38 $^{i-k}$	25.36 $^{g-i}$	63.60 ^{cd}	2.25 ^{gh}	18.66 ^{gh}
10	1	10	1.78 $^{e-g}$	8.52 $^{a-f}$	25.76 $^{d-g}$	29.40 $^{d-g}$	68.93 ^{ab}	2.48 ^{de}	20.88 $^{c-f}$
		25	1.89 $^{d-f}$	8.71 $^{a-f}$	26.26 $^{c-g}$	31.83 $^{a-f}$	69.07 ^{ab}	2.48 ^{de}	21.19 $^{c-e}$
		50	2.39 ^{a}	9.51 ^{a}	32.74 ^{a}	35.81 ^{a}	72.78 ^{a}	2.69 ^{ab}	22.56 ^{a}
	2.5	10	1.82 ^{ef}	8.32 $^{c-g}$	25.33 $^{d-h}$	30.55 $^{b-f}$	68.22 $^{a-c}$	2.45 $^{d-f}$	20.88 $^{c-f}$
		25	1.96 $^{c-f}$	8.43 $^{b-g}$	27.20 $^{b-g}$	33.10 $^{a-e}$	69.91 ^{ab}	2.50 $^{c-e}$	21.51 $^{a-e}$
		50	1.94 $^{c-f}$	8.82 $^{a-e}$	29.30 $^{a-d}$	32.64 $^{a-e}$	69.87 ^{ab}	2.50 $^{c-e}$	21.43 $^{a-e}$
	5	10	1.75 $^{e-g}$	7.77 $^{f-j}$	22.98 $^{f-j}$	29.08 $^{e-g}$	67.39 $^{b-d}$	2.43 $^{d-g}$	20.24 ^{ef}
		25	1.90 $^{d-f}$	8.24 $^{c-h}$	25.68 $^{d-g}$	32.19 $^{a-e}$	69.78 ^{ab}	2.50 $^{b-e}$	21.34 $^{a-e}$
		50	1.91 $^{d-f}$	8.91 $^{a-e}$	28.51 $^{a-e}$	32.18 $^{a-e}$	69.82 ^{ab}	2.50 $^{c-e}$	21.35 $^{a-e}$
15	1	10	2.03 $^{b-e}$	8.87 $^{a-e}$	29.63 $^{a-d}$	33.37 $^{a-d}$	70.40 ^{ab}	2.57 $^{a-e}$	21.70 $^{a-d}$
		25	2.20 $^{a-c}$	9.13 $^{a-c}$	31.20 ^{ab}	34.47 ^{ab}	71.40 ^{ab}	2.67 $^{a-c}$	22.07 $^{a-c}$
		50	2.28 ^{ab}	9.35 ^{ab}	32.81 ^{a}	35.56 ^{a}	72.41 ^{a}	2.71 ^{a}	22.48 ^{ab}
	2.5	10	1.83 ^{ef}	8.43 $^{b-g}$	25.53 $^{d-g}$	29.97 $^{c-f}$	68.57 $^{a-c}$	2.50 $^{c-e}$	20.90 $^{c-e}$
		25	1.97 $^{c-e}$	8.60 $^{a-f}$	28.20 $^{a-e}$	32.47 $^{a-e}$	69.50 ^{ab}	2.47 $^{d-f}$	21.33 $^{a-e}$
		50	2.17 $^{a-d}$	9.17 $^{a-c}$	31.00 $^{a-c}$	34.23 $^{a-c}$	71.33 ^{ab}	2.60 $^{a-d}$	21.97 $^{a-c}$
	5	10	1.77 $^{e-g}$	8.13 $^{d-i}$	24.27 $^{e-h}$	29.53 $^{d-g}$	68.00 $^{a-d}$	2.47 $^{d-f}$	20.57 $^{d-f}$
		25	1.93 $^{c-f}$	8.40 $^{b-g}$	26.93 $^{b-g}$	32.30 $^{a-e}$	69.60 ^{ab}	2.50 $^{c-e}$	21.37 $^{a-e}$
		50	2.00 $^{b-e}$	9.07 $^{a-d}$	29.77 $^{a-d}$	33.23 $^{a-e}$	70.57 ^{ab}	2.53 $^{a-e}$	21.67 $^{a-d}$

RBC, Red blood cell count; Hb, Hemoglobin; HCT, Hematocrit; WBC, White blood cell count.

Discussion

Crowding is a common aquaculture-related stressor with diverse effects on fish, including reduced growth and health [46,47]. Factors influencing fish physiology under crowding conditions include the carrying capacity of water for oxygen provision and waste dilution, along with the need for adequate space [48,49]. Oxygen consumption and ammonia excretion are critical factors limiting water-carrying capacity. Mismanaged ammonia levels adversely affect fish health, growth, and survival in production systems [13,50]. Zeolite treatment effectively mitigated the adverse effects of increased stocking density on water quality and fish performance [51]. This study investigates the optimal effects of zeolite, stocking density, and water exchange on the growth, feed utilization, water quality, and biochemical parameters of juvenile European seabass (*D. labrax*). Zeolites among the most vital basic materials, have gained popularity as feed additives. Zeolites are used to do things like stop pollution in pools and help marine animals grow faster [52]. Stocking density is a typical aquaculture-related stressor that has a wide range of consequences on aquatic animals, including lower

Table 3-B
Means of the main impact and ANOVA for hematological factors of European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days.

Factors			Parameters						
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	RBC ($\times 10^6$/mm^3)	HB (g/dl)	HCT (%)	WBC (10^3/mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Means of main impact									
0			1.62 ^{C}	7.36 ^{C}	21.20 ^{C}	27.22 ^{B}	63.57 ^{B}	2.35 ^{C}	19.24 ^{B}
10			1.93 ^{B}	8.59 ^{B}	27.03 ^{B}	31.87 ^{A}	69.53 ^{A}	2.50 ^{B}	21.26 ^{A}
15			2.02 ^{A}	8.79 ^{A}	28.82 ^{A}	32.79 ^{A}	70.19 ^{A}	2.56 ^{A}	21.56 ^{A}
	1		1.99 ^{A}	8.71 ^{A}	27.88 ^{A}	32.34 ^{A}	70.11 ^{A}	2.55 ^{A}	21.44 ^{A}
	2.5		1.88 ^{B}	8.30 ^{B}	25.93 ^{B}	31.11 ^{B}	68.87 ^{B}	2.48 ^{B}	20.93 ^{B}
	5		1.69 ^{C}	7.73 ^{C}	23.29 ^{C}	28.43 ^{C}	64.33 ^{C}	2.37 ^{C}	21.44 ^{C}
		10	1.71 ^{C}	7.74 ^{C}	23.17 ^{C}	28.26 ^{C}	65.23 ^{C}	2.41 ^{C}	19.85 ^{C}
		25	1.87 ^{B}	8.16 ^{B}	25.40 ^{B}	31.11 ^{B}	68.14 ^{B}	2.47 ^{B}	20.82 ^{B}
		50	1.99 ^{A}	8.84 ^{A}	28.53 ^{A}	32.51 ^{A}	69.94 ^{A}	2.53 ^{A}	21.39 ^{A}
Analysis of variance (ANOVA, *p-values*)									
Z			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
D			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
W			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Z x D			0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001
Z x W			ns	0.0001	ns	ns	0.0001	ns	0.0001
D x W			ns	ns	ns	ns	0.0004	ns	0.02
Z x D x W			0.0002	ns	ns	0.007	0.0001	0.0008	0.0001

RBC, Red blood cell count; Hb, Hemoglobin; HCT, Hematocrit; WBC, White blood cell count; Z, Zeolite; D, Density; W, Water exchange; ns = $p > 0.05$.

growth, health, and feed conversion ratios [53]. Water's carrying ability to deliver oxygen and dilute metabolic wastes can operate as a stressor. Furthermore, the need for proper room inhibits fish health. The rate of oxygen consumption and ammonia excretion are the elements that limit the water's carrying capacity [54].

In the present study we observed that FBW, WG, ADG, SGR, SR, FCR, and PPV were significantly affected by different dietary levels of zeolite. Our results were likewise in agreement with those of Lanari et al. who found that zeolite additions of 2.5 and 5 % improved weight increase and feed efficiency in rainbow trout [55]. Similar findings were made by Yildirim et al. who discovered that fish fed zeolite-supplemented diets at 1 and 2 % had higher WG, SGR, protein efficiency rate, and FCR than fish fed zeolite-free diets [56]. The interaction between zeolite, density and water exchange ($Z \times D \times W$) also improved FBW, SR, FCR of *D. labrax*. The interaction between density and water (D x W) did not have a significant effect on growth indices WG, ADG, SGR, FCR, and PPV. In accordance with our result above, it has been demonstrated that the interaction of water treatment with zeolite and *Yucca schidigera* (YE) extract can enhance the health and production of seabass fish, in favor of zeolite [32].

The study of hematological factors in fish is crucial for understanding their physiological adaptations and overall health. Hematological parameters provide valuable insights into the functional status of the circulatory system and reflect the ability of fish to cope with environmental stressors [57]. The current data show that zeolite and water exchange treatment considerably enhanced RBC count, HB, WBC, lymphocytes, monocytes, and neutrophils, and so reduced the effects of stocking density on various haematological parameters. It has been reported that adding YE to water or feeding it to European seabass enhanced their haematological health [33, 58]. Also, zeolite assisted the fish that had been exposed to low and high levels of ammonia to get improved, but their RBCs, Hb, and Ht were the same as those of the control group [59]. Recently, it has been discovered that zeolite and YE extract water treatment in favor of Z15 significantly reduced the detrimental effects of increased stocking density on various hematological parameters [32]. The enhancement in RBC and Hb content observed in the present study as a result of varying zeolite and water exchange may partially explain the enhanced growth and feed utilization observed. Normal hematopoiesis ensures that RBCs have adequate Hb levels to aid in the delivery of oxygen to healthy body tissues.

The analysis of cholesterol, cortisol, AST, ALT, urea, and uric acid in fish provides valuable insights into their physiological and metabolic functions. Cholesterol is an essential component of cell membranes and plays a crucial role in various biological processes, including hormone production [60]. The measurement of cholesterol levels in fish helps assess their overall health status and lipid metabolism [61]. Cortisol, a stress hormone, is involved in the regulation of physiological responses to stressors in fish. Elevated cortisol levels indicate the presence of stress or environmental disturbances [62,63]. AST and ALT are enzymes primarily associated with liver function and can serve as indicators of liver damage or disease in fish [64]. Monitoring these enzyme levels aids in assessing the health and well-being of fish populations. Urea and uric acid are waste products resulting from protein and nitrogen metabolism. Evaluating their concentrations provides insights into the renal function and metabolic state of fish [65]. Overall, analyzing cholesterol, cortisol, AST, ALT, urea, and uric acid levels in fish contributes to a comprehensive understanding of their physiological adaptations, health status, and responses to environmental factors.

The plasma biochemical and immune-related parameters in *D. labrax* treated with different levels of zeolite were improved, while the stocking density enhanced the level of cholesterol, cortisol, AST, ALT, urea, and uric acid. On the other hand, the water exchange boosted the TP, ALB, GLOB, LYZ, and GH while the levels of cholesterol, cortisol, AST, ALT, urea, and uric acid significantly declined in *D. labrax*. Studies demonstrated that the plasma cortisol level is also an excellent indicator of stress. Plasma cortisol levels typically increase at the onset of a protracted stress situation, such as high stocking density, and return to baseline values within a few days,

Table 4-A
Plasma biochemical and immune-related indexes in European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days (mean [$n = 3$]).

Factors			Parameters										
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	TP ((g/dl)	ALB (g/dl)	GLOB (g/dl)	CL (mg/dl)	LYZ (U/ml)	Cortisol (ng/ml)	GH (PG/ml)	AST (U/ml)	ALT (U/ml)	Urea (mg/dl)	Uric acid (mg/dl)
Contributed (Pooled) treatment means													
0	1	10	3.54 $^{e-g}$	1.98 ^{bc}	1.56 $^{f-h}$	202.55 $^{b-d}$	0.15 ^{kl}	425.48 ^{ab}	402.00 ^{k}	33.74 ^{bc}	30.90 ^{bc}	38.17 ^{b}	2.29 ^{b}
		25	3.99 $^{d-g}$	2.20 $^{a-c}$	1.79 $^{e-h}$	186.39 $^{b-f}$	0.19 $^{j-l}$	409.18 ^{ab}	510.67 $^{h-j}$	32.46 $^{b-d}$	27.13 $^{b-e}$	38.00 ^{b}	2.42 ^{b}
		50	4.44 $^{c-g}$	2.48 $^{a-c}$	1.96 $^{d-h}$	182.00 $^{b-f}$	0.25 $^{h-j}$	306.48 $^{d-f}$	536.33 $^{d-h}$	32.37 $^{b-e}$	27.21 $^{b-e}$	38.50 ^{b}	2.75 ^{b}
	2.5	10	3.33 ^{fg}	1.92 ^{bc}	1.41 ^{gh}	211.63 ^{b}	0.11 ^{l}	436.81 ^{ab}	360.00 ^{kl}	34.18 ^{b}	33.95 ^{b}	37.67 ^{b}	2.27 ^{b}
		25	3.60 $^{e-g}$	1.90 ^{bc}	1.70 $^{f-h}$	193.47 $^{b-e}$	0.12 ^{l}	442.29 ^{ab}	403.33 ^{k}	33.31 $^{b-d}$	27.85 $^{b-e}$	38.67 ^{b}	2.33 ^{b}
		50	3.95 $^{e-g}$	2.10 $^{a-c}$	1.85 $^{e-h}$	186.55 $^{b-f}$	0.13 ^{l}	419.74 ^{ab}	415.67 ^{jk}	31.62 $^{b-f}$	26.42 $^{b-e}$	37.33 ^{b}	2.50 ^{b}
	5	10	3.15 ^{g}	1.90 ^{bc}	1.25 ^{h}	293.17 ^{a}	0.11 ^{l}	455.59 ^{a}	310.67 ^{l}	45.60 ^{a}	48.86 ^{a}	49.17 ^{a}	4.25 ^{a}
		25	3.48 ^{fg}	1.90 ^{bc}	1.57 $^{f-h}$	279.71 ^{a}	0.12 ^{l}	429.29 ^{ab}	335.33 ^{l}	42.28 ^{a}	45.29 ^{a}	48.00 ^{a}	3.83 ^{a}
		50	3.63 $^{e-g}$	1.93 ^{bc}	1.70 $^{f-h}$	211.67 ^{b}	0.13 ^{l}	424.59 ^{ab}	357.00 ^{kl}	32.08 $^{b-f}$	29.33 $^{b-d}$	37.00 ^{b}	2.30 ^{b}
10	1	10	3.71 $^{e-g}$	1.94 ^{bc}	1.77 $^{e-h}$	180.78 $^{c-f}$	0.34 $^{e-h}$	311.00 $^{d-f}$	511.33 $^{g-i}$	31.83 $^{b-f}$	26.76 $^{b-e}$	37.67 ^{b}	2.29 ^{b}
		25	3.87 $^{e-g}$	1.53 ^{bc}	2.35 $^{c-f}$	174.88 $^{d-f}$	0.43 $^{c-e}$	261.67 $^{f-i}$	567.67 $^{c-g}$	28.98 $^{b-f}$	23.76 $^{c-e}$	39.81 ^{b}	2.79 ^{b}
		50	6.41 ^{ab}	3.42 ^{a}	2.99 ^{c}	165.26 ^{ef}	0.47 $^{b-d}$	255.89 $^{f-j}$	621.67 ^{bc}	26.79 ^{f}	21.46 ^{e}	38.33 ^{b}	2.50 ^{b}
	2.5	10	3.70 $^{e-g}$	1.95 ^{bc}	1.75 $^{e-h}$	208.00 ^{bc}	0.31 $^{g-i}$	327.66 $^{c-e}$	496.00 ^{hi}	28.49 $^{c-f}$	23.27 $^{c-e}$	40.00 ^{b}	2.80 ^{b}
		25	4.04 $^{d-g}$	1.74 ^{bc}	2.30 $^{c-f}$	202.75 $^{b-d}$	0.32 $^{f-i}$	271.22 $^{e-h}$	528.33 $^{e-i}$	29.46 $^{b-f}$	24.25 $^{c-e}$	39.63 ^{b}	2.79 ^{b}
		50	4.87 $^{b-f}$	2.34 $^{a-c}$	2.54 $^{c-e}$	195.83 $^{b-e}$	0.41 $^{c-f}$	244.93 $^{g-k}$	570.33 $^{c-g}$	30.43 $^{b-f}$	25.24 $^{c-e}$	39.25 ^{b}	2.78 ^{b}
	5	10	3.62 $^{e-g}$	1.97 ^{bc}	1.66 $^{f-h}$	205.28 $^{b-d}$	0.24 $^{i-k}$	381.67 ^{bc}	466.33 ^{ij}	31.12 $^{b-f}$	27.08 $^{b-e}$	39.08 ^{b}	2.55 ^{b}
		25	4.01 $^{d-g}$	1.97 ^{bc}	2.05 $^{d-h}$	194.57 $^{b-e}$	0.27 $^{h-j}$	346.67 ^{cd}	499.67 ^{hi}	30.96 $^{b-f}$	25.69 $^{c-e}$	38.81 ^{b}	2.60 ^{b}
		50	4.66 $^{c-g}$	2.41 $^{a-c}$	2.25 $^{c-f}$	188.92 $^{b-f}$	0.38 $^{d-g}$	299.33 $^{d-g}$	510.67 $^{h-j}$	31.40 $^{b-f}$	26.22 $^{b-e}$	38.88 ^{b}	2.76 ^{b}
15	1	10	4.97 $^{b-f}$	2.42 $^{a-c}$	2.53 $^{c-e}$	180.37 $^{c-f}$	0.40 $^{c-g}$	232.67 $^{h-k}$	660.67 ^{ab}	28.91 $^{b-f}$	23.68 $^{c-e}$	39.26 ^{b}	2.71 ^{b}
		25	6.07 $^{a-c}$	1.87 ^{bc}	4.20 ^{b}	168.50 ^{ef}	0.45 ^{cd}	214.00 $^{h-l}$	700.00 ^{a}	27.93 $^{d-f}$	22.66 ^{de}	38.29 ^{b}	2.48 ^{b}
		50	7.16 ^{a}	1.33 ^{c}	5.83 ^{a}	162.67 ^{f}	0.58 ^{a}	192.00 ^{kl}	717.33 ^{a}	26.95 ^{ef}	21.63 ^{de}	37.33 ^{b}	2.25 ^{b}
	2.5	10	3.73 $^{e-g}$	1.65 ^{bc}	2.07 $^{d-h}$	194.40 $^{b-e}$	0.39 $^{c-g}$	220.33 $^{h-l}$	578.33 $^{c-f}$	30.16 $^{b-f}$	25.01 $^{c-e}$	38.83 ^{b}	2.55 ^{b}
		25	4.27 $^{d-g}$	1.96 ^{bc}	2.33 $^{c-f}$	188.80 $^{b-f}$	0.48 ^{bc}	168.00 ^{l}	596.00 ^{cd}	29.22 $^{b-f}$	24.01 $^{c-e}$	39.72 ^{b}	2.79 ^{b}
		50	5.63 $^{a-d}$	2.88 ^{ab}	2.77 ^{cd}	180.57 $^{c-f}$	0.55 ^{ab}	196.67 $^{j-l}$	584.33 $^{c-e}$	28.61 $^{c-f}$	23.35 $^{c-e}$	38.79 ^{b}	2.64 ^{b}
	5	10	3.67 $^{e-g}$	1.57 ^{bc}	2.10 $^{d-g}$	199.83 $^{b-d}$	0.39 $^{c-g}$	229.00 $^{h-k}$	521.33 $^{f-i}$	30.64 $^{b-f}$	26.05 $^{c-e}$	38.96 ^{b}	2.55 ^{b}
		25	4.13 $^{d-g}$	1.63 ^{bc}	2.53 $^{c-e}$	191.70 $^{b-f}$	0.48 ^{bc}	206.00 $^{i-l}$	588.33 $^{c-e}$	30.09 $^{b-f}$	24.85 $^{c-e}$	39.27 ^{b}	2.69 ^{b}
		50	5.17 $^{b-d}$	2.40 $^{a-c}$	2.73 ^{cd}	184.73 $^{b-f}$	0.55 ^{ab}	203.67 $^{i-l}$	621.00 ^{bc}	30.01 $^{b-f}$	24.79 $^{c-e}$	38.83 ^{b}	2.70 ^{b}

TP, Total protein; ALB, Albumin; GLOB, Globulin; CL; Total cholesterol; LYZ, Lysozyme; GH, Growth hormone; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase.

Table 4-B
Means of the main impact and ANOVA for plasma biochemical and immune-related indexes in European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days.

Factors			Parameters										
Zeolite (ppt)	Density (Kg/ m^3)	Water exchange (%)	TP ((g/ dl)	ALB (g/ dl)	GLOB (g/ dl)	CL (mg/ dl)	LYZ (U/ ml)	Cortisol (ng/ ml)	GH (PG/ ml)	AST (U/ ml)	ALT (U/ ml)	Urea (mg/ dl)	Uric acid (mg/ dl)
Means of main impact													
0			3.68 ^{C}	2.03 ^{A}	1.64 ^{C}	216.35 ^{A}	0.15 ^{C}	416.61 ^{A}	403.44 ^{C}	35.29 ^{A}	32.99 ^{A}	40.28 ^{A}	2.77 ^{A}
10			4.42 ^{B}	2.14 ^{A}	2.18 ^{B}	190.69 ^{B}	0.35 ^{B}	300.00 ^{B}	530.22 ^{B}	29.94 ^{B}	24.86 ^{B}	39.05 ^{A}	2.65 ^{B}
15			4.97 ^{A}	1.97 ^{A}	3.01 ^{A}	183.51 ^{C}	0.48 ^{A}	206.93 ^{C}	618.59 ^{A}	29.17 ^{B}	24.00 ^{B}	38.81 ^{A}	2.59 ^{B}
	1		4.90 ^{A}	2.13 ^{A}	2.78 ^{A}	178.16 ^{C}	0.36 ^{A}	289.82 ^{C}	580.85 ^{A}	29.99 ^{B}	25.02 ^{B}	38.37 ^{B}	2.49 ^{C}
	2.5		4.12 ^{B}	2.05 ^{A}	2.08 ^{B}	195.78 ^{B}	0.31 ^{B}	303.07 ^{B}	503.59 ^{B}	30.61 ^{B}	25.93 ^{B}	38.88 ^{B}	2.60 ^{B}
	5		3.95 ^{B}	1.96 ^{A}	1.98 ^{B}	216.62 ^{A}	0.30 ^{B}	330.65 ^{A}	467.82 ^{C}	33.79 ^{A}	30.91 ^{A}	40.89 ^{A}	2.92 ^{A}
		10	3.71 ^{C}	1.92 ^{B}	1.79 ^{C}	208.44 ^{A}	0.27 ^{C}	335.58 ^{A}	478.52 ^{C}	32.74 ^{A}	29.51 ^{A}	39.87 ^{A}	2.69 ^{AB}
		25	4.16 ^{B}	1.85 ^{B}	2.31 ^{B}	197.86 ^{B}	0.32 ^{B}	305.37 ^{B}	525.48 ^{B}	31.63 ^{B}	27.28 ^{B}	40.02 ^{A}	2.75 ^{A}
		50	5.10 ^{A}	2.36 ^{A}	2.74 ^{A}	184.24 ^{C}	0.38 ^{A}	282.59 ^{C}	548.26 ^{A}	30.03 ^{C}	25.07 ^{C}	38.25 ^{B}	2.58 ^{B}
Analysis of variance (ANOVA, *p-values*)													
Z			0.0001	ns	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.01	0.04
D			0.0001	ns	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
W			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.002	0.04
Z x D			0.002	ns	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Z x W			0.005	ns	0.0001	0.0004	0.0001	0.001	ns	0.0005	0.0001	0.01	0.03
D x W			ns	ns	0.0001	0.004	ns	0.04	0.002	0.02	0.02	0.01	0.003
Z x D x W			ns	0.0005	0.0001	0.0005	ns	0.001	0.002	0.0001	0.0001	0.0004	0.0001

TP, Total protein; ALB, Albumin; GLOB, Globulin; CL; Total cholesterol; LYZ, Lysozyme; GH, Growth hormone; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; Z, Zeolite; D, Density; W, Water exchange; ns = $p > 0.05$.

Table 5-A
Physicochemical parameters of water for European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days (mean [$n = 3$]).

Factors			Parameters						
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	DO (mg/L)	pH	NH_4 (mg/L)	NH_3 (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	ARRS%
Contributed (Pooled) treatment means									
0	1	10	5.40 $^{g-i}$	6.78 ^{cd}	3.30 ^{d}	0.029 $^{a-c}$	0.110 ^{ab}	0.13 ^{i}	29.44 ^{i}
		25	5.53 $^{g-j}$	7.14 ^{bc}	3.06 ^{de}	0.015 $^{d-j}$	0.029 $^{e-g}$	0.38 $^{b-e}$	34.67 ^{hi}
		50	6.11 $^{b-g}$	7.53 ^{ab}	1.89 ^{ij}	0.009 $^{f-k}$	0.010 ^{fg}	0.40 $^{b-e}$	59.41 ^{cd}
	2.5	10	4.69 ^{kl}	6.24 ^{ef}	4.59 ^{b}	0.036 ^{ab}	0.082 ^{bc}	0.21 $^{g-i}$	16.72 ^{k}
		25	5.28 ^{ij}	6.65 $^{d-f}$	3.99 ^{c}	0.016 $^{c-h}$	0.054 $^{c-e}$	0.24 $^{f-i}$	16.73 ^{j}
		50	5.57 $^{f-j}$	6.93 ^{cd}	3.73 ^{c}	0.009 $^{f-k}$	0.030 $^{d-g}$	0.33 $^{c-g}$	20.39 ^{j}
	5	10	4.37 ^{l}	6.18 ^{f}	5.89 ^{a}	0.037 ^{a}	0.123 ^{a}	0.19 ^{hi}	16.71 ^{k}
		25	5.03 ^{jk}	6.29 ^{ef}	4.57 ^{b}	0.026 $^{a-d}$	0.055 $^{c-e}$	0.24 $^{f-i}$	16.28 ^{k}
		50	5.37 ^{ij}	6.48 $^{d-f}$	3.85 ^{c}	0.016 $^{d-i}$	0.035 $^{d-g}$	0.31 $^{c-h}$	17.76 ^{j}
10	1	10	6.18 $^{b-e}$	6.68 $^{c-e}$	2.79 ^{ef}	0.010 $^{f-k}$	0.010 ^{fg}	0.37 $^{b-e}$	40.34 ^{gh}
		25	6.35 $^{b-e}$	7.76 ^{a}	2.49 ^{fg}	0.012 $^{e-k}$	0.014 ^{fg}	0.33 $^{c-g}$	46.69 ^{fg}
		50	6.54 $^{a-d}$	7.86 ^{a}	1.19 ^{k}	0.008 $^{f-k}$	0.004 ^{fg}	0.53 ^{a}	74.50 ^{b}
	2.5	10	5.81 $^{e-i}$	6.47 $^{d-f}$	2.88 ^{e}	0.017 $^{c-g}$	0.038 $^{d-f}$	0.33 $^{c-g}$	38.54 ^{h}
		25	6.21 $^{b-e}$	7.59 ^{ab}	2.14 ^{hi}	0.016 $^{c-i}$	0.024 $^{e-g}$	0.31 $^{c-h}$	54.33 ^{de}
		50	6.14 $^{b-f}$	7.88 ^{a}	1.79 ^{j}	0.009 $^{f-k}$	0.012 ^{fg}	0.47 ^{ab}	61.73 ^{c}
	5	10	5.99 $^{d-h}$	6.51 $^{d-f}$	3.28 ^{d}	0.024 $^{b-e}$	0.067 ^{cd}	0.28 $^{e-h}$	30.00 ^{i}
		25	5.86 $^{e-i}$	6.61 $^{d-f}$	2.44 ^{gh}	0.019 $^{c-f}$	0.035 $^{d-g}$	0.28 $^{e-h}$	47.91 ^{ef}
		50	6.07 $^{c-g}$	6.72 $^{c-e}$	1.99 ^{ij}	0.012 $^{e-k}$	0.019 $^{e-g}$	0.42 $^{a-c}$	57.52 ^{cd}
15	1	10	6.51 $^{a-d}$	7.94 ^{a}	0.97 ^{kl}	0.001 ^{k}	0.008 ^{fg}	0.30 $^{c-h}$	79.27 ^{ab}
		25	6.68 ^{ab}	7.63 ^{a}	1.00 ^{kl}	0.001 ^{k}	0.002 ^{fg}	0.33 $^{c-g}$	78.63 ^{ab}
		50	6.66 $^{a-c}$	8.01 ^{a}	0.75 ^{l}	0.001 ^{k}	0.001 ^{g}	0.34 $^{c-f}$	84.02 ^{a}
	2.5	10	6.63 $^{a-c}$	7.67 ^{a}	1.14 ^{k}	0.003 $^{i-k}$	0.002 ^{fg}	0.27 $^{e-h}$	75.57 ^{b}
		25	6.65 $^{a-c}$	7.89 ^{a}	1.13 ^{k}	0.002 ^{jk}	0.002 ^{fg}	0.35 $^{b-f}$	75.71 ^{b}
		50	6.53 $^{a-d}$	7.99 ^{a}	1.02 ^{kl}	0.001 ^{k}	0.002 ^{fg}	0.41 $^{a-d}$	78.21 ^{ab}
	5	10	6.40 $^{a-e}$	7.58 ^{ab}	1.19 ^{k}	0.005 $^{h-k}$	0.003 ^{fg}	0.33 $^{c-g}$	74.50 ^{b}
		25	6.64 $^{a-c}$	7.78 ^{a}	1.14 ^{k}	0.004 $^{h-k}$	0.002 ^{fg}	0.29 $^{d-h}$	75.57 ^{b}
		50	6.96 ^{a}	7.99 ^{a}	1.06 ^{kl}	0.002 ^{k}	0.001 ^{g}	0.37 $^{b-f}$	77.28 ^{ab}

DO, Dissolved oxygen; NH_4, Ammonium; NH_3, Ammonia; NO_2, Nitrite; NO_3, Nitrate; ARRS, Alkaline reserve.

Table 5-B
Means of the main impact and ANOVA for water physicochemical parameters of European seabass (*Dicentrarchus labrax*) juveniles treated with different levels of zeolite, density, and water exchange for 75 days.

Factors			Parameters						
Zeolite (ppt)	Density (Kg/m^3)	Water exchange (%)	DO (mg/L)	pH	NH_4 (mg/L)	NH_3 (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	ARRS%
Means of main impact									
0			5.26 ^{C}	6.69 ^{C}	3.88 ^{A}	0.022 ^{A}	0.059 ^{A}	0.27 ^{C}	17.15 ^{C}
10			6.13 ^{B}	7.12 ^{B}	2.33 ^{B}	0.014 ^{B}	0.025 ^{B}	0.37 ^{A}	50.17 ^{B}
15			6.63 ^{A}	7.83 ^{A}	1.05 ^{C}	0.002 ^{C}	0.003 ^{C}	0.33 ^{B}	77.64 ^{A}
	1		6.22 ^{A}	7.48 ^{A}	1.94 ^{C}	0.009 ^{C}	0.021 ^{C}	0.35 ^{A}	58.55 ^{A}
	2.5		5.95 ^{B}	7.26 ^{B}	2.49 ^{B}	0.012 ^{B}	0.027 ^{B}	0.32 ^{B}	46.77 ^{B}
	5		5.86 ^{B}	6.91 ^{C}	2.82 ^{A}	0.016 ^{A}	0.038 ^{A}	0.30 ^{C}	39.64 ^{C}
		10	5.78 ^{C}	6.89 ^{C}	2.89 ^{A}	0.018 ^{A}	0.049 ^{A}	0.27 ^{C}	38.15 ^{C}
		25	6.03 ^{B}	7.26 ^{B}	2.44 ^{B}	0.013 ^{B}	0.024 ^{B}	0.31 ^{B}	47.84 ^{B}
		50	6.22 ^{A}	7.49 ^{A}	1.92 ^{C}	0.008 ^{C}	0.013 ^{C}	0.39 ^{A}	58.98 ^{A}
Analysis of variance (ANOVA, *p-values*)									
Z			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
D			0.0001	0.0001	0.0001	0.0001	0.0001	0.0007	0.0001
W			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Z x D			0.0001	0.0001	0.0001	ns	0.002	0.01	0.0001
Z x W			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
D x W			ns	0.0001	0.0001	ns	ns	ns	0.0001
Z x D x W			0.03	0.0001	0.0001	ns	0.009	0.001	0.0001

DO, Dissolved oxygen; NH_4, Ammonium; NH_3, Ammonia; NO_2, Nitrite; NO_3, Nitrate; ARRS, Alkaline reserve; Z, Zeolite; D, Density; W, Water exchange; ns = $p > 0.05$.

indicating that the fish have adapted to their new environment [66–68].

The physicochemical parameters of water play a crucial role in the survival and well-being of fish species [69]. The results obtained from our study highlight the significance of monitoring and maintaining optimal levels of these parameters in aquatic environments. In the current investigation, increasing stocking density had a negative impact on various water quality measures, particularly oxygen levels and ammonia nitrogen derivatives NH_4, NH_3, NO_2 and NO_3 with zeolite addition. However, low stocking density and water

exchange reduced the levels of the following ammonia derivatives, NH_4, NH_3, and NO_2, but increased as stocking density increased. According to the previous findings, raising European seabass at a high stocking density (200 fish/m^3) led to a significant decline in water quality parameters like dissolved oxygen, NH_3—N, NH_4—N, NO_2—N, and NO_3—N by 11.11%, 49.5 %, 33.33 %, 38.46 %, and 9.09 %, respectively, when compared to the low stocking density [32].

Conclusions

Over a 75-day period, the incorporation of zeolite, optimization of stocking density, and strategic water exchange significantly enhanced water quality for European seabass. This improvement was marked by a reduction in ammonia derivatives and the stabilization of dissolved oxygen levels, particularly beneficial for seabass cultured at both low and high stocking densities. Furthermore, the supplementation of zeolite, adoption of lower stocking density, and increased water exchange demonstrated positive impacts on growth performance, efficient feed utilization, and mitigated the adverse effects of high stocking density on immune-related, hematobiochemical, and plasma biochemical parameters. In conclusion, our findings recommend the application of 15 ppt level of zeolite, stocking density of 1 kg/m^3, and 50 % water exchange regimen for 75 days as an effective strategy to enhance the overall growth, feed utilization, water quality, and biochemical parameters of juvenile European seabass. The results may offer insights for fish rearing development, but a comprehensive understanding of the molecular mechanisms influencing fish health and aquaculture management requires further research.

Data availability statement

The corresponding author can provide the supporting data sets used and/or analyzed during the current study upon request.

CRediT authorship contribution statement

Ghada R. Sallam: Conceptualization, Data curation, Formal analysis, Methodology. **Yusuf Jibril Habib:** . **Mohammed F. El Basuini:** Writing – original draft, Writing – review & editing. **Walied M. Fayed:** Conceptualization, Writing – review & editing. **Akram Ismael Shehata:** Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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