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Research Article

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The Applicability of Activated Carbon, Natural Zeolites, and Probiotics (EM®) and Its Effects on Ammonia Removal Efficiency and Fry Performance of European Seabass *Dicentrarchus labrax*

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Abstract

Ammonia is toxic to fish if allowed to accumulate and not-properly managed in fish production systems. Six treatments were studied to evaluate the effectiveness of applying three commercial Ammonia Removal Products (Activated Carbon, Natural Zeolite and Effective Micro-organisms (EM®)). These treatments are: (1) C, Control, (2) AC5, activated carbon at 5ppt, (3) AC10, activated carbon at 10 ppt, (4) Z5, Zeolite at 5 ppt, (5) Z10, zeolite at 10 ppt, and (6) EM400, EM at 400 ppm. European Seabass fry (240.74 mg/fish IW) were stocked into glass aquaria (50 litres each) at density of 20 fry/aquarium. Water exchange rate was 20% daily and the experiment continued for 35 days. Fish were fed on experimental diet contained 51.37% crude protein, three meals daily, and six days weekly. Data of water quality, survival and growth performance were recorded weekly.

The results revealed that, ammonia removal efficiency of the tested products was significantly ($P \leq 0.05$) better than control, with no significant differences ($P>0.05$) between the evaluated products. The best ammonia removal rate (76.60%) was obtained at Z10 treatment. Fish survival (%) ranged between 37.78% to 90% with highly significant ($P \leq 0.05$) differences between treatments. The best survival (%) was obtained at EM400 (90%), while the lowest (37.78%) was obtained at AC5 and AC10 treatments. Growth performance was significantly ($P \leq 0.05$) higher at (EM400, Z10, and Z5), compared with treatments (AC10, AC5, and C).

It could be clearly concluded that, using Probiotics (EM®) and Zeolite for ammonia removal might be a good potential alternative choice, while activated carbon cannot be recommended for marine fish rearing tanks in terms of low survival and growth performance and also the higher expected production cost.

Keywords: *Dicentrarchus labrax*; Ammonia removal products; Ammonia removal efficiency; Survival; Growth

Introduction

Of all the water quality parameters that affect fish performance, ammonia is the most important effluent after oxygen concentration, especially in intensive systems [1]. Fish consume oxygen and excrete ammonia and carbon dioxide. Ammonia is a major excretion product of the fish reared under intensive feeding regime of high nitrogen containing feeds [2]. Ammonia is primarily excreted across the gill membrane with only a small amount excreted in the urine [3]. Also, ammonia is produced in fish ponds by the bacterial ammonification process of the nitrogenous organic matter in water column under aerobic conditions, as well as in pond sediment under anaerobically conditions. Ammonia present in the water either in the form of un-ionized ammonia (NH_3) and/or the ammonium ion (NH_4^+). The relative proportion of the two forms present in water is mainly affected by pH and temperature. Increasing both pH [4] and temperature [5] will increase the percentage of NH_3. Un-ionized ammonia is the toxic form. It is 300 to 400 times more toxic than NH_4^+ [6,7]. Ammonia is toxic to fish and other aquatic organisms in very low concentration, about 0.2 mg/L [8]. Ammonia at relatively low concentration can have negative effects on fish tissues such as gill damage and physiological factors such as poor growth, higher oxygen consumption and more susceptible to bacterial infections [1,9] and can restrict yields in intensive fish culture [10]. When ammonia accumulates to toxic levels, fish cannot extract energy from feed efficiently. If the ammonia concentration gets high enough, the fish will become lethargic and eventually fall into a coma and die [11]. European seabass is one of the most important cultivated fish species in the Mediterranean countries. In seabass juveniles with average weight of 11 gm, mortality of 28.9% and 42.6% occurred within the first 8 days at un-ionized ammonia nitrogen (UIA-N) concentrations of 0.90 and 0.88 mg/L, respectively, while the 0.26 mg/L UIA-N concentration, under an average pH (8.0), temperature (21.8°C), salinity (37.0 ppt) can be considered as a safe long-term limit [12]. Person-Le Ruyet et al. [13] have been reported that the 96-h LC50 was 1.7 mg/L UIA-N in seabass juvenile.

There are four methods of ammonia removal technologies, three methods are common in aquaculture systems; (1) nitrification, and (2) ion exchange and (3) Air stripping, while the other method is not common; (4) Breakpoint Chlorination [14]. Nitrification is a two-step oxidation of ammonia to nitrate by autotrophic bacteria, and is an essential part of the recirculating fish culture system [15]. For nitrification, materials such as oyster shell, rock, sand, plastic, etc. are used to prepare a substrate for bacteria. Nitrification process requires applying probiotics. There is a considerable interest in use of probiotics to improve water quality in pond aquaculture but the positive influence is still in infancy [16]. Improving water quality by the addition of

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probiotic strains especially of the gram-positive genus *Bacillus* sp. has been documented through few studies [17-21]. Probably, since this bacterial group is more efficient than gram-negative in transforming organic matter to CO_2 [22]. It is suggested that maintaining high levels of probiotics in production ponds, fish farmers can minimize the accumulation of dissolved and sedimentary organic matter during the growing season, and thus ammonia level. The addition of probiotics to aquaculture exert multiple advantages as reduction in nitrogen and phosphorus concentrations; enhance decomposition of organic matter, increase algal growth, abundance of dissolved oxygen, decrease in toxic algae, control of toxic metabolites such as ammonia and nitrite and increase profit margin of shrimp and fish farms [16].

Ion exchange method is a process in which ions of an exchanger (synthetic or natural material) are exchanged with certain ions in wastewater, such as ammonia and/or heavy metals. Some natural materials, such as zeolite, and activated carbon are being used in removing ammonia from wastewater culture systems. One of the best zeolites in ammonia removal is Clinoptilolite [23]. Zeolite is a naturally occurring rock that has a fairly unique structure which has large internal cavities and entry channels which easily fill with water, air, and other molecules. The adsorptive surface area is several hundred meters per gram of zeolite and can adsorb up to 30% of their weight of gases and other molecules [24]. They have strong capacities to adsorb and desorb molecules that allows for rapid uptake and loosing of charged particles. Therein lies the relevance of zeolite to aquaculture. Zeolite eradicate ammonia out of the water and holds it inside its' porous structure [25,26]. The ability of zeolite to adsorb ammonia is not unlimited and once it reaches saturation, it can be placed into a salt water solution to be recharged. This charging and removing of ammonia from zeolite can be repeated many times prior to the zeolite become clogged and useless. In aquaculture, zeolite can be used in filters for removing ammonia from fish holding tanks [27]. The other benefits of zeolite are adsorbing toxic gases, regulate pH level of pond water, provide micro nutrients, adsorb odors, bacteria, suspended solids, waste and organic matter in fish ponds.

Activated carbon is the generic term used to describe the family of carbonaceous adsorbents with an extensively developed internal pore structure. A wide variety of activated carbon products are available, exhibiting markedly different characteristics. They are commonly made from wood, coal, lignite and coconut shell. Activated carbon is a form of carbon which has been treated in a special way that makes its surfaces highly adsorbent [28]. Activated carbon has the strongest physical adsorption forces or the highest volume of adsorbing porosity. It is relatively inexpensive with an enormous specific surface area, typically about 1000 $m^2 g^{-1}$ [29]. Adsorption occurs when molecules in the fluid phase are held for a period of time by forces emanating from an adjacent surface [30]. Activated carbon are being used in aquaculture to take impurities out of water; remove halogens such as ozone, chlorine and bromine, and remove color and metabolic by-products in recirculating systems. Activated carbon's adsorptive characteristics are based on the principle that the greater the surface area, the higher the number of adsorptive sites available.

Thus, published evidence for improving water quality is limited, except for the nitrification [16,31]. No studies have yet been carried out to compare activated carbon, zeolite and probiotic (EM®) as a direct application of ammonia removal products to mariculture systems. Therefore, this study was performed to evaluate the effect of these three commercial ammonia removal products on the ammonia removal efficiency and nursery performance of seabass fry reared under intensive culture system using saline underground water.

Materials and Methods

Experimental place

This experiment was carried out in Fish Rearing Lab., El-Max Research Station, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt during the period from 10 June to 14 July, 2015.

Experimental fish and rearing conditions

European Seabass, *Dicentrarchus labrax* fry with average initial weight of 240.74 ± 2.7mg/fish, obtained from the Marine Fish Hatchery, NIOF, Alexandria, Egypt, were used in this experiment. Seabass fry were stocked into 18 glass aquaria (each 50 litres of water) at initial density of 20 pcs/aquarium. Each aquarium was supported with artificial aeration through air blower. The water exchange rate was 20% per day and 80% every week during the periodical fish samples.

Ammonia removal products

Activated carbon (AC): It is used in this experiment is a product of Jacobi Company, Sweden, http://www.jacobi.net/water-treatment/ and was purchased from the Egyptian distributor. The price is 5 US $/kg.

Natural zeolite (Z): The natural Clinoptilolite was purchased and used as an adsorbent for ammonia. Table 1 shows the chemical composition of Clinoptilolite which is purchased from Yemen (http://alixzeolite.com/en/). The price is 0.9 US $/kg.

Probiotic product (EM): It is used in this experiment is "Effective Micro-organisms (EM®)" which is a liquid product of EMRO Japan https://emrojapan.com/aquaculture/ with co-operation with the Ministry of Agriculture and Land Reclamation, Egypt. EM technology is used in more than 100 countries around the world in numerous fields including agriculture, animal husbandry, aquaculture, environmental purification, etc. The benefits of using EM Technology in aquaculture include improvement of water quality in farm ponds and prevention of accumulation of sludge. The price is 0.8 US $/litre.

Experimental design

Six treatments were performed to evaluate the effectiveness of adding activated carbon, zeolite and Probiotics, Effective Micro-organisms (EM®) into fish rearing water on the nursery performance of seabass fry reared in saline underground water with high content of ammonia. These treatments are: (1) C, Control, (2) AC5, activated carbon at 5ppt, (3) AC10, activated carbon at 10 ppt, (4) Z5, Zeolite at 5 ppt, (5) Z10, zeolite at 10 ppt, and (6) EM400, EM at 400 ppm. Both Natural zeolite and activated carbon were put inside PVC nets, fixed on the wall of tank, and suspended in the water column. EM was added in the morning after changing 20% of water volume every day. Each treatment was performed in three replicates. The treatment of EM400 was chosen based on the recommendation of Lotfy [32] with Gilthead seabream. The experiment lasted for 35 days. Activated carbon and zeolite materials were being removed and replaced every week. EM product was being compensated after each time of water exchange to keep the concentration of EM at 400 ppm.

Element	%	Element	%	Element	%
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_2	0.112	SrO	0.047
CaO	3.583	Cl	0.025	MnO	0.120

Table 1: Chemical composition of Yemen natural zeolite.

Experimental diets and feeding regime

The experimental diet was formulated and prepared in El-Max Research Station with crude protein content of 51.37%. The chemical Analyses of the diet is shown in Table 2. This experimental diet was produced with feed particle sizes ranged from 800-1500 µm based on fish size. The feeding protocol was performed according to Moretti et al. [33]. Fish were fed 3 times daily, six days per week. The feeding rate was re-adjusted depending on live fish weights every 7 days.

Measured parameters

Water quality parameters: Water quality parameters (temperature, pH, total ammonia nitrogen (TAN), and dissolved oxygen) were monitored three times per week to study the effect of ammonia removal products on water quality parameters. Temperature and pH were measured using portable pH Meter (pH-8424) (HANNA Instrument). Dissolved oxygen was measured by HI-9142 (HANNA Instrument). The concentration of total ammonia nitrogen (TAN) was analyzed using YSI 9300 photometer and YSI Professional Plus. The concentration of un-ionized ammonia was calculated as a percentage of TAN according to U.S. Environmental Protection Agency [34].

Growth performance parameters: At the end of the experiment, final body weight (FW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), and survival rate, were calculated according to the following equations:

$$Weight\ gain\ (g/fish):\ WG = W_t - W_0$$

Where: W_0: initial mean weight of fish in grams.

W_t: final mean weight of fish in grams.

Average daily gain (g/fish/day): $ADG = W_t - W_0 / n$

Where: n: experiment period (days).

$$Specific\ growth\ rate\ (\%/day):\ SGR = 100 \times (\ln W_t - \ln W_0) / \ days$$

Where ln: natural logarithm.

$$Survival\ rate\ (\%) = 100 \times (final\ number\ of\ fish / initial\ number\ of\ fish)$$

Feed analytical methods

Feed samples were taken to determine the proximate composition analyses of the diet including moisture, protein, lipid and ash contents according to AOAC [35] methodology.

Energy content

Gross energy (GE) content of the diet was estimated according to the following equation:

GE in (MJ / kg DM)= [CP*23.6+39.4+NFE*17.2]

Where: CP = Crude Protein; CL = Crude Lipids; and NFE = Nitrogen Free Extract.

GE: gross energy calculated on the basis of 23.6 k joule, 39.4 k joule and 17.2 k joule gross energy g^{-1} for protein, ether extract and NFE respectively [36].

Statistical analysis

Data were subjected to statistical analysis according to the software program SPSS version 16 (Standard Version 16 SPSS Inc. Chicago, Illinois, USA). Analysis of variance, one-way ANOVA was used to evaluate the effect of ammonia removal products on the water quality, survival and growth performance of European seabass. The differences within each experimental treatment were evaluated using Duncan test at 0.05 probability.

Results

Water quality and ammonia removal efficiency

Weekly results of total ammonia nitrogen, (TAN) during five weeks' experimental period have been shown in Table 3 and Figure 1. During the experimental weeks, there were highly significant ($P \leq 0.05$) differences between the tested treatments and the control one. The overall results can be arranged from the best to worst as follows: Z10, Z5, EM 400, AC 10, AC5 and C, respectively.

Final results of water quality (temperature (°C), pH, total ammonia nitrogen (TAN), ammonia removal rate as % of the source water and ammonia removal rate as % of the control treatment) have been collected and shown in Table 4 and Figure 2. There were no significant ($P>0.05$) differences in the data of pH and water temperature during the experimental period. However, a highly significant ($P \leq 0.05$) differences in the average final content of TAN and un-ionized ammonia (NH_3) were observed between the tested treatments and the control one. The results can be arranged from the best to the lowest as follows: Z10, Z5, EM400, AC10, AC5, and C. Values of ammonia removal rate as % of

Chemical Composition (%) on DM basis	
Dry matter (DM)	92.24
Crude protein (CP)	51.37
Ether extract (EE)	14.29
Crude fibre (CF)	1.81
Ash	9.30
NFE[1]	17.28
Gross energy (kJ /g)[2]	20.74

[1]NFE = 100 – (% Moisture + % Ash + % lipid + % protein)
[2]GE: Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g^{-1} protein, ether extract and NFE respectively (NRC, 1993).

Table 2: Chemical analyses of the experimental diet.

Treatments*	Week 1	Week 2	Week 3	Week 4	Week 5
C	0.170 ± 0.065^b	0.132 ± 0.034^b	0.145 ± 0.030^b	0.294 ± 0.199^b	0.173 ± 0.009^c
AC5	0.090 ± 0.000^a	0.103 ± 0.009^{ab}	0.105 ± 0.010^{ab}	0.105 ± 0.011^a	0.116 ± 0.022^b
AC10	0.074 ± 0.006^a	0.085 ± 0.008^{ab}	0.067 ± 0.015^a	0.133 ± 0.058^a	0.083 ± 0.015^a
Z5	0.097 ± 0.003^a	0.090 ± 0.001^a	0.081 ± 0.007^a	0.072 ± 0.004^a	0.077 ± 0.009^a
Z10	0.082 ± 0.002^a	0.071 ± 0.007^a	0.057 ± 0.004^a	0.074 ± 0.002^a	0.067 ± 0.009^a
EM400	0.191 ± 0.002^b	0.088 ± 0.008^{ab}	0.073 ± 0.014^a	0.080 ± 0.010^a	0.095 ± 0.013^{ab}

*C = Control; AC5 = Activated Carbon, 5 ppt; AC10 = Activated Carbon, 10 ppt; Z5 = Zeolite, 5 ppt; Z10 = Zeolite, 10 ppt; and EM400 = Effective Micro-organism (EM) at 400 ppm.

Table 3: The weekly results of total ammonia nitrogen (TAN) in the experimental tanks of European Seabass fry, tested with different ammonia removal products during five weeks' experimental period.

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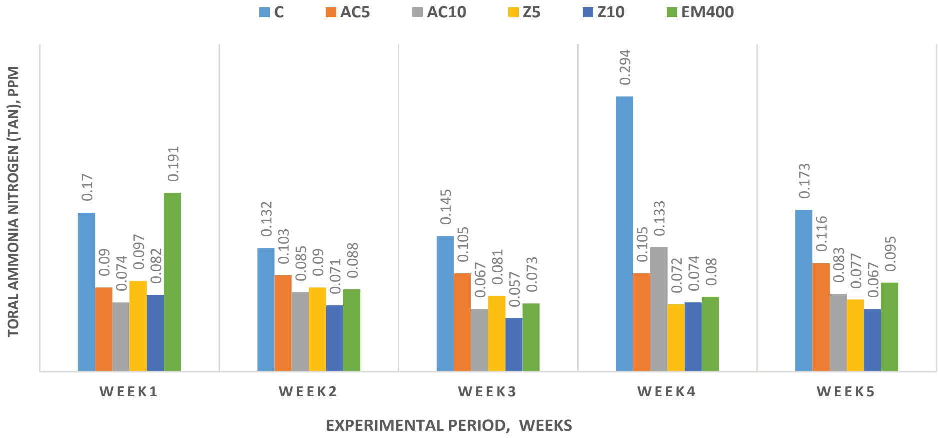

Figure 1: Total ammonia nitrogen (TAN) in the rearing tanks of European seabass fry tested with different ammonia removal products during five weeks' experimental period.

Treatments*	pH	Water Temperature, (°C)	Total Ammonia Nitrogen (TAN), (ppm)	Un-ionized Ammonia (NH_3), (ppm)	Ammonia Removal Rate; as % of the Source (ARRS)**	Ammonia Removal Rate; as % of the Control (ARRC)***
C	8.00 ± 0.01	26.42 ± 0.08	0.1830 ± 0.043^{b}	0.0107 ± 0.002^{c}	39.00 ± 14.50^{b}	0
AC5	8.03 ± 0.07	26.39 ± 0.20	0.1039 ± 0.002^{a}	0.0063 ± 0.001^{b}	65.38 ± 0.79^{a}	67.64 ± 2.03^{c}
AC10	8.06 ± 0.06	26.90 ± 0.04	0.0887 ± 0.013^{a}	0.0063 ± 0.001^{b}	70.44 ± 4.36^{a}	80.63 ± 11.18^{bc}
Z5	7.89 ± 0.03	26.68 ± 0.33	0.0832 ± 0.001^{a}	0.0040 ± 0.000^{ab}	72.27 ± 0.24^{a}	85.31 ± 0.63^{ab}
Z10	7.90 ± 0.01	26.52 ± 0.19	0.0702 ± 0.002^{a}	0.0030 ± 0.000^{a}	76.60 ± 0.61^{a}	96.40 ± 1.56^{a}
EM400	7.95 ± 0.01	26.24 ± 0.04	0.1055 ± 0.003^{a}	0.0053 ± 0.000^{ab}	64.85 ± 0.93^{a}	66.28 ± 2.38^{c}

*C = Control; AC5 = Activated Carbon, 5 ppt; AC10 = Activated Carbon, 10 ppt; Z5 = Zeolite, 5 ppt; Z10 = Zeolite, 10 ppt; and EM400 = Effective Micro-organism (EM) at 400 ppm.
**Total ammonia nitrogen (TAN) content of the source water was 0.3 ± 0.06 ppm.
**Ammonia Removal Rate; as % of the Source (ARRS) = (TAN source – TAN treatment) ˙100/TAN source.
***Ammonia Removal Rate; as % of the Control (ARRC) = (ARRS treatment-ARRS control) ˙100/ARRS control.

Table 4: Water quality parameters for Seabass rearing tanks tested with different ammonia removal products.

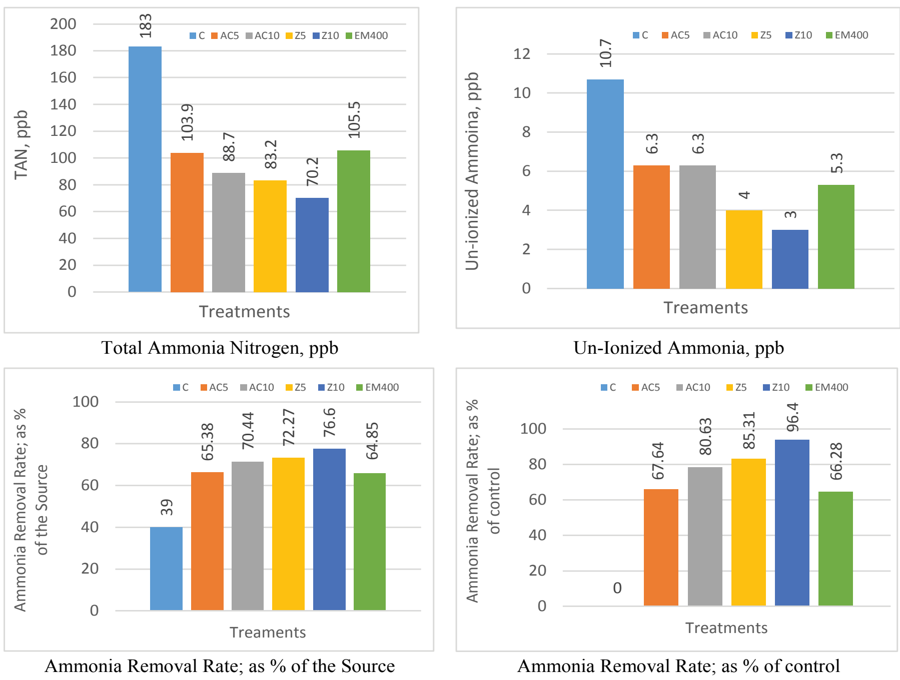

Figure 2: Effect of using different ammonia removal products on the total ammonia nitrogen (TAN), un-ionized ammonia, ammonia removal rate as % of the source water and ammonia removal rate as % of the control in the rearing tanks of seabass fry.

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the source water revealed that all the tested products achieved better results than the control and the best result was achieved at Z10 with ammonia removal rate 76.6%. The concentration of dissolved oxygen was within the safe limits (5.1 ppm to 5.8 ppm).

Survival rate

The survival rate of seabass fry at the end of this experiment was shown in Table 5 and Figure 3. Values of survival rate ranged between 37.78% to 90%. The results clearly showed that, there was highly significant difference ($P \leq 0.05$) between the treatments. The best survival rate was obtained at EM400, while the lowest ones were obtained at AC5 and AC10 with average percent of 37.78 for both previous treatments.

Growth performance

Final body weight, weight gain (WG), average daily gain (ADG), and specific growth rate (SGR, %) were shown in Table 5 and Figure 3. Data of Growth performance showed that the best growth rate was achieved at EM400 treatment with final weight (1.207 gm/fish), while the lowest one was for the control treatment (0.953 gm/fish) with significant ($P \leq 0.05$) differences between treatments (EM400, Z10, and Z5) and the other treatments (AC5, AC10, and C). The results clearly showed that adding activated carbon has no influence on the growth rate of seabass fry.

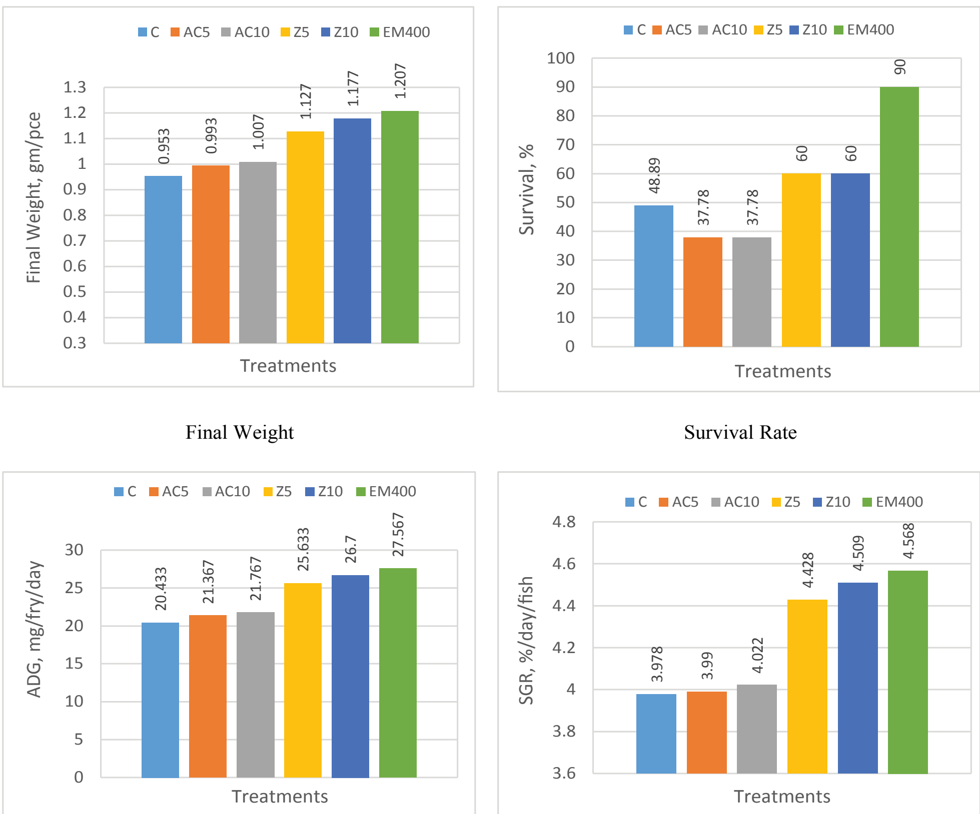
Discussion

The most widely used methods for ammonia removal from

Treatments*	Initial Weight, gm/fish	Final Weight, gm/fish	Weight Gain, gm/fish	Average Daily Gain, mg/fish/day	Specific Growth rate, %/day	Survival Rate, %
C	0.236 ± 0.007	0.953 ± 0.055^{c}	0.715 ± 0.063^{c}	20.433 ± 1.82^{c}	3.978 ± 0.254^{c}	48.89 ± 5.88^{bc}
AC5	0.246 ± 0.007	0.993 ± 0.053^{bc}	0.749 ± 045^{bc}	21.367 ± 1.27^{bc}	3.990 ± 0.064^{bc}	37.78 ± 4.44^{c}
AC10	0.247 ± 0.010	1.007 ± 0.044^{bc}	0.762 ± 0.053^{bc}	21.767 ± 1.50^{bc}	4.022 ± 0.222^{bc}	37.78 ± 9.69^{c}
Z5	0.231 ± 0.004	1.127 ± 0.038^{ab}	0.897 ± 0.033^{ab}	25.633 ± 0.93^{ab}	4.428 ± 0.056^{ab}	60.00 ± 3.47^{b}
Z10	0.242 ± 0.005	1.177 ± 0.062^{a}	0.935 ± 0.067^{a}	26.700 ± 1.93^{a}	4.509 ± 0.209^{a}	60.00 ± 3.85^{b}
EM400	0.243 ± 0.005	1.207 ± 0.073^{a}	0.965 ± 0.072^{a}	27.567 ± 2.09^{a}	4.568 ± 0.166^{a}	90.00 ± 5.77^{a}

*C = Control; AC5 = Activated Carbon, 5 ppt; AC10= Activated Carbon, 10 ppt; Z5= Zeolite, 5 ppt; Z10 = Zeolite, 10 ppt; and EM400 = Effective Micro-organism (EM) at 400 ppm.
** The mean difference is significant at the 0.05 level.

Table 5: Growth performance and survival rate of European seabass fry tested with different ammonia removal products**.



Final Weight

Survival Rate

Average Daily Gain

Specific Growth rate

Figure 3: Effect of using different ammonia removal products on the survival rate, final weight, average daily gain, and specific growth rate of European seabass fry reared under intensive nursery system.

polluted water are air stripping, ion exchange with natural zeolite and biological nitrification/denitrification [37]. During the first week of this experiment, it was noticed that there were no significant ($P>0.05$) differences between the control and EM400 treatments. This may be attributed to that the beneficial micro-organisms in EM product have been transferred from the media that has been grown in to the fish rearing tanks with low concentration of nutrient and high salinity. This explanation is in compatible with other researchers [14,23,38,39]. Caglia [38] found that ammonia removal efficiency using biological process varied between 28.31% to 92.46% depending on water temperature, quantity of organic matter, retention time, species and density of micro-organisms. The organic matter will be converted to inorganic forms through the decomposition process using the beneficial microorganisms. This process occurs by different specific of bacteria that convert the ammonia (NH_3) that is present in the organic matter into ammonium salts (NH_4). Subsequently, ammonium is converted to nitrite (NO_2) by specific autotrophic bacteria (*Nitrosomonas*), following by the release of nitrate (NO_3) from nitrite through another particular type of bacteria (*Nitrobacter*) [40]. The union of these processes goes under the name of Nitrification.

Activated carbon and natural zeolite adsorbents have each been used to adsorb Volatile Organic Compounds (VOC) and other pollutants from relatively dilute concentrations in both water and air to control pollution [41]. The differences in values of TAN between AC10 and AC5 treatments during the experimental period were relatively high, while the differences in values of TAN between Z10 and Z5 treatments were relatively low. This may be attributed to that, for Activated Carbon, its efficiency will be adversely affected by the time and cannot be reactivated (Desorbing/regeneration) easily because regeneration process requires high temperature up to 830°C. So, it must be changed alternately every one or two weeks depending on the amount of ammonia excreted. While, zeolite can be reactivated easily though washing in clean salt water and re-used many times before being 100% clogged. This conclusion agrees with the results of [42]. It was found that the loss of clinoptilolite capacity to be regenerated may occur after 10-11 regenerations [42-44]. Also, regeneration can be done biologically [45,46] or chemically [42]. Zeolite is cheaper than activated carbon and does not need conditioning before use. This makes use of zeolite in fish culture facilities a better option for reducing ammonia concentration [23]. Both activated carbon and zeolite are adsorbers not absorbers because the pollutant is adsorbed on the internal surface of a granule, bead, or crystal of adsorbent material. It is not absorbed by a chemical reaction. This is an important difference [41].

Ammonia removal rate for the control treatment was 39% compared with the source water. This value can be explained by the theory that water movement up to the header tank and down from the header tank to the inlet pipes and also artificial aeration surely helped in removing this percentage of ammonia. This explanation is compatible with Tchobanoglous and Urton [37] about using air stripping as a common method of ammonia removal. Generally, the recorded values of pH, TAN, and un-ionized ammonia in this experiment were within the acceptable rage of seabass hatcheries [12,13,47-50].

Results of survival rate obtained in this experiment were surprising, precisely for AC5 and AC10 treatments with the lowest values (37.78%). Also, one of the most amazing observations that have been seen and confirmed only for treatments with activated carbon is the attacking of zeolite containing bags by the experimental fish and feeding on them. It is likely that, this strange behavior is due to the lack of certain necessary micro-nutrients that fish need for vital activities to do the best growth and to preserve their life. This scientific theory may be supported by Sigworth and Smith [51] whom found that the use of activated carbon can remove trace metals and compounds. Also, Caglia [38] found that the activated carbon may adsorb low concentrations of some micro-elements from the rearing water that can be considered very important for fish livelihood. Whilst, this phenomenon may be less potential for zeolite treatments. The previous opinion may explain the lowest rate of survival under activated carbon treatment. The best survival rate was achieved under EM treatment. This may be attributed to the role of the beneficial micro-organisms for improving both of the water quality criteria and immunity system for the cultivated marine fishes [32,39]. The overall results obtained of this experiment agree with the findings of many authors [32,39,52-54]. Lotfy [32] found that adding 400 ppm (EM) in the rearing water of Gilthead seabream increased the survival rate from 26.1% in the control treatment to 54.97% at EM 400 ppm. The same trend was observed by El-Okaby [39] when added the symbiotic MICROPAN AQUA® in the rearing water of seabream. Survival rate is the best evaluating way for the efficiency of fish hatcheries in general and marine fish hatcheries in particular.

The same trend was observed in growth performance, but without significant differences between EM treatment and Zeolite treatments in spite of better ammonia removal efficiency for Zeolite treatments compared with EM treatments. This may be attributed to the significant role of micro-organisms in improving the digestive system [32,55]. Appropriate probiotic applications were shown to improve intestinal microbial balance, thus leading to improve feed absorption [56], and reduce pathogenic problems in the gastrointestinal tract [32,39]. Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible components [57]. Tovar-Ramírez et al. [58] noticed that the growth of seabass larvae fed 1.1% live yeast as a probiotic was increased than the control group. Also, Lotfy [32] found that adding probiotic (EM) in the rearing water of Gilthead seabream at concentration 400 ppm improved average daily gain of fish fry from 41.71 (control) to 55.75 mg/fish/day (at EM 400 ppm). Also, El-Okaby [39] studied the effect of different levels of MICROPAN AQUA® as a symbiotic in the rearing water aquaria on water quality and performances of gilthead seabream, *Sparus aurata*, fingerlings. The author found that gilthead seabream fingerlings showed higher rates in growth performance, condition factors, feed utilization and survival rate in treatments supported with symbiotic compared with control.

On the other hand, growth performance of seabass under control treatment in this study was the lowest. This attributed to the highest concentration of ammonia compared with the other treatments. Ammonia causes stress and damages of gills and other tissues, even in small amounts. Fish exposed to low levels of ammonia over time are more susceptible to bacterial infections, have poor growth, and will not tolerate routine handling [1,5,12].

Conclusion

It could be concluded from the results of this experiment that, using Zeolite and Effective-Micro-Organisms (EM) gave the best results and might be a good alternative choice for each other, while, activated carbon cannot be recommended into fish rearing tanks in terms of lower survival and growth performance and also the highest expected ammonia removal cost. Other research works should be carried out to clarify many vague issues about the best way to make use of both zeolite and probiotic in marine aquaculture using underground saline water.

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Ethical Issues

We certify that all data collected during this study is presented in this manuscript and no data from the study has been or will be published separately.

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