Rule of dietary activated wood charcoal on the growth and biochemical composition of Gilthead Seabream (*Sparus aurata*) reared under different stocking densities

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Abstract: A 60-days' two-way factorial design study was performed to evaluate the role of dietary activated wood charcoal (AWC) at various fish rearing densities on growth performance, body composition, and water quality of Gilthead Seabream (*Sparus aurata*) fingerlings. Two levels of AWC (20 and 40 g kg^{-1}) were added to the control diet (T1; 0 g kg^{-1} AWC) and fed to Gilthead Seabream [initial weight (IW) of 0.5±0.1 g] at three different density levels (D1; 20, D2; 40 and D3; 60 fish m^{-3}). At the end of the experiment, survival (S), fish weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and energy retention (ER) of the fish groups fed 20 and 40 g kg^{-1} (T2 and T3 AWC) in diet were significantly ($P<0.05$) better compared to the control fish group at all density levels (D1, D2 and D3). Moreover, some proximate composition such as crude protein (CP) of the fish groups fed on 20 and 40 g kg^{-1} AWC diets at different density levels showed higher values ($P<0.05$) than those of control fish groups (T1; 0 g kg^{-1} AWC) at the three density levels (D1, D2 and D3 respectively). These data were powered by the data of the water quality that showed significant ($P<0.05$) enhancement in both dissolved oxygen and ammonia concentrations by the increment in dietary charcoal levels even at higher density levels (D2 and D3). The above-mentioned parameters' data suggested that 40 g kg^{-1} dietary AWC can be considered as a suitable level to maintain optimal growth of Gilthead Seabream juveniles especially at high density levels (D2 and D3) as well as to enhance water quality of the rearing tanks.

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Key Words: Gilthead Seabream, Charcoal, Nutrition, Growth, Proximate composition, stocking densities.

1. Introduction

Gilthead seabream species is one of the most important marine fish species since the early twentieth century till the present time. Gilthead seabream and seabass are the two marine fish species, which have characterized the development of marine aquaculture in the Mediterranean basin in the last two decades. The substantial increase in production levels of these two high value species is referred to the progressive improvement in the technologies involved in the production of fry in hatcheries. As a result of this technological progress more than one hundred hatcheries have been built in the Mediterranean basin. At present the farmed production of these two species that is derived from hatchery produced fry is far greater than the supply coming from the wild. The development of these techniques, based originally on Japanese hatchery techniques has followed its own evolution and has resulted in what could be called a Mediterranean hatchery technology that is still evolving to provide higher quality fish and to reduce cost of production [1].

Marine fish require high protein levels in their feeds to maintain optimal growth and survival. However, the presence of elevated protein levels in fish feeds loads more nitrogenous wastes such as ammonia [2] to the aquatic environment. Total ammonia nitrogen (TAN) is considered a toxic factor to fish limiting their survival and growth [3]. Fish can be reared under different density conditions as extensive; 1-5 fish m^{-2}, semi-intensive; 5-30 fish m^{-2} and intensive; > 30 fish m^{-2} [4]. The most economical systems are the semi-intensive and intensive, however, these two systems may result in producing large amounts of toxic metabolites such as; TAN and carbon dioxide (CO_2) to the water environment [5]. High fish numbers in a rearing tank will definitely result in higher levels of nitrogenous waste products. The high toxicity of ammonia comes from the un-ionized form (NH_3-N) and low toxicity results from the ionized form (NH_3). However, wet chemical tests measure TAN; the ionized and un-ionized forms [6].

Charcoal has been utilized in the feeds of some terrestrial animals [7; 8; 9; 10]. Dietary charcoal showed a good influence on growth performance of piglets, goats, ducks and broiler chickens [10; 11; 12; 13 respectively]. Charcoal was found to have a good adsorbing surface for many toxins and unwanted gases due to its long and folded surface area [14].

Charcoal was proven to be a good feed additive in fish diets of Tiger puffer fish (Takifugu rubripes) and Japanese flounder, Paralichthys olivaceus [15; 16], both specis developed better growth when fed charcoal based diets. Another study found that 20 g kg^{-1} bamboo charcoal could decrease ammonia loading of Pangasius hypophthalmus [17]. A different study found that only 10 g kg^{-1} could maintain optimal growth and lower ammonia levels in the culture medium of Pangasianodon hypophthalmus [18]. Nile tilapia was found to develop better growth by feeding on 20 - 30 g kg^{-1} charcoal [19]. In a previous study 30 - 40 g kg^{-1} charcoal showed the best growth, biochemical composition as well as improving the water quality of red tilapia hybrid [20].

The present work aims to estimate the suitable AWC level in the feeds of gilthead seabream reared at different stocking densities to improve their growth performances and enhancing the water quality of the rearing tanks.

2. Materials and Methods

2.1. Experimental Design and Diet Preparation:

Gilthead Seabream fingerlings were obtained from El-Araby fish farm (Damietta Governorate, Egypt). The fish had an average initial weight of 0.5 ± 0.1 g $fish^{-1}$ and randomly stocked in flow-through circular fiber tanks (1 m^3 water-capacity with a daily water exchange ratio of 10 % of the total water volume of each tank). After 7 days of acclimation on a control diet (without charcoal) the fish were divided based on stocking density into three groups; D1: 20 fish m^{-3}, D2: 40 fish $m^{-3}$and D3: 60 fish m^{-3}. Each fish stocking density group (D1, D2 and D3) was fed on three different dietary treatments (T1: 0, T2: 20 and T3: 40 g kg^{-1}) of AWC powder (60-µm in diameter, 7.8 % moisture, 6.5 % crude ash, and pH 8.3). Three AWC diets were formulated to contain approximately 48 % crude protein and 15 % crude lipid as shown in Table 1. All dry ingredients were grinded, sieved and thoroughly blended together, oil was then added. The mix was moistened; cold-pelleted with a laboratory mincer, and then dried in a convection oven at 55 °C for 3 hours to approximately 10 % moisture then stored at -20°C until used. The used ingredients and proximate analyses of the test diets were also shown in Table 1. The test diets were fed twice a day, at 09.00 and 17.00 h, at 6 % of fish biomass for 6 days a week. Fish were weighed biweekly throughout the study to adjust the ration size according to the updated biomass.

2.2. Evaluation of growth performance and feed utilization efficiency

Fish growth performance and feed utilization were analyzed in terms of survival (S %), weight gain (WG, g), percentage weight gain (WG %), specific growth rate (SGR, % day^{-1} $fish^{-1}$), feed conversion ratio (FCR), protein efficiency ratio (PER), and energy retention (ER, %).

The following formulae were used:

Survival (S %) = (final fish count / initial fish count) × 100

WG (g) = final fish weight (FW) (g) - initial fish weight (IW) (g)

WG (%) = 100 × [(FW – IW)/IW]

SGR = 100 × [(ln FW) - (ln IW)]/experimental days

FCR = feed fed (g)/weight gain (g)

PER = weight gain (g)/protein fed (g)

Energy retention (ER) = 100 [Energy gain (kcal/kg)/ Energy intake (kcal kg^{-1})]

2.3. Proximate Analyses

Ten fish were randomely selected from each tank then pooled together and homogenized to be analyzed for fish proximate composition at the beginning and end of the experiment. Moisture, protein and ash contents were all determined by standard Association of Official Analytical Chemist [21] methodology. Triplicates of diet samples were used for proximate analyses Table 1.

1: Vitamins and minerals premix (mg kg^{-1}),; p-amino benzoic acid (9.48); D-Biotin (0.38); Inositol (379.20); Niacin (37.92); Ca-pantothenate (56.88); Pyridoxine-HCl (11.38); Riboflavin (7.58); Thiamine-HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); Folic acid (0.76); Cyanocobalamine (0.08); Menadione (3.80), Vitamin A-palmitate (17.85); α-tocopherol (18.96); Calciferol (1.14). K_2PO_4 (2.011); $Ca_3(PO_4)_2$ (2.736); Mg SO_47H_2O (3.058); NaH_2PO_4 $2H_2O$ (0.795).

2: Nitrogen-free extracts (NFE) = 1000 - [Ash + lipid + protein + Fiber] (g kg^{-1}).

Analytical procedures: Analysis of dry matter (by oven drying at 80 °C for 24 h), crude protein (Kjeldahl apparatus, nitrogen X 6.25), crude fiber (by an automatic analyzer, Fibertec, Tecator, Sweden) and ash (incineration in a muffle furnace at 600 °C for 4 h) were performed for both feed and fish.

2.4. Determination of total lipid and Fatty Acids

The extraction of total lipids from the homogenized fish samples was carried out according to the method of Folch *et al.* [22]. Fatty acids were determined in total lipids by gas chromatography (HP Agilent 6890N GC, Hewlett Packard, Palo Alto, CA, USA). Injecting fatty acid methyl esters in hexane/chloroform (4/1, v/v) were carried out according to the method of Guler *et al.* [23] in GC, fitted with an HP-88 capillary column (0.2 µm thickness, 0.25 mm ID, and 100 m length) using a flame ionization detector.

2.5. Water Quality

DO was analyzed every 15 days according to Azide method that was approved by AOAC methods (1995). Total ammonia nitrogen (TAN) concentrations were analyzed every 15 days. TAN was determined by the method of ammonia salicylate using ammonia reagent kit from Hach Co. and measured in a spectrophotometer (DR-3800 Hach Co. USA). The standard curve of ammonia was obtained using three different ammonium sulfate solutions (0.2, 0.4, 0.6 ml). The water parameters measured before starting the experiment (before putting the fish) were as follows: Temp, 28.5±0.15 ºC; salinity, 32.0±1.32 ppt; pH, 8.3±0.21; D.O., 7.8±0.61 mg l^{-1} and ammonia, 0.06±0.01 mg l^{-1}.

2.6. Statistical analysis

All data were statistically evaluated using Super ANOVA version 1.11 statistical package for Macintosh (Abacus Concepts, Berkeley, CA, USA). Two-way analysis of variance followed by Tukey Kramer test (Abacus Concepts, Berkeley, CA, USA) was applied to all treatments to test the statistical significance of the pure and interactive effects of AWC & stocking density among all dietary treatments. When probability level was lower than 0.05 ($P<0.05$), the difference between treatments was considered significant. Percent survival was arc-sin-square-root transformed before statistical analysis based on the method of Sokal and Rohlf [24].

Table 1: Dietary ingredients and proximate composition of the test diets with different AWC inclusion levels

Ingredients (g kg^{-1}) (Protein %)	Dietary Treatments T1 0 % AWC	 T2 2 % AWC	 T3 4 % AWC
Fish meal (60%)	350	350	350
Soybean meal (46%)	120	120	120
Wheat Gluten (70%)	180	180	180
Wheat flour	180	160	140
Wheat bran	50	50	50
Fish oil	100	100	100
Vit. & Min. premix¹	20	20	20
Charcoal	00	20	40
Total	1000	1000	1000
Proximate composition (%)			
Crude Protein	48.6±2.1	48.3±3.6	48.1±4.2
Total Lipids	14.6±0.9	14.8±0.4	14.7±0.9
Crude Fiber	2.7±0.1	2.6±0.3	2.5±0.1
Ash	8.3±0.2	8.4±0.7	8.7±0.2
Moisture	10.1±0.5	9.9±1.1	10.2±0.6
NFE	25.9±3.2	25.9±2.1	25.9±4.6

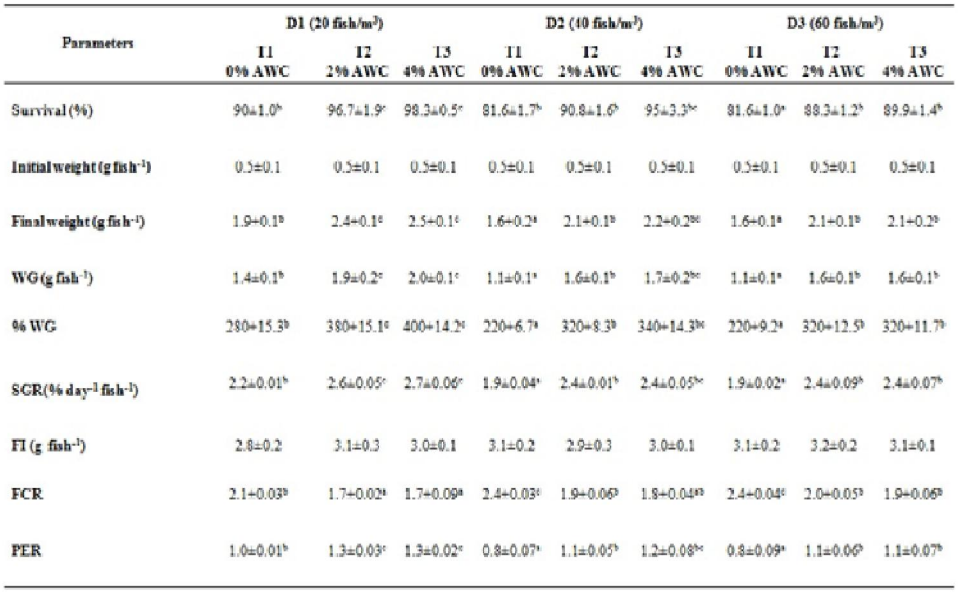
3. Results

All the data obtained in the present study were evaluated using two-way analysis of variance, and if interaction between the two factors (AWC level and stocking level) was found, the difference in means was tested within each factor. The pure and interactive effect of the two factors were significant ($P<0.05$) in terms of percent survival (S %), percent weight gain (WG %), specific growth rate (SGR, %/day), feed conversion ratio (FCR) and protein efficiency ratio (PER). AWC inclusion at 2 and 4 % supplementation levels significantly ($P<0.05$) improved S %, WG %, SGR, FCR and PER when compared with the control fish group at all tested stocking groups (D1, D2 and D3) that did not receive charcoal (T1; 0 g kg^{-1} AWC) as shown in Table 2.

Inclusion of 20 and 40 g kg^{-1} AWC (T2 and T3) at D3 stocking density fish group significantly improved survival (S %) as 88 and 90 % respectively compared with T1 at the same stocking level (D3) of lower survival percentage (81.6 %). The incorporation of AWC in gilthead seabream feeds at 20 and 40 g kg^{-1} significantly increased WG % (320 %) than the fish group that received 0 g kg^{-1} AWC (220 %) at D3 stocking level. Same trend was repeated in case of SGR as shown in Table 2. Although, in case of FI no significant difference among all treatments could be detected, FCR and PER showed a significant improvement by increasing dietary AWC level (20 and 40 g kg^{-1}) than the control fish group (0 g kg^{-1} AWC) within each stocking group as demonstrated in Table 2. Moreover, the recorded values of S % at lower stocking fish group (D1) showed a significant improvement ($P<0.05$) than S % of D3 at the same AWC inclusion levels (0, 20 and 40 g kg^{-1}). However, S % did not change significantly when the stocking density increased from 20 to 40 fish m^{-3} (D1 and D2) at T3 (40 g kg^{-1} AWC) inclusion level but in lower inclusion levels of AWC (0 and 20 g kg^{-1}) the effect of stocking density increment from 20 to 40 fish m^{-3}

showed a significant negative impact on survival rates. The values of WG and SGR at the lowest stocking density (D1) were significantly better ($P<0.05$) than those recorded for the higher stocking fish groups (D2 and D3) at similar AWC inclusion levels (T1; 0 and T2; 20 g kg^{-1}). However, at T3 (40 g kg^{-1} AWC) dietary inclusion level, no significant difference could be detected in WG and SGR when the stocking density level increased from D1 to D2 but by increasing the stocking level to D3 a significant decrease of both WG and SGR than D1 and D2 was monitored as shown in Table 2.

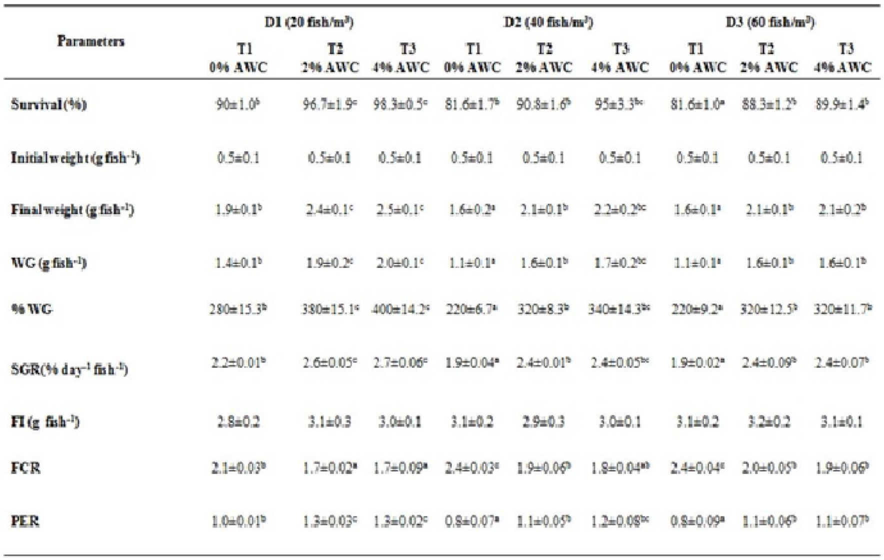
Table 2: Growth parameters of gilthead Seabream fed different levels of AWC at different stocking densities.



Parameters	D1 (20 fish/m^3)			D2 (40 fish/m^3)			D3 (60 fish/m^3)		
	T1 0% AWC	T2 2% AWC	T3 4% AWC	T1 0% AWC	T2 2% AWC	T3 4% AWC	T1 0% AWC	T2 2% AWC	T3 4% AWC
Survival (%)	$90±1.0^b$	$96.7±1.9^c$	$98.3±0.5^c$	$81.6±1.7^b$	$90.8±1.6^b$	$95±3.3^{bc}$	$81.6±1.0^a$	$88.3±1.2^b$	$89.9±1.4^b$
Initial weight (g $fish^{-1}$)	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Final weight (g $fish^{-1}$)	$1.9±0.1^b$	$2.4±0.1^c$	$2.5±0.1^c$	$1.6±0.2^a$	$2.1±0.1^b$	$2.2±0.2^{bc}$	$1.6±0.1^a$	$2.1±0.1^b$	$2.1±0.2^b$
WG (g $fish^{-1}$)	$1.4±0.1^b$	$1.9±0.2^c$	$2.0±0.1^c$	$1.1±0.1^a$	$1.6±0.1^b$	$1.7±0.2^{bc}$	$1.1±0.1^a$	$1.6±0.1^b$	$1.6±0.1^b$
% WG	$280±15.3^b$	$380±15.1^c$	$400±14.2^c$	$220±6.7^a$	$320±8.3^b$	$340±14.3^{bc}$	$220±9.2^a$	$320±12.5^b$	$320±11.7^b$
SGR(% day^{-1} $fish^{-1}$)	$2.2±0.01^b$	$2.6±0.05^c$	$2.7±0.06^c$	$1.9±0.04^a$	$2.4±0.01^b$	$2.4±0.05^{bc}$	$1.9±0.02^a$	$2.4±0.09^b$	$2.4±0.07^b$
FI (g $fish^{-1}$)	2.8±0.2	3.1±0.3	3.0±0.1	3.1±0.2	2.9±0.3	3.0±0.1	3.1±0.2	3.2±0.2	3.1±0.1
FCR	$2.1±0.03^b$	$1.7±0.02^a$	$1.7±0.09^a$	$2.4±0.03^c$	$1.9±0.06^b$	$1.8±0.04^{ab}$	$2.4±0.04^c$	$2.0±0.05^b$	$1.9±0.06^b$
PER	$1.0±0.01^b$	$1.3±0.03^c$	$1.3±0.02^c$	$0.8±0.07^a$	$1.1±0.05^b$	$1.2±0.08^{bc}$	$0.8±0.09^a$	$1.1±0.06^b$	$1.1±0.07^b$

Different l resents no significanc

Table 3: P rent levels of AWC a



Parameters	D1 (20 fish/m^3)			D2 (40 fish/m^3)			D3 (60 fish/m^3)		
	T1 0% AWC	T2 2% AWC	T3 4% AWC	T1 0% AWC	T2 2% AWC	T3 4% AWC	T1 0% AWC	T2 2% AWC	T3 4% AWC
Survival (%)	$90±1.0^b$	$96.7±1.9^c$	$98.3±0.5^c$	$81.6±1.7^b$	$90.8±1.6^b$	$95±3.3^{bc}$	$81.6±1.0^a$	$88.3±1.2^b$	$89.9±1.4^b$
Initial weight (g $fish^{-1}$)	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Final weight (g $fish^{-1}$)	$1.9±0.1^b$	$2.4±0.1^c$	$2.5±0.1^c$	$1.6±0.2^a$	$2.1±0.1^b$	$2.2±0.2^{bc}$	$1.6±0.1^a$	$2.1±0.1^b$	$2.1±0.2^b$
WG (g $fish^{-1}$)	$1.4±0.1^b$	$1.9±0.2^c$	$2.0±0.1^c$	$1.1±0.1^a$	$1.6±0.1^b$	$1.7±0.2^{bc}$	$1.1±0.1^a$	$1.6±0.1^b$	$1.6±0.1^b$
% WG	$280±15.3^b$	$380±15.1^c$	$400±14.2^c$	$220±6.7^a$	$320±8.3^b$	$340±14.3^{bc}$	$220±9.2^a$	$320±12.5^b$	$320±11.7^b$
SGR(% day^{-1} $fish^{-1}$)	$2.2±0.01^b$	$2.6±0.05^c$	$2.7±0.06^c$	$1.9±0.04^a$	$2.4±0.01^b$	$2.4±0.05^{bc}$	$1.9±0.02^a$	$2.4±0.09^b$	$2.4±0.07^b$
FI (g $fish^{-1}$)	2.8±0.2	3.1±0.3	3.0±0.1	3.1±0.2	2.9±0.3	3.0±0.1	3.1±0.2	3.2±0.2	3.1±0.1
FCR	$2.1±0.03^b$	$1.7±0.02^a$	$1.7±0.09^a$	$2.4±0.03^c$	$1.9±0.06^b$	$1.8±0.04^{ab}$	$2.4±0.04^c$	$2.0±0.05^b$	$1.9±0.06^b$
PER	$1.0±0.01^b$	$1.3±0.03^c$	$1.3±0.02^c$	$0.8±0.07^a$	$1.1±0.05^b$	$1.2±0.08^{bc}$	$0.8±0.09^a$	$1.1±0.06^b$	$1.1±0.07^b$

Different letters represent significant difference ($P<0.05$) within each row of data, while no letters represents no significance ($P>0.05$). No. of replications = 3. (Ash, TL, CP and ER were calculated in dry-weight basis).

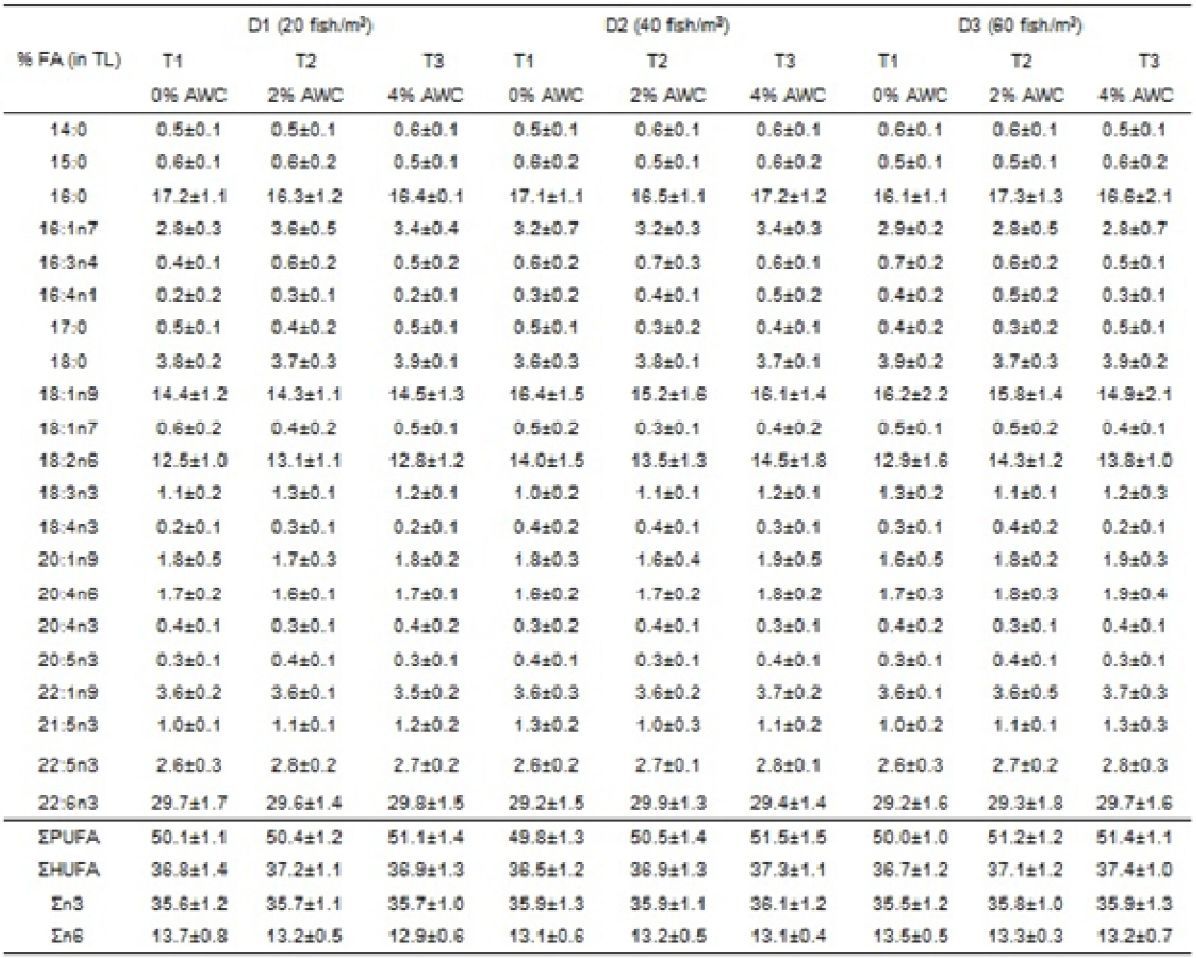
In the terms of FCR and PER, the fish group of the lowest density (D1) showed better ($P<0.05$) values at all AWC inclusion levels (T1, T2 and T3) than those of higher stocking fish groups (D2 and D3) as mentioned in Table 2. However, similar to the data of WG and SGR of the fish group received 4 % AWC at

D2 stocking density did not significantly change than those of the fish groups received 20, 40 g kg^{-1} AWC at D1 stocking level.

No significant (P>0.05) difference in the pure and interactive effects between the two factors (AWC level and stocking density level) in case of moisture, ash and total lipid (TL) body contents among all dietary treatments as shown in Table 3. However, in terms of crude protein (CP) and energy retention (ER), the pure effect of AWC inclusion level was significant (P<0.05). All the fish groups fed AWC (T2; 20 and T3; 40 g kg^{-1}) at all stocking densities (D1, D2 and D3) displayed a significant improvement than those of the control fish group (T1; 0 g kg^{-1} AWC) that did not receive AWC in their diet. Increasing the stocking density from D1 – D3 (20, 40 and 60 fish m^{-3}) showed no significant difference (P>0.05) at all AWC inclusion levels (0, 20 and 40 g kg^{-1}) in terms of both CP and ER as shown in Table 3.

Total fatty acids in total lipids of all fish groups fed AWC at different stocking densities (D1, D2 and D3) did not show any significant differences (P>0.05) than the control fish group (fed on 0 g kg^{-1} AWC) (Table 4). In terms of n3/n6 ratio showed a trend of higher ratio (2.8±0.1) at D1 and D2 fed on T3; 4 g kg^{-1} AWC than the other fish groups fed lower AWC levels in their feeds. However, no significant difference could be measured among treatments as shown in Fig. 1.

Table 4: Fatty acid profile of gilthead Seabream fed different levels of AWC at different stocking densities



	D1 (20 fish/m^{3})			D2 (40 fish/m^{3})			D3 (60 fish/m^{3})		
% FA (in TL)	T1	T2	T3	T1	T2	T3	T1	T2	T3
	0% AWC	2% AWC	4% AWC	0% AWC	2% AWC	4% AWC	0% AWC	2% AWC	4% AWC
14:0	0.5±0.1	0.5±0.1	0.6±0.1	0.5±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1
15:0	0.6±0.1	0.6±0.2	0.5±0.1	0.6±0.2	0.5±0.1	0.6±0.2	0.5±0.1	0.5±0.1	0.6±0.2
16:0	17.2±1.1	16.3±1.2	16.4±0.1	17.1±1.1	16.5±1.1	17.2±1.2	16.1±1.1	17.3±1.3	16.6±2.1
16:1n7	2.8±0.3	3.6±0.5	3.4±0.4	3.2±0.7	3.2±0.3	3.4±0.3	2.9±0.2	2.8±0.5	2.8±0.7
16:3n4	0.4±0.1	0.6±0.2	0.5±0.2	0.6±0.2	0.7±0.3	0.6±0.1	0.7±0.2	0.6±0.2	0.5±0.1
16:4n1	0.2±0.2	0.3±0.1	0.2±0.1	0.3±0.2	0.4±0.1	0.5±0.2	0.4±0.2	0.5±0.2	0.3±0.1
17:0	0.5±0.1	0.4±0.2	0.5±0.1	0.5±0.1	0.3±0.2	0.4±0.1	0.4±0.2	0.3±0.2	0.5±0.1
18:0	3.8±0.2	3.7±0.3	3.9±0.1	3.6±0.3	3.8±0.1	3.7±0.1	3.9±0.2	3.7±0.3	3.9±0.2
18:1n9	14.4±1.2	14.3±1.1	14.5±1.3	16.4±1.5	15.2±1.6	16.1±1.4	16.2±2.2	15.8±1.4	14.9±2.1
18:1n7	0.6±0.2	0.4±0.2	0.5±0.1	0.5±0.2	0.3±0.1	0.4±0.2	0.5±0.1	0.5±0.2	0.4±0.1
18:2n6	12.5±1.0	13.1±1.1	12.8±1.2	14.0±1.5	13.5±1.3	14.5±1.8	12.9±1.6	14.3±1.2	13.8±1.0
18:3n3	1.1±0.2	1.3±0.1	1.2±0.1	1.0±0.2	1.1±0.1	1.2±0.1	1.3±0.2	1.1±0.1	1.2±0.3
18:4n3	0.2±0.1	0.3±0.1	0.2±0.1	0.4±0.2	0.4±0.1	0.3±0.1	0.3±0.1	0.4±0.2	0.2±0.1
20:1n9	1.8±0.5	1.7±0.3	1.8±0.2	1.8±0.3	1.6±0.4	1.9±0.5	1.6±0.5	1.8±0.2	1.9±0.3
20:4n6	1.7±0.2	1.6±0.1	1.7±0.1	1.6±0.2	1.7±0.2	1.8±0.2	1.7±0.3	1.8±0.3	1.9±0.4
20:4n3	0.4±0.1	0.3±0.1	0.4±0.2	0.3±0.2	0.4±0.1	0.3±0.1	0.4±0.2	0.3±0.1	0.4±0.1
20:5n3	0.3±0.1	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1
22:1n9	3.6±0.2	3.6±0.1	3.5±0.2	3.6±0.3	3.6±0.2	3.7±0.2	3.6±0.1	3.6±0.5	3.7±0.3
21:5n3	1.0±0.1	1.1±0.1	1.2±0.2	1.3±0.2	1.0±0.3	1.1±0.2	1.0±0.2	1.1±0.1	1.3±0.3
22:5n3	2.6±0.3	2.8±0.2	2.7±0.2	2.6±0.2	2.7±0.1	2.8±0.1	2.6±0.3	2.7±0.2	2.8±0.3
22:6n3	29.7±1.7	29.6±1.4	29.8±1.5	29.2±1.5	29.9±1.3	29.4±1.4	29.2±1.6	29.3±1.8	29.7±1.6
ΣPUFA	50.1±1.1	50.4±1.2	51.1±1.4	49.8±1.3	50.5±1.4	51.5±1.5	50.0±1.0	51.2±1.2	51.4±1.1
ΣHUFA	36.8±1.4	37.2±1.1	36.9±1.3	36.5±1.2	36.9±1.3	37.3±1.1	36.7±1.2	37.1±1.2	37.4±1.0
Σn3	35.6±1.2	35.7±1.1	35.7±1.0	35.9±1.3	35.9±1.1	36.1±1.2	35.5±1.2	35.8±1.0	35.9±1.3
Σn6	13.7±0.8	13.2±0.5	12.9±0.6	13.1±0.6	13.2±0.5	13.1±0.4	13.5±0.5	13.3±0.3	13.2±0.7

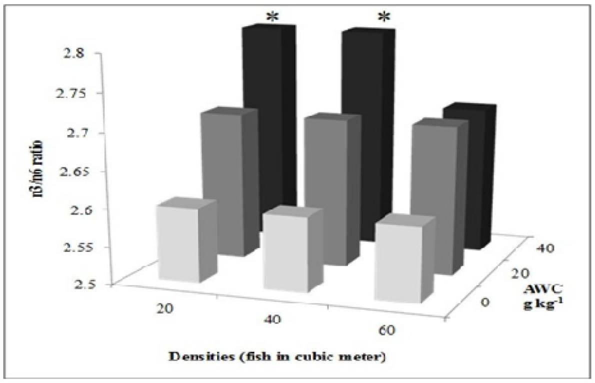


Figure 1: n3 / n6 ratio of the different fish groups fed different levels of AWC at different stocking densities

The water parameters all over the experimental period were monitored. Temperature (25.3±0.5 °C), salinity (26.9±0.15 ppt), and pH (8.1±0.17), no significant differences (P>0.05) could be detected among treatments when measured inside all fish tanks in the terms of the above-mentioned water parameters. However, other water parameters such as; Dissolved Oxygen (DO) and ammonia (TAN), were significantly enhanced (P<0.05) by inclusion of AWC (20 and 40 g kg^{-1}) in the feeds of gilthead seabream at D2 and D3 stocking fish groups than the fish groups that did not receive AWC in their feeds (0 g kg^{-1} AWC). However, no change could be detected by increasing AWC level at the lowest stocking density group (D1). Best DO and ammonia (TAN) levels were monitored in D1 stocking fish group at all AWC inclusion levels (0, 20 and 40 g kg^{-1}) with no significant difference than the other higher stocking density fish groups (D2 and D3) at 2 and 40 g kg^{-1} AWC inclusion level. Absence of AWC (T1) in the diets of the higher stocking density fish groups (D2 and D3) significantly (P<0.05) decreased the water quality of the rearing tanks (decreased DO and increased TAN) as shown in Table 5.

Table 5: Dissolved oxygen (DO) and ammonia levels (mg L^{-1}) within the rearing tanks of gilthead Seabream fed different levels of AWC at different stocking densities

Parameters	D1 (20 fish/m^3)			D2 (40 fish/m^3)			D3 (60 fish/m^3)		
	T1 0% AWC	T2 2% AWC	T3 4% AWC	T1 0% AWC	T2 2% AWC	T3 4% AWC	T1 0% AWC	T2 2% AWC	T3 4% AWC
DO	8.0±0.2^b	8.2±0.1^b	8.3±0.1^b	7.2±0.1^a	8.0±0.3^b	8.2±0.4^b	6.7±0.4^a	7.7±0.3^b	8.1±0.3^b
Ammonia	0.08±0.01^a	0.07±0.02^a	0.07±0.01^a	0.11±0.01^b	0.07±0.01^a	0.06±0.02^a	0.11±0.01^b	0.07±0.01^a	0.06±0.02^a

Different letters represent significant difference (P<0.05) within each row of data, while no letters represents no significance (P>0.05). No. of replications = 3.

4. Discussion

Through the history of aquaculture, the research and development attempted to formulate an ideal fish diet (especially for semi-intensive and intensive types of aquaculture) to meet the rising demand of cultured fish as well as to decrease the environmental loading resulting from these types of aquaculture. Previously, such feeds despite their capabilities to maintain high fish growth rates, they have a negative environmental impact due to their high nitrogenous wastes especially TAN that constitutes almost 80% of these wastes [2].

The present study aimed to evaluate different levels of activated wood charcoal (0, 20 and 40 g kg^{-1} AWC) in the feeds of gilthead seabream (Sparus aurata) fingerlings at different levels of stocking densities (20, 40 and 60 fish m^{-3}) to enhance their growth, feed utilization, and biochemical composition with lowering the environmental loading of waste nitrogenous products such as TAN even within high density rearing tanks.

In terms of growth performance parameters such as S, WG, SGR, FCR and PER, the present study showed that 40 g kg^{-1} AWC (T3) in the feeds of gilthead seabream reared under stocking density of 40 fish m^{-3} could maintain best S, WG, SGR, FCR, and PER. These data came in parallel with the data of a previous study on red hybrid tilapia that showed that 30 – 40 g kg^{-1} dietary charcoal can maintain optimal growth and survival of the tested fish species [20]. Similarly, the marine species such as Tiger puffer fish showed that 40 g kg^{-1} is optimal inclusion level of bamboo charcoal (BC) in the feeds of tiger puffer fish [15]. However, in case of fresh water Nile tilapia (Oreochromis niloticus), the fish needed lower dietary charcoal (20 g kg^{-1}) to maintain their optimal growth and survival [19]. Meanwhile, Japanese flounder (a slow eater fish species) seemed to need less dietary charcoal (5 – 10 g kg^{-1}) to fulfill their requirements [25 and 16]. Cheremishinoff and Moressi [26] suggested that the adsorption capacity of charcoal depends mainly on the contacting period with the adsorbed substances. Therefore, slow eater fish can optimize low amount of dietary charcoal to maintain good growth [16]. In case of fast eater fish such as tiger puffer fish, red tilapia and gilthead seabream shorter contacting time of charcoal with unwanted substances such as toxins and gases may lead to the elevation of charcoal requirement as was explained by Moe Thu *et al.* [15]. The enhanced growth and feed utilization parameters in the present study may be explained due to the adsorptive capability of AWC to remove unwanted gases and toxins and hence improving the digestion and metabolism of the feeds [27 and 28]. Also, charcoal and some vinegar compounds activate the intestinal function at both villi and cellular levels, and increase the feed efficiency as well as growth performance of piglets [10], Nile tilapia [19] and red hybrid tilapia [20].

Moreover, charcoal can form complexes with phenol compounds to prevent hydrolysable tannins interfering with protein formation, therefore, increases the availability of proteins [29 and 11]. The present study showed a higher fish protein content at all stocking levels (20 to 60 fish/m^3) fed on 20 and 40 g kg^{-1} AWC than the control fish group that received no charcoal (0 g kg^{-1} AWC). As well, illustrated that adding charcoal at a level of 1 g kg^{-1} of body weight increased energy retention (ER) of growing goats, that came in parallel with the measured levels of ER in the present study that showed significant increase of ER at all stocking levels (D1 to D3) fed 20 and 40 g kg^{-1} AWC than the control (T1; 0 g kg^{-1} AWC) fish group [11]. Nevertheless, other proximate parameters such as dry matter, total lipids and ash were not affected by the inclusion of AWC in the feeds of gilthead seabream as was recorded for Japanese flounder by Moe Thu *et al.* [16] and red hybrid tilapia by Michael *et al.* [20].

No significant difference among all treatments in terms of measured fatty acids in the total lipids which came in parallel with the results of total lipids in the same study that showed no effect of both AWC and stocking density among all fish treatments.

A good and well-designed feed results in high fish production with low environmental impact. In terms of TAN and DO, the present study showed the positive effect of AWC when added to gilthead seabream feeds at 20 and 40 g kg^{-1} at all stocking levels more than 20 fish m^{-3} (D2 and D3). A gradual decrement in ammonia levels in the rearing tanks of fish groups stocked at 40 and 60 fish m^{-3} by AWC inclusion at 20 and 40 g kg^{-1} levels in gilthead seabream feeds which empowered the crude protein results of the same study. Same trend of ammonia results was obtained when tiger puffer fish, Japanese flounder and red hybrid tilapia were fed different levels of charcoal. The authors found a depleted trend of total ammonia nitrogen (TAN) excretion with elevated dietary charcoal in fish feeds [15; 16; 17; 20]. Adequate dissolved oxygen (DO) is needed for all forms of aquatic life. The growth of many fish species is affected by water oxygen levels such as tilapia [30] and Atlantic salmon [31; 32; 33]. The results of the present study showed that DO levels were gradually increased at D2 and D3 stocking fish groups by the increment of AWC levels in gilthead seabream feeds (20 and 40 g kg^{-1}). This might be resulted from the accumulative levels of AWC excretion in the water for 60 successive days, which resulted in reduction of ammonia concentrations in the water of fish tanks that received elevated dietary AWC levels (20 and 40 g kg^{-1}).

5. Conclusion

The overall results of the present study indicated that activated wood charcoal (AWC) at 4 % can be used as a useful feed additive in the diets of gilthead seabream at high stocking rates (40 - 60 fish m^{-3}) to enhance their growth, biochemical parameters and simultaneously decrease the environmental loadings to improve the water quality of the rearing tanks.

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