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Growth Performance, Survival, Hematological Parameters, and Water Quality of European Seabass (*Dicentrarchus labrax*) Fed on Two Different Types of Probiotics as a Feed Supplement

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ABSTRACT

This study aimed to investigate the effects of using two probiotic formulations on the European seabass (*Dicentrarchus Labrax*). Three diets (C= control diet without any probiotics, P_1= probiotics, and P_2= probiotics + enzymatic additives) and three fish densities (20, 40, and 80 F/m3) were used in 3 replicates. Healthy European seabass juveniles (IBW =5g) were obtained from a commercial farm in Damietta, Egypt. Fish were acclimated for 1 week before experiment. Diets with 45% crude protein were tested for 12 weeks. Water quality parameters were measured bi-weekly. Five fish per hapa were randomly taken to collect the blood sample for hematological assessment. Final body weight was significantly higher (P<0.05) in group P_2, with no significant variance based on densities. Groups P_1 and P_2 exhibited the highest significant specific growth rate (SGR) (P<0.05), with no significant differences based on densities. Furthermore, both P_1 with 20 fish/m^3 and P_2 groups demonstrated significantly higher survival rates (P<0.05). Group P_2 achieved best FCR followed by P_1 and control. Density was recorded with a further significant effect on PER and ER, at densities of 80 fish/m3. Group P_2 (80 fish/m3) was displayed with the highest significant percentage of HCT compared to other groups. Levels of MCH and MCV were significantly high in P_1 with 40 fish/m3. The highest significant RBCs were recorded in P_1 with 80 fish/m^3. The lowest significant WBCs was recorded in the control group with 40 fish/m^3. The total protein was improved by the inclusion of two types of probiotics at different densities. The highest glucose level was recorded in the control with 80 fish/m^3. The control group experienced a significant increase in cholesterol levels compared to the other groups. No significant differences were observed between the types of probiotics and the level of density in the triglyceride values. pH, DO and TAN were significantly improved for the groups of P_1 and P_2 compared to the control group. Notably, probiotics' induction improves the host's health and water quality.

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INTRODUCTION

The European seabass (*Dicentrarchus labrax*) is a major species in the Mediterranean aquaculture industry, with a global production of nearly 300,000 tons valued at USD 1.8 million in 2021 **(FAO, 2023)**. Seabass is particularly sensitive to water quality standards. Despite this, it is very sensitive to certain stress factors in aquaculture conditions, leading to significant losses **(Carbone & Vagio, 2016)**. With the expansion of the aquaculture sector and the increase in production intensity and marketing, numerous diseases have spread, which have become a significant challenge for the aquaculture industry **(Hai, 2015)**.

The use of probiotics in aquaculture has attracted considerable attention in recent years due to their potential positive impact on fish health and growth **(Hamka *et al*., 2020)**. Probiotics, as live microorganisms, when given in adequate amounts, confer health benefits to the host. In the realm of fish farming, probiotics have demonstrated their ability to strengthen the immune system, enhance nutrient absorption, and combat the effects of harmful microorganisms **(Huang *et al*., 2020; El-Saadony *et al*., 2021)**. Furthermore, they have been associated with a reduced environmental impact by minimizing nutrient excretion from fish farms **(Merrifield & Carnevali, 2014)**. Research on the effects of probiotics on various farmed fish species has shown promising results. For instance, *Lactobacillus* spp. and *Bacillus* spp. have been found to enhance the growth rates and disease resistance in the rainbow trout (*Oncorhynchus mykiss*) **(Nayak *et al*., 2007)**. Similarly, adding certain probiotic strains to the diet has resulted in improved feed utilization efficiency and disease resistance in carp species like common carp, *Cyprinus carpio* **(Ringø & Gatesoupe , 1998)**. The potential benefits of probiotics in aquaculture extend beyond the direct effects on fish health. Probiotics can also play a vital role in maintaining water quality by influencing the composition and activity of the microbial communities in aquaculture systems. For instance, **Liu *et al*. (2021)** found that the addition of *Bacillus*-based probiotics effectively reduced ammonia and nitrite levels in high-density common carp (*Cyprinus carpio*) ponds, indicating their potential to mitigate the negative impacts of stocking density on water quality.

The stocking density for fish, whether it is extremely low or high, can have either negative **(Adams & Wolfe, 2007; Millán-Cubillo *et al*., 2016)** or positive effects on fish welfare and growth **(Liu *et al*., 2019; Yang *et al*., 2020)** depending on the species. In intensive aquaculture systems, stocking density is crucial in determining production efficiency **(Riche *et al*., 2013)**. It has an impact on the growth, water quality, and welfare of the fish. Finding the optimal stocking densities is challenging due to the complex relationship between fish welfare and stocking density **(Ellis *et al*., 2002)**. The response of a species to stocking density can vary significantly based on fish size and the farming system **(Ellis *et al*., 2002)**. Therefore, stocking density is a critical factor that affects the health of commercially farmed fish and is a particular concern for fish welfare **(Li *et al*., 2021)**. In recent studies, the combined effect of probiotics and stocking density on fish health and performance has been investigated. For instance, **Smith *et al*. (2020)** showed that adding *Lactobacillus*-based probiotics improved growth performance and disease resistance in the high-density-reared rainbow trout (*Oncorhynchus mykiss*). Similarly, **Ahmed *et al*. (2019)** found that dietary administration of *Bacillus*-based probiotics reduced oxidative stress and enhanced survival rates in the densely stocked Nile tilapia (*Oreochromis niloticus*).

This study aimed to investigate the effects of using two probiotic formulations on the European seabass (*Dicentrarchus Labrax*). One formulation consisted of bacteria without additives, while the other contained bacteria with added vitamins and enzymes. The study examined the impact of these formulations at three different densities on the growth performance, survival rates, hematological parameters, and water quality parameters of the European seabass.

MATERIALS AND METHODS

Experimental design

Healthy European seabass juveniles (mean body weight =5g) were obtained from commercial farm in Damietta- Egypt. The obtained fish were free from any infection and were placed in 27 /hapas (1x1x1.5m). The water was treated with chlorine and was allowed to stand for at least three days with aeration, and seabass samples were stocked. The fish were acclimated for 1 week before a random distribution in each /hapas with three replications.

Experimental diet

Three experimental diets (45% crude protein) containing 2 different types of probiotics and three fish densities (20, 40, and 80 F/m^3) were tested in 2 treatments (+ control diet) × 3 densities × 3 replicates. The daily ration was offered two times a day (09.00 and 14.00 h) 6 days a week until satiation level for 12 weeks.

The water salinity was set at 19 ± 2 ‰, and photoperiod was 12-h light: 12-h dark
C diet = control diet without any probiotics
P_1 = diet with probiotics
P_2 = diet with probiotics + Enzymatic additives

Table 1. Composition and chemical analysis of the experimental diets

Ingredient	C	P1	P2
shrimp meal	200.0	200.0	200.0
Rice bran	70.0	67.5	67.5
Soybean meal	300.0	300.0	300.0
Probiotic	0.0	2.5	2.5
Fish meal	350.0	350.0	350.0
Fish oil	45.0	45.0	45.0
CMC	20.0	20.0	20.0
***Vit. &Min. Mix**	8.0	8.0	8.0
min mix	7.0	7.0	7.0
Proximate Analysis			
Dry mater	87.97	88.25	87.86
Moisture	12.03	11.75	12.14
Crude protein (N × 6.25)	45.1	45.1	45.1
Crude fat	17.89	17.44	17.95
Crude fiber	2.11	2.07	2.31
Ash	7.22	7.13	7.41
****Carbohydrate (NFE)**	15.61	16.50	15.08
*****Gross energy (GE) kcal/100g^3**	489.465	488.709	487.681

*Each 100 gram of vitamin and mineral contained: Mineral: Zn, 2.50 mg; Mn, 16.00 mg; Fe, 31.50 mg; Cu, 5.50; I, 0.55 mg; Ca, 1.15 g and P, 450 mg. Vitamins: A, 7500000 IU; Bi, 100 mg; B3, 500 mg; B6, 150 mg; B12, 2.5 mg; E, 100 mg; K, 100 mg; Pantothenic acid, 275 mg; Folic acid, 100 mg and vit. D3, 7500 IU.

**NFE = 100 - [% Ash + % lipid + % protein + % Fiber]

***GE (kcal/100 g DM) = CP x 5.64 + EE x 9.44 + NFE x 4.11 calculated according to **McDonald *et al.* (1973)**.

Water quality parameters

Water quality parameters were measured bi-weekly. Total ammonia nitrogen (TAN) was measured using analytical kits (Lovebird®, Multidirect, co 210070 England). Dissolved oxygen was measured using a portable oxygen meter (DO-5510). Other water quality parameters including pH were measured every two days by pH meter (Extech pH / temp pen model pH 60), and the pH range was 7.3 ± 0.7 throughout the experimental period.

Blood sample

Ten fish per hapa were randomly taken to collect the blood sample from the caudal vasculature with a 1-ml syringe. The collected blood was divided into two tubes, one containing heparin (1600 UI/ml) as anticoagulant agent for hematological assessment.

Hematological analysis

The erythrocytic count was carried out by using a hemocytometer and special diluting fluid (Natt–Herrick) for fish blood. Hemoglobin (Hb) was determined by using centrifugation, followed by the cyanmethemoglobin colorimetric method according to **Stoskopf (1993)**. Packed cell volume (PCV) was measured as described by **Dacie and**

Lewis (1991) through using the microhematocrit tubes method. The erythrocytic indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the method of **Stoskopf (1993)**.

Statistical analysis

Data of the investigated traits (growth performance, feed utilization, hematological analyses and water quality) were analyzed as factorial experiment with 3 × 3 two-way analysis of variance (ANOVA) using IBM SPSS Statistics 25 statistical package to evaluate the differences between the tested treatments.

RESULTS

Growth performance

In Table (2), the effects of two types of probiotics at three stocking densities on growth performance parameters {Final body weight (FBW), weight gain (WG), specific growth rate (SGR) and survival (S, %)} are presented. The findings revealed that FBW was significantly higher ($P \leq 0.05$) in group P_2, with no significant variance based on densities. The groups fed P_1 and P_2 exhibited the highest significantly specific growth rate (SGR), ($P \leq 0.05$) with no significant differences based on densities. Conversely, the control group had the significantly lowest SGR value ($P \leq 0.05$).

Furthermore, both P_1 (at a density of 20 fish/m^3) and P_2 groups demonstrated significantly higher survival rates ($P \leq 0.05$). In contrast, the control treatment at a fish density of 80 fish/m^3 exhibited the significantly lowest survival rate ($P \leq 0.05$), followed by densities of 40 fish/m^3 and 20 in the control treatment and densities of 40 f/m^{2}and 80 fish/m^3 in P_1.

Table 2. Growth parameters (mean ± SE) of the European seabass fed on two types of probiotics, with three stock densities (**D**) for 12 weeks

Probiotic	D	Final weight	Weight gain	SGR	S%
C	20	21.78 ± 0.06^c	16.78 ± 0.06^c	1.64 ± 0.003^b	86.67 ± 1.67^b
	40	21.03 ± 0.07^d	16.03 ± 0.07^d	1.60 ± 0.004^b	81.67 ± 1.67^b
	80	20.23 ± 0.10^e	15.23 ± 0.10^e	1.55 ± 0.005^c	70.00 ± 2.89^c
P_1	20	23.70 ± 0.07^a	18.70 ± 0.07^a	1.73 ± 0.003^a	91.67 ± 1.67^a
	40	23.57 ± 0.12^a	18.57 ± 0.12^a	1.72 ± 0.01^a	86.67 ± 1.67^b
	80	23.29 ± 0.25^b	18.29 ± 0.25^b	1.71 ± 0.01^a	86.67 ± 1.67^b
P_2	20	23.76 ± 0.05^a	18.76 ± 0.05^a	1.73 ± 0.002^a	93.33 ± 1.67^a
	40	23.62 ± 0.03^a	18.62 ± 0.03^a	1.73 ± 0.001^a	90.00 ± 2.89^a
	80	23.45 ± 0.06^a	18.45 ± 0.06^a	1.72 ± 0.003^a	88.33 ± 1.67^a
Pooled means					
Probiotics	C	21.01 ± 0.83^b	16.01 ± 0.63^b	1.60 ± 0.06^b	79.44 ± 2.69^b
	P_1	23.52 ± 0.92^a	18.52 ± 0.73^a	1.72 ± 0.07^a	88.33 ± 1.18^a
	P_2	23.61 ± 0.93^a	18.61 ± 0.73^a	1.73 ± 0.07^a	90.56 ± 1.30^a
Densities (Fish/m^3)	20	23.08 ± 0.91^a	18.08 ± 0.71^a	1.70 ± 0.07^a	90.56 ± 1.30^a
	40	22.74 ± 0.90^b	17.74 ± 0.70^b	1.68 ± 0.07^a	86.11 ± 1.62^b
	80	22.32 ± 0.88^c	17.32 ± 0.69^b	1.66 ± 0.07^a	81.67 ± 3.12^c

Different superscript letters within the same row are significantly different ($P<0.05$)

FBW: Final body weight; **WG:** weight gain; **SGR:** specific growth rate; **S:** survival.

Feed utilization

The effects of two types of probiotics with three levels of stocking density on feed utilization parameters are shown in Table (3). The feed conversion ratio was significantly affected by the addition of probiotics to the feed besides stocking density, the treatment that was enhanced with probiotics P_2 achieved the best FCR followed by treatment P_1 followed by the control treatment. Density also had a significant effect on PER and ER, as fish at densities of 80 fish/m^3 achieved the highest significance than the rest groups.

Table 3. Feed utilization parameters (mean ± SE) of the European seabass fed on two types of probiotics, with three stock densities for 12 weeks

Probiotic	Density	FCR	PPV	PER	ER
C	20	1.81±0.01^b	21.70±0.11^c	1.23±0.004^d	12.11±0.51^c
	40	1.84±0.01^b	21.54±0.37^c	1.20±0.01^d	11.83±0.16^c
	80	1.89±0.01^a	20.61±0.22^d	1.17±0.01^e	11.14±0.21^d
P_1	20	1.58±0.01^c	24.84±0.31^a	1.40±0.01^b	14.41±0.20^a
	40	1.58±0.01^c	24.32±0.15^a	1.41±0.01^b	14.31±0.15^a
	80	1.62±0.03^c	24.31±0.66^b	1.36±0.03^c	14.13±0.36^b
P_2	20	1.53±0.01^d	25.51±0.18^a	1.45±0.01^a	14.76±0.11^a
	40	1.52±0.01^d	25.72±0.22^a	1.46±0.01^a	14.91±0.06^a
	80	1.54±0.01^d	25.28±0.20^a	1.44±0.01^a	14.72±0.04^a
Pooled means					
probiotics	C	1.85±0.07^a	21.28±0.84^c	1.20±0.05^c	11.69±0.46c
	P_1	1.59±0.06^b	24.65±0.97^b	1.39±0.05^b	14.28±0.56b
	P_2	1.53±0.06^c	25.50±1.00^a	1.45+0.06^a	14.80±0.58a
Densities (Fish/m^3)	20	1.64±0.06^b	24.02±0.95^a	1.36±0.05^a	13.76±0.54^a
	40	1.65±0.07^b	24.03±0.95^a	1.36±0.05^a	13.69±0.54^a
	80	1.68±0.07^a	23.40±0.93^b	1.33±0.05^b	13.33±0.54^b

- Different superscript letters within the same row are significantly different (P<0.05)
- **FCR:** Feed conversion ratio; **PPV:** protein productive value; **PER:** protein efficiency ratio; **ER:** Energy retention.

Blood hematological parameters

Red blood cell parameters contained hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), erythrocyte count (RBC) and white blood cells (WBCs), as shown in Table (4). Adding probiotics led to significant differences in hemoglobin, as fish fed on probiotic treatments achieved significantly higher values than control fish. The fish of P_2 at a density of 80 fish/m^2 displayed the highest significant percentage of hematocrit (HCT) compared to the other groups. Conversely, treatments P_1 and P_2, at a density of 20 fish/m^3, exhibited the lowest significant percentage of hematocrit. Table (2) indicates that differences in pooled means in hematocrit are attributable to varying density levels, with higher densities correlating with increased hematocrit percentages. Additionally, MCH levels were notably high for treatment P_1 at a density of 40 fish/m^3; while, group P_2 at a density of 20 fish/m^3 recorded the lowest MCH levels. Control fish at a density of 40 fish/m^3 achieved the lowest mean of MCHC; wheresas, the highest rate was recorded for group P_1 at density of 20 fish/m^2. The group P_2 with a density of 20 fish/m^3 achieved the lowest rate of MCV; on the other hand, the highest rate was recorded in group P_1 at a density 40 fish/m^3. Meanwhile, the control group with a density of 20 had the lowest significant rate of RBCs, while the highest significant values were recorded in group P_1at density 80 fish/m^3, followed by P_2 at density 20 fish/m^3. The control group with a density of 40 fish/m^3 experienced the lowest significant value in WBCs compared to the other groups.

Table 4. Hematological parameters (mean ± SE) of European seabass fed on two types of probiotics, with three stock densities (D) for 12 weeks

Probiotic	D	HB (g/dL)	HCT	MCH	MCHC	MCV	RBCs	WBCs
C	20	9.50±0.10^{de}	49.49±0.51^{bc}	25.19±0.51^{ab}	19.20±0.36^{bc}	131.34±3.68^{ab}	2.56±0.06^{b}	25.92±0.34^{ab}
	40	9.37±0.08^{e}	49.81±0.21^{abc}	25.52±0.53^{ab}	18.81±0.23^{c}	135.64±1.45^{a}	2.67±0.05^{b}	25.60±0.67^{b}
	80	9.56±0.11^{cde}	50.24±0.24^{ab}	25.30±0.90^{ab}	19.03±0.12^{bc}	132.88±3.89^{ab}	2.79±0.10^{ab}	26.92±0.25^{a}
P1	20	9.83±0.06^{ab}	49.26±0.18^{c}	25.92±0.32^{ab}	19.96±0.10^{a}	129.86±1.10^{ab}	2.79±0.02^{ab}	27.32±0.21^{a}
	40	9.66±0.04^{bcd}	49.86±0.48^{abc}	26.56±0.71^{a}	19.37±0.18^{abc}	137.18±4.54^{a}	2.64±0.09^{b}	27.06±0.45^{a}
	80	9.87±0.04^{a}	50.38±0.41^{ab}	25.32±0.20^{ab}	19.59±0.23^{ab}	129.32±1.83^{ab}	2.90±0.03^{a}	27.22±0.38^{a}
P2	20	9.65±0.05^{bcd}	49.21±0.28^{c}	24.57±0.24^{b}	19.62±0.19^{ab}	125.22±0.34^{b}	2.93±0.02^{a}	26.99±0.46^{a}
	40	9.70±0.03^{abc}	49.99±0.07^{abc}	25.57±0.41^{ab}	19.41±0.03^{ab}	131.73±2.11^{ab}	2.80±0.06^{ab}	27.37±0.47^{a}
	80	9.75±0.06^{abc}	50.46±0.26^{a}	26.06±0.77^{ab}	19.32±0.16^{bc}	134.98±4.81^{a}	2.75±0.12^{ab}	27.22±0.38^{a}
Pooled means								
Probiotics	C	9.48±0.06^{b}	49.85±0.20^{a}	25.34±0.34^{a}	19.01±0.14^{c}	133.29±1.72^{a}	2.67±0.05^{a}	26.15±0.80^{b}
	P1	9.78±0.04^{a}	49.83±0.25^{a}	25.93±0.29^{a}	19.64±0.12^{a}	132.12±1.92^{a}	2.78±0.05^{a}	27.20±0.24^{a}
	P2	9.70±0.03^{a}	49.89±0.21^{a}	25.40±0.34^{a}	19.45±0.09^{a}	130.65±2.09^{a}	2.82±0.05^{a}	27.19±0.15^{a}
Densities (Fish/m^3)	20	9.66±0.06^{ab}	49.32±0.18^{b}	25.23±0.27^{a}	19.59±0.16^{a}	128.81±1.44^{b}	2.76±0.04^{a}	26.74±0.43^{a}
	40	9.58±0.06^{b}	49.88±0.15^{a}	25.88±0.33^{a}	19.20±0.13^{b}	134.85±1.71^{a}	2.70±0.04^{a}	26.68±1.01^{a}
	80	9.73±0.06^{a}	50.36±0.16^{a}	25.56±0.37^{a}	19.31±0.12^{ab}	132.39±2.04^{ab}	2.81±0.05^{a}	27.12±1.28^{a}

- Different superscript letters within the same row are significantly different (P<0.05)
- **Hb:** hemoglobin concentration; **HCT:** hematocrit; **MCH:** mean corpuscular hemoglobin; **MCHC:** mean corpuscular hemoglobin concentration; **MCV:** mean corpuscular volume; **RBCs:** erythrocyte count; **WBCs:** white blood cells.

The blood biochemical parameters (total protein, glucose, cholesterol and triglycerides) are shown in Table (5). The results revealed that the total protein was improved via the inclusion of two types of probiotic at different densities, while the lowest was observed in the control treatment at fish densities of 20, 40, and 80 fish/m^3. The highest glucose level was recorded in the control treatment at a density of 80 fish/m^3, while it was significantly lower in treatment P_2 at both densities of 20 and 40 fish/m^3 compared to the control. Additionally, the control group experienced a significant increase in cholesterol levels compared to the other groups. Fish in the control treatment at 40 fish/m^3 achieved the highest significant level of triglyceride, while no significant differences were observed between the types of probiotic and the level of density in the triglyceride values.

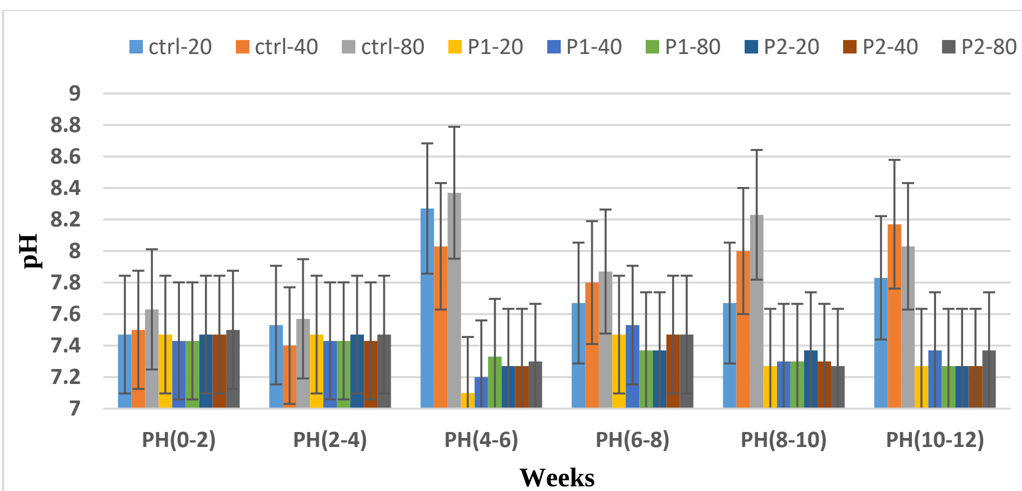
Table 5. Blood biochemical parameters (mean + SE) of the European seabass fed two types of probiotic, with three stock densities for 12 weeks

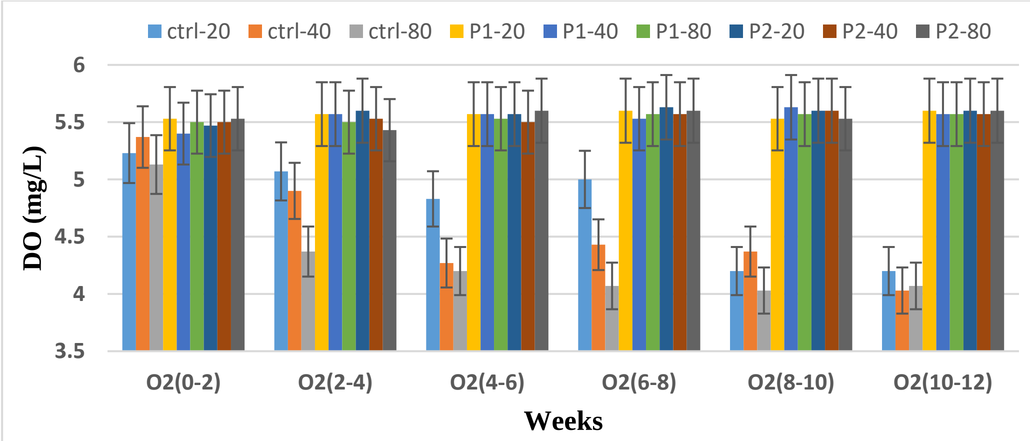
Probiotic	D	Total protein	Glucose	Cholesterol	Triglyceride
C	20	4.59±0.10^{c}	99.47±0.52^{c}	251.92±3.14^{a}	116.78±2.30^{ab}
	40	4.56±0.09^{c}	121.31±0.63^{b}	252.25±3.25^{a}	117.99±2.66^{a}
	80	4.62±0.20^{c}	129.23±0.68^{a}	255.74±0.05^{a}	113.71±0.33^{b}
P_1	20	5.13±0.11^{b}	93.01±1.26^{e}	223.58±0.31^{b}	114.18±0.86^{ab}
	40	5.83±0.07^{a}	94.51±0.97^{de}	224.96±0.25^{b}	114.08±1.09^{b}
	80	5.42±0.11^{b}	97.43±1.25^{cd}	225.10±1.12^{b}	113.48±0.15^{b}
P_2	20	5.91±0.07^{a}	92.76±0.52^{e}	224.50±0.31^{b}	113.27±0.51^{b}
	40	5.84±0.06^{a}	92.75±1.87^{e}	224.50±0.36^{b}	113.93±0.25^{b}
	80	5.76±0.16^{a}	94.69±0.85^{de}	224.87±0.51^{b}	113.27±0.56^{b}
Pooled means					
Probiotics	C	4.59±0.08^{c}	116.67±4.46^{a}	253.30±1.44^{a}	116.16±1.20^{a}
	P_1	5.46±0.12^{b}	94.98±0.87^{b}	224.55±0.42^{b}	113.91±0.42^{b}
	P_2	5.84±0.06^{a}	93.40±0.69^{b}	224.62±0.21^{b}	113.49±0.26^{b}
Densities (Fish/m^3)	20	5.21±0.20^{y}	95.08±1.18^{z}	233.33±4.74^{x}	114.74±0.89^{x}
	40	5.41±0.22^{x}	102.86±4.66^{y}	233.90±4.68^{x}	115.33±1.07^{x}
	80	5.27±0.14^{xy}	107.12±5.56^{x}	235.23±5.14^{x}	113.49±0.20^{x}

- Different superscript letters within the same row are significantly different ($P < 0.05$).

Water quality

At the end of this study, the results illustrated in Fig. (1) showed that the water quality parameters were affected by the addition of probiotic to the fish feed, where the pH, DO (mg/L) and TAN (mg/L) were significantly improved for the group that treated with P_1 and P_2 compared to the control group, even with an increase density is up to 80 fish/m^3.





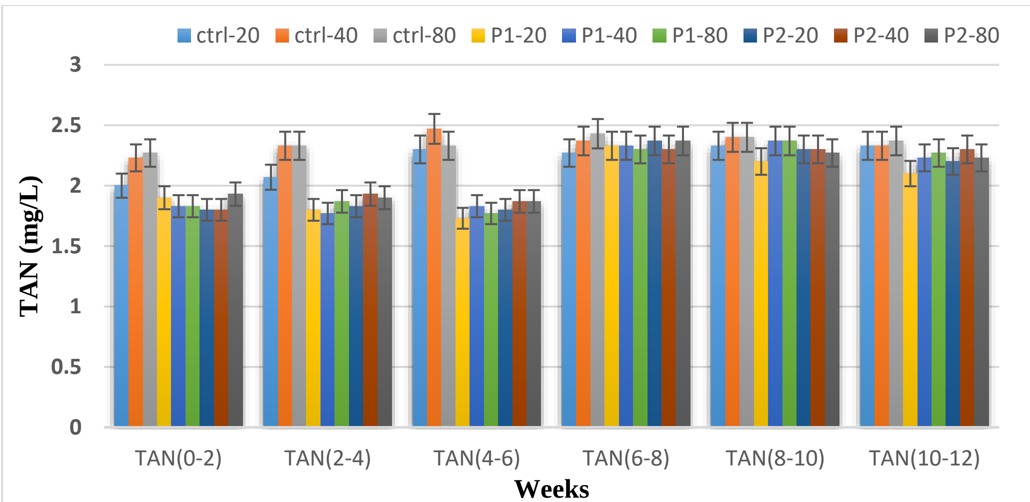


Fig. 1. Effects of two types of probiotics, and three fish densities on culture water quality of European seabass during the experiment period (12 weeks)

DISCUSSION

The use of probiotics is one of the most common and safe methods of use with the aim of improving the growth of cultured species and improving the quality of cultured water parameters. In this study, two types of probiotics were used with three levels of density, and the result at the end of the experiment was to improve growth performance even with high levels of density, namely a very good result from an economic point of view, and a significant improvement in water quality characteristics was also observed. It was found that the final body weight of the seabass in the P_1 and P_2 treatment was significantly higher than the level of the fish treated with the control diet so that the fish that were treated with probiotics had a extremely high values.

The same is the case with FCR, where the best value was for group P2 followed by P1, as for the control group, the highest value of FCR was recorded at a density of 80 fish/m^3. It was observed that an increase in FCR was followed by a decrease in SGR, and this corresponds to the results reached by some authors **(Zehra & Khan 2012; Amin *et al*., 2015)**. The group of fish that consumed the control diet (probiotic-free diet) was the lowest in terms of PPV, PER, ER and survival% values than the rest of the groups, and this is in agreement with **Lin *et al*. (2017)** who found that the use of a mixture of microorganisms led to an improved growth of the Asian bass. **Lus *et al*. (2016)** noted that the use of *B. subtilis* as a nutritional supplement has led to a very significant improvement in the growth performance, nutritional utilization, digestion and survival of white seabass.

Parameters of hematological are important indicators that give a clear assessment of the nutritional and health status of fish and their response to diverse environmental stresses **(Burgos-Aceves *et al*., 2019)**. **Davis *et al*. (2008)** detected a decrease in the number of white blood cells at high stock densities as a result of excessive stress. This is due to the change in the proportion of lymphocytes, moreover this is consistent with the findings of **Kpundeh *et al*. (2013)** and **Zaki *et al*. (2020)**, who noticed a significant change in the red blood cells, white blood cells, platelets and hemoglobin of tilapia fish when the density rates were higher than the normal limit.

The leukocyte count (WBC) is commonly used to assess the immune potential of fish. Toxic agents can affect the leukocyte count. An increase in WBC (leukocytosis) is usually seen as an activation of the immune response due to tissue damage by a toxic agent, often leading to neutrophilia and/or monocytosis, indicating an inflammatory response. Conversely, leukopenia (a decrease in WBC) is attributed to a toxicity-induced general stress response, causing lymphopenia and an increase in the neutrophil to lymphocyte ratio, or to a specific toxic action affecting circulating leukocytes or leukopoiesis, resulting in immunosuppression. **Ligina *et al*. (2022)** reported leukocytosis in *Anabas testudineus* during intoxication with acrylamide, while exposure to the insecticide chlorpyrifos caused leukocytosis in *Oreochromis niloticus* **(Zahran *et al*., 2018)**.

The results of this study indicated a decrease in blood glucose levels with the use of both types of probiotics, and this is in full agreement with many studies that suggested that prebiotic bacteria added to feed reduce blood glucose levels in fish, among these studies in that of **Mohapatra *et al*. (2014)**. In addition, blood parameters improved despite the increase in density, with differences between the control treatment and the rest of the treatments, and all proportions remained within the normal rates for fish.

CONCLUSION

In conclusion, the results demonstrated that supplementing fish feed with probiotics enhanced growth performance, food utilization, blood parameters, and water quality, even when fish densities were increased. This improvement in water quality was attributed to the presence of bacteria in fish waste and feed residues. Furthermore, the study confirmed that there was no significant difference between using commercially fortified probiotics with enzymes and those containing bacteria only.

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