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The synergistic effect of *Bacillus coagulans* and/or β glucan on growth performance and Immune responses in seabream (*Sparus aurata*) exposed to sub-optimal temperature

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Abstract:

The objective of this trial was to assess the impact of dietary *B. coagulans* (BC) and/or *β*-glucan (*β*G) supplements on growth, serum biochemical and hematological indices, antioxidant activity, immune parameters, digestive enzyme, and immune-related gene expression of seabream fingerlings (*Sparus aurata*) under sub-optimal temperature. A total of 405 healthy seabream fingerlings with an average weight of 22.95 ± 1.2 g were randomly allocated equally into 27 enclosures (hapa), with 15 fish per hapa. Nine fish groups were fed balanced diets containing 45% crude protein and 488 MJ/kg Gross energy, and supplemented with tested levels of BC (0.0, 1 or 2g/kg diet) or *β*G (0.0, 0.5 or 1g/kg diet), and their combined doses for 12 weeks then subjected to suboptimal temperature for two additional weeks. The results showed that the group fed a diet supplemented with 2g of BC and 1g of *β*G per kg diet exhibited higher specific and relative growth rates compared to the control group. Also, the same treated groups recorded the lowest mortality rate. Meanwhile, the groups supplemented with BC alone or in combination with *β*G significantly improved all estimated biochemical and hematological parameters compared to the control group. Similarly, the groups supplemented with BC and/or *β*G showed significant improvements in immunological parameters such as lysozyme, immunoglobulin M (IgM) levels, phagocytic index, and phagocytic activity. At the same tend, the dietary treatments significantly improved antioxidant capacity indices (CAT, MDA, and SOD), lipid profiles (triglyceride, cholesterol, glucose), cortisol concentrations, and gene expressions. In conclusion, the research findings indicate that the use of BC and/or *β*G supplements enhances the growth and boosts the resistance of seabream fingerlings against the negative impacts of sub-optimal temperatures.

INTRODUCTION

Seabream is a highly valued marine fish species that is predominantly found in the Mediterranean Sea regions. This species is also considered to be of great interest for aquaculture purposes due to its ability to tolerate a wide range of temperatures and salinity levels (**Pavlidis & Mylonas, 2011**). Seabream typically lives in environments ranging from 11°C in the winter to 26°C in the summer. Over the past few decades, the global production of seabream has consistently increased. Specifically, the worldwide production of gilthead seabream in 2020 reached a staggering 282,100 tons **(FAO, 2022)**. Nowadays, this species is thought to be a promising global instead of a capital asset for expanding the potential for marine fish farming **(Waite *et al.*, 2014)**. The gilthead seabream, *Sparus aurata*, is highly vulnerable to chronic

low temperatures. However, several reports suggest that seabream in their natural habitat tend to move to deeper and warmer waters when surface temperatures start to decrease **(Davis, 1988). Ravagnan (1978) and Barnabé (1990)** found that this species has a low lethal temperature of around 5°C. Prolonged exposure to low temperatures can lead to "winter disease" in these fish **(Mininni *et al.*, 2014)**. Since fish in intensive cultivation cannot migrate to warmer waters, temperature drops become a critical issue. Fish activity and growth performance are significantly reduced below 13°C **(Ibarz *et al.*, 2003; Tort *et al.*, 1998a; Sarusic, 1999)**. During winter, there is also a noticeable decrease in feeding habits **(Tort *et al.*, 1998)**. In an aquaculture farm located in the northern Mediterranean, this natural fasting phase under low ambient temperature conditions can last for several months, resulting in weight loss and huge drawbacks in final biomass output **(Mhalhel *et al.*, 2023)**. In addition, there have been regular occurrences of significant mortalities during specific winter months in various regions of the Mediterranean in recent years **(Vezzulli *et al.*, 2010)**. Typically, mortality rates within fish stocks range from 7 to 10%; however, under extremely undesirable ambient temperature degrees, they can reach up to 80% **(Padrós *et al.*, 1998)**, causing significant economic losses.

Several reports have shown that probiotics, prebiotics, their combination known as "symbiotic," and natural feed supplements can be effective additives in aquafeed, with potential immunostimulant features (**Dawood *et al.*, 2020a & Zarantoniello *et al.*, 2018)**. In particular, Symbiotics is believed to offer the combined advantages of probiotic and prebiotic supplements, including boosting both overall immunity and intestinal immunity **(Akhter et al., 2015)**. In addition, recent studies have investigated the potential benefits of synbiotics in areas such as immunostimulatory, antioxidative effects, and growth enhancement **(Modanloo *et al.*, 2017; Hasan *et al.*, 2018; Cao *et al.*, 2019; Devi *et al.*, 2019)**. Additionally, synbiotics have been identified as effective anti-inflammatory agents that can mitigate the negative impacts of several types of stress on the performance of finfish species (**Dawood *et al.*, 2020a, Hoseinifar 2016, Dawood and Koshio, 2018).** Specifically, including dietary-inactivated Lactobacillus cells in aquafeed has been shown to stimulate the growth, digestibility, and general health of various aquatic animals **(Van Nguyen *et al.*, 2019 Yassine *et al.*, 2021)**. Furthermore, βG derived from yeast cells has been demonstrated to function as an immunostimulant when added to aquafeed **(Leyva-López *et al.*, 2020)**. After multiple trials, the combination of *Lactobacillus* and *β* glucan has been approved as an active immunobiotic of Nile tilapia **(Dawood *et al.*, 2020 – Dawood *et al.*, 2015)**.

Numerous studies have examined the biological effects of BC or *βG* on various fish species (**Fath El-Bab *et al.*, 2022)**. However, there is limited knowledge about their combined impact on seabream under sub-optimal temperature conditions. Hence, the primary aim of this trial was to assess the synergistic advantages of including BC and *βG* in seabream diets. This combination was found to enhance the utilization of *βG*, resulting in improved growth performance, blood biochemical indices, immune responses, antioxidant status, and regulation of gene expression in *Sparus aurata*.

Materials and Methods:

Experimental Procedures:

2.1. Feed Additives

In this feeding trial study, two available commercial products were employed. *B. coagulans* DSM 32016 (Technospore®; Biochem co., Germany) commercial products containing $2.5x10^9$ CFU/g have been used as a safe feed additive. Also, *β*-glucan powder extract (Batch No:2809115, Pharma Health Co. Egypt) was applied as a prebiotic compound.

2.2. Prepared Experimental Diets

To prepare the experimental diets, the dry ingredients were thoroughly mixed and then added 200 mL of water per kg of diet. Once the mixture became a paste, it was pelleted using a laboratory pellet machine with a 1 mm diameter. The prepared pellets were then dried at room temperature and stored in plastic bags at -4 °C until they were ready to be used. It's important to note that all diets were formulated based on the literature (**Izquierdo *et al.*, 2015**) to meet the macronutrient and essential amino acid requirements for seabream **(Table, 1)**.

Table 1. Diet formulation and chemical composition of the experimental diets.

Ingredient	%
Fishmeal (CP, 62%)	34
Corn gluten meal (CP, 60%)	6
Extruded wheat (CP, 12%)	12

Soybean meal (CP, 48%)	29
Fish oil	9.6
Soybean oil	6.4
[a] Mineral & Vitamin premix	3
Total	100
Chemical composition	
Dry matter	90.1
Crude protein	43.4
Crude lipid	18.3
Fiber	9
Ash	12
[b] NFE	17.3
[c] Gross energy, MJ/kg	489.248

[a] Providing, per kg of mix: Vitamin E, 5.8 g; vitamin K_3, 3.3 g; thiamin, 3.3 g; riboflavin, 6.6 g; pyridoxine (as pyridoxine hydrochloride), 3.3 g; niacin, 16.6 g; folic acid, 3.3 g; vitamin B_{12} (cyanocobalamin), 0.01 g; D-biotin, 0.1 g; vitamin c (ascorbic acid), 33.3 g, calcium pantothenate, 13.3 g; Cu as copper sulfate, 3 g; I as calcium iodine, 0.4 g; Co as cobalt carbonate, 0.3 g; Mn as manganese sulfate, 10 g; zinc oxide, 30 g; sodium selenite, 0.08 g; calcium, 0.8 g.
[b] NFE = 100- (CP + EE + CF + Ash).
[c] GE = protein × 23.62 kJ/g + lipid × 39.52 kJ/g + carbohydrates × 17.2 kJ/g.

2.3. Experimental design and feeding regime:

Healthy *S. aurata* fingerlings weighing 22.95 ± 1.2 g were obtained from a private farm in Shatta, Damietta, Egypt. Following their transfer to the experimental hapa on the same farm, the fingerlings of *S. aurata* were randomly assigned into 27 enclosures (hapa), which corresponded to nine treatments. Fingerlings of *S. aurata* were not afforded feed during the transported day to alleviate any stress. Each treatment consists of three hapas (1 x 2 x 1.25 m) with 15 fish each. A fish feed was adjusted at a rate equivalent to 3% of biomass by weight. The fingerlings were hand-fed the different experimental diets twice per day, at 9:00 and 15:00, and their daily feed intake was recorded. Every 14 days, seabream fish were picked from each hapa and weighed, and the quantity of feed was adjusted to account for body weight differences during the study.

BC and *β*G were subjected to nine isonitrogenous and isocaloric diets, containing 45% protein and 488 kcal/100 g. The first group received a basal diet with no feed additives (T1) and served as a control group. Other treatment groups included a basal diet supplemented with *BC* at 1g (T2) or 2g (T3), or *β*G at levels of 0.5g (T4) and 1g (T5), and combinations of *BC* 1g+ *β*G 0.5g (T6) or BC 1g+ *β*G 1.0g (T7) and BC 2g+ *β*G 0.5g (T8) and BC 2g+ *β*G 0.1g (T9) per kg diet. During the 12-week feeding experiment, the rearing water temperature was appropriate for seabream production and health. However, during the next two weeks, the temperature was under suboptimal conditions at 9.50 ± 0.50 ◦C.

2.4. Water Quality Parameters

The fish were reared under a natural temperature of 16± 0.50 °C for 12 weeks and then naturally subjected to winter temperature (9± 0.40°C) for two weeks. The fish group was kept under a natural photoperiod regime (12 light:12 dark). The water temperature, pH, dissolved oxygen level, and salinity were measured daily. The mean values for all estimated water quality criteria were found to be suitable for fish production and health in all experimental treatments. The daily estimated water quality measurements were included pH, salinity, nitrite (NO^{2-}), nitrate (NO^{3-}), ammonia (NH^{3+}), dissolved oxygen, potassium (K^{+}), sodium (Na^{+}), magnesium (Mg^{2+}), calcium (Ca^{2+}), sulfur (SO_4), chloride (Cl^{-}), and bicarbonate (HCO_3^{-}), which were maintained at 7.44±0.05, 26.42 ppt, 0.026 mg/L, 0.031 mg/L, 0.029 mg/L, 5.2 mg/L, 2.41 mg/L, 211.22 mg/L, 55.16 mg/L, 69.74 mg/L, 162.4 mg/L, 168.23 mg/L, and 4.16 mg/L, respectively.

2.5. Growth performance indices:

The surviving fish were regularly fed and weighed every two weeks to determine their diets and measure their growth. Various parameters, such as Weight Gain (WG), Specific Growth Rate (SGR), Relative Growth Rate (RGR), and mortality percentage (MR%), were considered to evaluate the performance as well as tolerance to suboptimal temperature stress. These parameters were calculated using the following formula:

Weight gain (WG)=final body weight (g) - initial body weight (g)

$$\text{Specific growth rate (SGR)} = 100 \times \frac{\ln \text{final body weight (g)} - \ln \text{initial body weight (g)}}{\text{Duration of feeding (day)}}$$

Relative growth rate (RGR)=
((FW-IW)/FW) ×100

Mortality rate (%) per hapa = (number of stocked fish per hapa - number of fish harvested per hapa) / number of stocked fish per hapa * 100 **(Ricker, 1976)**

2.6. Blood Sampling:

According to **Feldman *et al.* (2000)**, blood samples were taken from the caudal vein of 10 fish per group. The fish were first anesthetized with 40 mg/L Clove oil. Each blood sample was divided into two equal portions and stored in separate tubes. One tube was used for hematological analysis (including EDTA), while the other tube was centrifuged at 4000 rpm for 15 minutes to separate the serum. The serum was then stored at -20 °C until required.

2.7. Hematological analysis:

The total count of red blood cells (RBCs) and white blood cells (WBCs) was determined using a hemocytometer **(Tsan and White, 1988).** Hemoglobin (Hb) values were determined following the method described by **Collier (1944)**, while hematocrit (HTC) values and mean corpuscular hemoglobin concentration (MCHC) were estimated as outlined by **Wintrobe (1934)**. The mean corpuscular volume (MCV) was measured directly using an automated Coulter LH 750 hematology analyzer (Beckman Coulter, Fullerton, CA) (**Dacie and Lewis, 1984)**. The differential white blood cell counts (WBCs) were performed using Klontz's method **(Klontz, 1994)**.

2.8. Serum biochemical parameters

Serum samples were analyzed using Bio-diagnostic commercial kits (Cairo, Egypt) to determine the levels of total protein, albumin, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, triglycerides, and cholesterol. The specific catalog numbers for the kits were TP 20 20, AB 10 10, GL 13 20, AS 10 61 (45), AL 10 31 (45), CR 12 50, UR 21 10, TR 20 30, and CH 12 20, respectively. The content of globulins was computed mathematically. All assays were performed in strict accordance with the manufacturers' instructions.

2.9. Digestive Enzymes Assay:

To assess the activities of amylase and lipase, the intestinal samples were homogenized in phosphate-buffered saline (pH 7.4), and then centrifuged. The resulting supernatant was used to measure the activities of lipase and amylase using colorimetric kits provided by Spin react (Spain; Cat. no. MX1001275) and Bio diagnostic (Cairo, Egypt; Cat. no. AY 10 50).

2.10. Immunological variables:

The phagocytic activity and index were determined using the procedure described **by Kawahara *et al.* (1991)**. To calculate the phagocytic index, the following formulas were used: Phagocytic activity = (Total number of macrophages containing yeast/total number of macrophages) × 100, and phagocytic index = phagocytized cell count/total phagocytic cell number. Serum lysozyme (LYZ) activity was assessed using a turbidimetric assay, as outlined by **Parry *et al.* (1965)**, with a suspension of *Micrococcus lysodeikticus* (EC 3.2.1.17) from Sigma-Aldrich, USA. LYZ activities in serum were then calculated using a standard curve established from LYZ extracted from chicken egg white, also from Sigma-Aldrich, USA. Immunoglobulin M (IgM) levels were measured using the Cusabio (China; cat. no. CSB-E12045Fh) ELISA kit. All assays were performed according to the manufacturer's instructions.

2.11. Determination of Redox Status

To assess the activities of serum glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), as well as the levels of malonaldehyde (MDA), reagent kits provided by Biodiagnostic (Egypt) were used. The specific kit catalog numbers used for each enzyme were GP 2524 for GPx, SD 25 21 for SOD, CA 25 17 for CAT, and MD 25 29 for MDA. All assays were performed following the manufacturer's instructions. Cortisol levels were determined using an ELISA kit obtained from MyBioSource (USA), with the catalog number MBS9424415.

2.12. Gene expression:

Total RNA was extracted from frozen liver samples (10 fish/group) using Trizol (Invitrogen, USA) according to the manufacturer's instructions. The RNA was quantified using a NanoDrop method, and the obtained samples were reverse transcribed into cDNA using an Invitrogen (USA) kit at an OD260/OD280 nm ratio of ≥ 1.8. The generated cDNA was then amplified using TOPreal™ PreMIX SYBR Green qPCR master mix (Enzynomics; cat. no. RT 500) and examined using the primers listed in Table 2. The β-actin gene was applied as the main housekeeping gene for normalizing cDNA loading. The resulting data were analyzed using the $2^{-\Delta\Delta Ct}$ applicable method **(Livak and Schmittgen, 2001)**.

Table 2. Specific applied primer sequences used for RT-qPCR analysis and their accession number

Target gene	Forward 5' →3'	Reverse 5' →3'	Gene Bank Accession No
IGF-1	AGTGCGATG TGCTGTATC	CAGCTCACA GCTTTGGAA G-	EF563837.1
HSP70	AATGTTCTG CGCATCATC AA	CCAACCTTTT TGTCCAATCC	EU805481.1
IL-1β	GGGCTGAAC AACAGCACT CTC	TTAACACTCT CCACCCTCCA	115592467
β-actin	CGACGGAC AGGTCATCA CCA	AGAAGCATT TGCGGTGGA CG	AF384096.1

2.13. Statistical analysis:

A two-way ANOVA was conducted to examine the impact of dietary BC, *β*G levels, and their interactions on alleviating adverse effects of low temperature. Before conducting the two-way ANOVA, Levene's test was employed to assess the normality and homogeneity of variance for all collected data. Additionally, percentage data were transformed using the arcsine transformation, but the original untransformed data are presented. The Statistical Analysis System **(SAS, 2012)** version (8.02) was used to analyze all data through the general linear model procedure. Differences among treatments were determined using Tukey's HSD test with a significance level of 5%. The analyzed data are presented as mean ± standard error (SE).

Results and Discussion

3.1. Growth performance:

Table 3 illustrates the growth performance of seabream fish that were fed the basal diet (CTR) and diets supplemented with two levels of BC alone and/or two other levels of *β*G for 12 weeks. After this period, the fish were exposed to a sub-optimal temperature for an additional two weeks. The two-way ANOVA analysis showed a significant effect between dietary BC or βG supplementation and the control group, as well as their interaction, on all growth including FBW, WG, SGR, and RGR values (Table 3). Furthermore, the combination of high levels of both BC and *β*G supplementation in the diet resulted in the highest FBW, WG, SGR, and RGR values among all groups ($P < 0.001$). Moreover, MR (%) was significantly decreased ($P < 0.001$) in all experimental groups compared to the CTR group, with the lowest MR% observed in the fish groups that were fed the combined supplementation with BC and *βG*.

Table 3. Growth Performance and mortality rate of seabream fish (*S. aurata*) fed diets supplemented with BC (1 and 2 g kg^{-1}) and/or *β*G (0.5 and 1 g kg^{-1}) for 12 weeks then exposed to sub-optimal temperature for two weeks.

	Parameters					
Treatments	**IBW (g)**	**FBW (g)**	**WG (g)**	**SGR (%/d)^2**	**RGR (g/g)**	**Mortality%**
CTR	22.95	99.57^{f}	76.62^{f}	1.75^{b}	76.95^{e}	65.33^{a}
BC1	23.30	115.63^{e}	92.33^{e}	1.91^{a}	79.85^{d}	29.67^{b}
BC2	24.25	119.63^{de}	95.38^{e}	1.90^{a}	79.73^{d}	26.67^{b}
***β*G1**	24.10	120.36^{d}	96.26^{e}	1.92^{a}	79.98^{d}	26.33^{b}
***β*G2**	23.02	122.54^{d}	99.52^{d}	1.99^{a}	81.22^{c}	22.53^{c}
BC1+*β*G1	23.50	126.64^{d}	103.14^{c}	2.01^{a}	81.44^{c}	15.50^{d}
BC1+*β*G2	22.95	130.35^{c}	107.40^{c}	2.07^{a}	82.39^{b}	12.00^{e}
BC2+*β*G1	23.79	138.18^{b}	114.40^{b}	2.10^{a}	82.79^{b}	9.67^{f}
BC2+*β*G2	22.18	140.24^{a}	118.06^{a}	2.20^{a}	84.19^{a}	9.33^{f}
PSE	0.12	0.22	0.25	0.13	0.11	0.25
Two-way ANOVA (P value)						
BC	0.514	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
***β*G**	0.354	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
BC*_β_G	0.754	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

CTR, fish group fed basal diets;
BC1, fish group fed diets supplemented with 1 g BC per kg;
BC2, fish group fed diets supplemented with 2 g BC per kg;
*β*G1, fish group fed diets supplemented with 0.5 g *β*G per kg;
*β*G2, fish group fed diets supplemented with 1 g *β*G per kg;
BC1+*β*G1, fish group fed diets supplemented with 1 g *BC*+ 0.5 g *β*G per kg diet;
BC1+*β*G2, fish group fed diets supplemented with 1 g *BC*+ 1 g *β*G per kg diet;
BC2+*β*G1, fish group fed diets supplemented with 2 g *BC*+ 0.5 g *β*G per kg diet;
BC2+*β*G2, fish group fed diets supplemented with 2 g *BC*+ 1 g *β*G per kg diet.
[a,b,c,d,e,f] Values within the same column having different superscripts are significantly different ($P < 0.05$). Data were presented as the mean ± mean pooled standard error (PSE).
IW=Initial weight (g); FW= Final weight (g); WG= Weight gain (g); DG= daily gain; SGR= Specific growth rate (%); RGR = Relative growth rate (g/g).

3.2. Blood Hematology:

Table 4 represents the hematological indices of seabream fish that were influenced by diets supplemented or un-supplemented with two levels of BC or *β*G alone, or in combination forum. The fish were fed these diets for a period of 12 weeks and then subjected to suboptimal temperature degrees for an additional two weeks. The total count of red blood cells (RBCs), white blood cells (WBCs), Monocytes, and lymphocytes significantly increased ($P < 0.001$) under the influence of BC, βG, and their interaction. The highest counts of RBCs, WBCs, and lymphocytes were observed with either BC, *β*G, or the interaction between a high level of BC and βG. Moreover, MCV values showed significant ($P < 0.05$) increases in BC treated group as well as decreases in *βG* supplemented group. Meanwhile, fish diets supplemented with a high level of BC or any level of BC combined with a high level of *β*G significantly ($P < 0.001$) increased hemoglobin (Hb) concentration compared to other treated groups. Conversely, the percentage values of Basophils and Eosinophils were not significantly affected by any of the examined diets.

Table 4. Blood hematological parameters of seabream fish (*S. aurata*) fed diets supplemented with *BC* (1 and 2 g kg-1) and/or *β*G (0.5 and 1 g kg-1) for 12 weeks then exposed to sub-optimal temperature for two weeks.

	Parameters								
Treatments	**RBC (10^6×µL)**	**Hb (g/dL)**	**MCV (fL)**	**MCHC (g/dL)**	**WBC (10^5×µL)**	**Lymphocytes**	**Monocytes**	**Basophil (%)**	**Eosinophils (%)**
CTR	3.91^{ab}	12.04^{b}	97.31^{b}	31.68^{a}	18.40^{d}	12.51^{d}	1.47^{c}	0.37	0.28
BC1	4.22^{a}	12.60^{b}	98.34^{a}	30.35^{b}	25.59^{b}	18.94^{c}	2.05^{b}	0.26	0.26
BC2	4.40^{a}	13.27^{a}	98.86^{a}	30.51^{ab}	27.46^{a}	21.28^{a}	2.34^{a}	0.28	0.41
***β*G1**	3.99^{ab}	12.05^{b}	97.75^{ab}	30.90^{ab}	23.42^{c}	17.68^{c}	1.75^{b}	0.35	0.23
***β*G2**	4.05^{a}	12.12^{b}	96.42^{c}	31.08^{a}	24.59^{b}	18.45^{c}	2.21^{a}	0.25	0.25
BC1+*β*G1	4.32^{a}	13.08^{a}	98.38^{a}	30.77^{ab}	27.04^{a}	20.55^{ab}	2.30^{a}	0.27	0.27
BC1+*β*G2	4.42^{a}	13.49^{a}	97.40^{b}	31.37^{a}	27.54^{a}	21.48^{a}	2.48^{a}	0.41	0.28
BC2+*β*G1	4.30^{a}	12.91^{ab}	96.62^{bc}	31.10^{a}	27.02^{a}	20.27^{b}	2.57^{a}	0.41	0.41
BC2+*β*G2	4.42^{a}	13.45^{a}	97.40^{b}	31.27^{a}	27.98^{a}	21.96^{a}	2.52^{a}	0.42	0.28
PSE	0.04	0.17	0.32	0.36	0.51	0.14	0.57	0.41	0.33
Two-way ANOVA (P value)									
BC	< 0.001	< 0.001	۰.018	۰.001	< 0.001	< 0.001	< 0.001	۰.106	۰.0۹9
***β*G**	< 0.001	0.243	0.0۱2	< 0.001	< 0.001	< 0.001	< 0.001	0.198	0.235
BC**β*G**	< 0.001	< 0.001	0.۰۸0	< 0.001	< 0.001	< 0.001	< 0.001	0.795	0.047

CTR, fish group fed basal diets;
BC1, fish group fed diets supplemented with 1 g BC per kg;
BC2, fish group fed diets supplemented with 2 g BC per kg;
*β*G1, fish group fed diets supplemented with 0.5 g *β*G per kg;
*β*G2, fish group fed diets supplemented with 1 g *β*G per kg;
BC1+*β*G1, fish group fed diets supplemented with 1 g *BC*+ 0.5 g *β*G per kg diet;
BC1+*β*G2, fish group fed diets supplemented with 1 g *BC*+ 1 g *β*G per kg diet;
BC2+*β*G1, fish group fed diets supplemented with 2 g *BC*+ 0.5 g *β*G per kg diet;
BC2+*β*G2, fish group fed diets supplemented with 2 g *BC*+ 1 g *β*G per kg diet.
[a,b,c,d,e,f] Values within the same column having different superscripts are significantly different ($P < 0.05$). Data were presented as the mean ± mean pooled standard error (PSE).
RBCs = red blood cell count; Hb = hemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin conc

3.3. Serum Biochemistry:

The total protein content (TP) was significantly increased (P < 0.001) by a higher dietary BC level alone or in combination with βG2, compared to the other experimental groups (Table, 5). Furthermore, the concentration of GLOB showed significant increases ($P < 0.001$) in the presence of all feed supplements and their interaction. Specifically, the highest level of GLOB was recorded in the BC2 fish group followed by the fish group that was fed combined supplements, namely BC1+ βG2, BC1+ *β*G2, and BC2+ *β*G2, respectively. Meanwhile, the ALT level showed a significant decrease ($P < 0.001$) in the fish group that received a low level of BC alone or in combination with any level of *β*G. Adversely, the ALT level increased significantly ($P < 0.01$) when *β*G supplementation was administered alone compared to the control groups. Finally, the AST levels were significantly decreased ($P < 0.001$) in the fish group that was fed a low level of BC, a high level of *β*G (*β*G2), and their combination, compared to the other groups. Moreover, the blood levels of ALB, urea, and creatinine were not significantly affected by any of the feed additives that were examined.

Table 5. Serum biochemical parameters of seabream fish (*S. aurata*) fed diets supplemented with BC (1 and 2 g kg-1) and/or *β*G (0.5 and 1 g kg-1) for 12 weeks then exposed to sub-optimal temperature for two weeks.

Treatments	Parameters						
	TP (g/dL)	ALB (g/dL)	GLOB(g/dL)	Urea (mg/dL)	Creatinine (mg/dL)	ALT(UL)	AST (UL)
CTR	5.05^{b}	1.56	3.49^{b}	1.84	0.35	41.23^{a}	25.93^{a}
BC1	5.56^{ab}	1.60	3.97^{ab}	1.80	0.30	38.18^{c}	24.50^{b}
BC2	5.70^{ab}	1.62	4.08^{a}	1.68	0.29	39.99^{b}	25.55^{a}
βG1	5.23^{b}	1.56	3.67^{ab}	1.87	0.30	40.83^{a}	24.73^{b}
βG2	5.24^{b}	1.58	3.66^{ab}	1.83	0.31	40.24^{ab}	22.07^{d}
BC1+βG1	5.62^{ab}	1.60	4.02^{a}	1.81	0.30	40.21^{ab}	24.67^{b}
BC1+βG2	5.76^{ab}	1.63	4.14^{a}	1.68	0.33	37.80^{c}	22.28^{d}
BC2+βG1	5.57^{ab}	1.64	3.94^{ab}	1.78	0.31	39.79^{b}	22.90^{c}
BC2+βG2	6.07^{a}	1.62	4.45^{a}	1.64	0.34	37.45^{c}	23.71^{c}
PSE	0.12	0.08	0.17	0.16	0.02	0.22	0.40
Two-way ANOVA (P value)							
BC	< 0.001	0.074	< 0.001	0.285	0.096	< 0.001	< 0.001
βG	0.061	0.247	< 0.001	0.095	0.081	٠.0١٢	< 0.001
BC*βG	< 0.001	0.106	< 0.001	0.204	0.124	< 0.001	< 0.001

CTR, fish group fed basal diets;
BC1, fish group fed diets supplemented with 1 g BC per kg;
BC2, fish group fed diets supplemented with 2 g BC per kg;
*β*G1, fish group fed diets supplemented with 0.5 g *β*G per kg;
*β*G2, fish group fed diets supplemented with 1 g *β*G per kg;
BC1+*β*G1, fish group fed diets supplemented with 1 g *BC*+ 0.5 g *β*G per kg diet;
BC1+*β*G2, fish group fed diets supplemented with 1 g *BC*+ 1 g *β*G per kg diet;
BC2+*β*G1, fish group fed diets supplemented with 2 g *BC*+ 0.5 g *β*G per kg diet;
BC2+*β*G2, fish group fed diets supplemented with 2 g *BC*+ 1 g *β*G per kg diet.
[a,b,c,d,e,f] Values within the same column having different superscripts are significantly different ($P < 0.05$). Data were presented as the mean ± mean pooled standard error (PSE).
TP=total protein; ALB=albumin; GLOB=globulin; ALT=alanine transaminase; AST=aspartate aminotransferase.

3.4. Antioxidant and immunological responses :

The data in Table 6 demonstrate that the dietary administration of BC and/or *β*G significantly (($P < 0.001$) stimulated antioxidant and immunological measurements. Specifically, the serum lysozyme and phagocytic activities were significantly stimulated ($P < 0.001$) by all the feed diets examined. The highest levels were observed in the fish group that received combined supplements of high levels of both BC and *β*G. At the same trend, supplementing fish diets with BC alone or in combination with βG significantly ($P < 0.001$) increased the phagocytic index and IgM values compared to the control group.

Regarding the antioxidant activity results, it was observed that the fish group that received diets supplemented with a high level of BC alone or in combination with a high level of *β*G significantly ($P < 0.001$) increased SOD and CAT activities compared to other fish groups. Meanwhile, the combined BC and *β*G groups exhibited a significant ($P < 0.001$) increase in glutathione peroxidase activity, while the fish groups that received BC-supplemented diets showed a significant ($P < 0.001$) reduction compared to the other fish groups. On the other hand, fortifying feed supplements to fish diets significantly ($P < 0.001$) reduced MDA levels compared to the control group. In particular, the fish group that was fed diets supplemented with a combination of BC and *β*G had the lowest MDA levels, followed by the fish group that was fed a high level of BC (BC_2).

Table 6. Serum antioxidant activities and immune responses of seabream fish (*S. aurata*) fed diets supplemented with BC (1 and 2 g kg^{-1}) and/or *β*G (0.5 and 1 g kg^{-1}) for 12 weeks then exposed to sub-optimal temperature for two weeks.

Treatments	Parameters							
	LYZ (µg/ml)	Phagocytic index %	Phagocytic activity %	IgM (µg/ml)	MDA (IU/L)	GPx (IU/L)	CAT (IU/L)	SOD (IU/L)
CTR	5.73^{e}	٠.78^{c}	8.17^{d}	1.21^{b}	18.85^{a}	9.29^{c}	11.21^{c}	6.85^{d}
BC1	7.35^{c}	1.05^{a}	9.53^{b}	2.02^{a}	14.58^{cd}	9.70^{b}	12.75^{b}	8.03^{b}
BC2	8.01^{b}	1.02^{a}	9.89^{b}	2.33^{a}	13.82^{d}	9.95^{b}	13.35^{a}	9.06^{a}
βG1	6.17^{d}	٠.89^{ab}	9.05^{c}	1.24^{b}	16.35^{b}	9.35^{c}	12.10^{b}	7.07^{c}
βG2	6.26^{d}	٠.90^{ab}	9.20^{c}	1.31^{b}	15.47^{c}	9.33^{c}	12.50^{b}	7.04^{c}
BC1+βG1	7.42^{c}	1.03^{a}	9.64^{b}	2.1١^{a}	13.79^{d}	9.88^{b}	12.96^{ab}	8.18^{b}
BC1+βG2	8.07^{b}	1.00^{a}	10.08^{ab}	2.61^{a}	13.80^{d}	9.95^{b}	13.41^{a}	9.11^{a}
BC2+βG1	7.33^{c}	1.08^{a}	9.63^{b}	2.06^{a}	13.67^{d}	9.75^{b}	13.07^{a}	8.08^{b}
BC2+βG2	9.07^{a}	1.06^{a}	10.96^{a}	2.50^{a}	13.13^{e}	10.31^{a}	13.50^{a}	9.63^{a}
PSE	0.56	0.45	0.26	0.22	0.54	0.48	0.33	0.03
Two-way ANOVA (P value)								
BC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
βG	< 0.001	< 0.001	< 0.001	0.078	< 0.001	0.063	< 0.001	< 0.001
BC*βG	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

CTR, fish group fed basal diets;
BC1, fish group fed diets supplemented with 1 g BC per kg;
BC2, fish group fed diets supplemented with 2 g *BC* per kg;
*β*G1, fish group fed diets supplemented with 0.5 g *β*G per kg;
*β*G2, fish group fed diets supplemented with 1 g *β*G per kg;
BC1+*β*G1, fish group fed diets supplemented with 1 g *BC* + 0.5 g *β*G per kg diet;
BC1+*β*G2, fish group fed diets supplemented with 1 g *BC* + 1 g *β*G per kg diet;
BC2+*β*G1, fish group fed diets supplemented with 2 g *BC* + 0.5 g *β*G per kg diet;
BC2+*β*G2, fish group fed diets supplemented with 2 g *BC* + 1 g *β*G per kg diet.
[a,b,c,d,e,f] Values within the same column having different superscripts are significantly different ($P < 0.05$). Data were presented as the mean ± mean pooled standard error (PSE).
LYZ=lysozyme; IgM=immunoglobulin M; MDA=malonaldehyde; GPx=glutathione peroxidase; CAT=catalase; SOD=super oxide dismutase

3.5. Serum stress biomarkers and digestive enzyme activities:

Table 7 illustrates the effects of feed supplements on blood stress biomarkers (cholesterol, triglycerides, and glucose) and digestive enzyme activities (lipase and amylase) under conditions of suboptimal temperature stress. In detail, the cortisol level was significantly ($P < 0.001$) decreased in the BC1+ *β*G2 fish group, followed by the *β*G2 and BC1 fish groups, when compared with the other treated groups. Meanwhile, the fish group that received a low level of BC combined with a high level of *β*G showed a significant ($P < 0.001$) increase in cholesterol and glucose levels compared to the other treated groups. Additionally, when fish diets were supplemented with high levels of either BC or *β*G, there was a significant ($P < 0.001$) increase in cholesterol levels compared to the control group. At the same trend, fish groups that were fed diets supplemented with BC combined with *β*G showed significant ($P < 0.001$) increases in triglyceride content compared to other treated groups. The highest levels of triglycerides were recorded in the BC2+ *β*G2, BC1+ *β*G1, and BC1+ *β*G2 groups, respectively.

The estimated activities of digestive enzymes (lipase and amylase) showed a significant increase ($P < 0.001$) in the BC or *β*G supplementation groups, as well as the control group reared under sub-optimal temperature. The fish group fed diets containing high levels of BC alone or in combination with high levels of *β*G, as well as the BC1+ *β*G2 fish group, showed the highest values compared to the control and other treated groups.

Table 7. Stress biomarkers and digestive enzyme activities of seabream fish (*S. aurata*) fed diets supplemented with BC (1 and 2 g kg^{-1}) and/or *β*G (0.5 and 1 g kg^{-1}) for 12 weeks then exposed to sub-optimal temperature for two weeks.

Treatments	Parameters					
	Cortisol (µg/dL)	**Cholesterol (mg/dL)**	**Triglycerides (mg/dL)**	**Glucose (mg/dL)**	**Lipase (IU/L)**	**Amylase (IU/L)**
CTR	28.23^{a}	89.55^{c}	81.95^{d}	10.32^{e}	30.60^{d}	49.56^{d}
BC1	28.72^{a}	96.22^{b}	101.33^{a}	12.90^{bc}	38.27^{b}	70.90^{b}
BC2	27.81^{ab}	99.86^{ab}	104.56^{a}	13.21^{b}	41.22^{a}	72.80^{a}
βG1	27.37^{ab}	88.98^{c}	92.89^{c}	11.22^{d}	35.31^{c}	58.52^{c}
βG2	28.12^{a}	90.86^{c}	95.84^{c}	11.98^{c}	36.25^{c}	59.95^{c}
BC1+βG1	27.75^{ab}	99.68^{ab}	100.74^{a}	12.99^{bc}	39.93^{a}	70.82^{b}
BC1+βG2	26.22^{c}	103.39^{a}	106.92^{a}	14.03^{a}	41.37^{a}	73.38^{a}
BC2+βG1	28.85^{a}	99.00^{ab}	99.98^{ab}	12.93^{bc}	39.99^{a}	70.70^{b}
BC2+βG2	26.24^{c}	103.67^{a}	106.49^{a}	13.10^{b}	41.70^{a}	73.47^{a}
PSE	0.44	0.41	0.37	0.51	0.28	0.29
Two-way ANOVA (P value)						
BC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
βG	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
BC*βG	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

CTR, fish group fed basal diets;
BC1, fish group fed diets supplemented with 1 g BC per kg;
BC2, fish group fed diets supplemented with 2 g BC per kg;
*β*G1, fish group fed diets supplemented with 0.5 g *β*G per kg;
*β*G2, fish group fed diets supplemented with 1 g *β*G per kg;
BC1+*β*G1, fish group fed diets supplemented with 1 g *BC* + 0.5 g *β*G per kg diet;
BC1+*β*G2, fish group fed diets supplemented with 1 g *BC* + 1 g *β*G per kg diet;
BC2+*β*G1, fish group fed diets supplemented with 2 g *BC* + 0.5 g *β*G per kg diet;
BC2+*β*G2, fish group fed diets supplemented with 2 g *BC* + 1 g *β*G per kg diet.
[a,b,c,d,e,f] Values within the same column having different superscripts are significantly different ($P < 0.05$). Data were presented as the mean ± mean pool

3.6. Gene transcription:

Figure 1 illustrates the effects of feed supplements on the transcription of target genes of seabream fish under suboptimal temperature stress. Supplementing fish feed diets with BC and/or *β*G has been found to significantly regulate the transcription of *HSP70*, *IL-1β*, and *IGF-1* in seabream fish (*S. aurata*) exposed to suboptimal temperature stress. Specifically, the fish group fed diets supplemented with a combination of BC and a high level of *β*G exhibited a highly significant (P <0.001) upregulation compared with the other treated groups. Furthermore, the transcription of the *IL-1β* gene showed significant (P <0.001) upregulation under the influence of *β*G supplementation, which indicated significant (P < 0.05) downregulation in fish group-fed diets supplemented with higher levels of both BC and *β*G. Furthermore, the transcription of the *IL-1β* gene demonstrated a significant (P < 0.001) increase when influenced by *β*G supplementation. In the meantime, the other groups that received combined treatment exhibited a noteworthy (P < 0.05) reduction in the transcription of *IL-1β* in fish that consumed diets containing higher levels of both BC and *β*G.

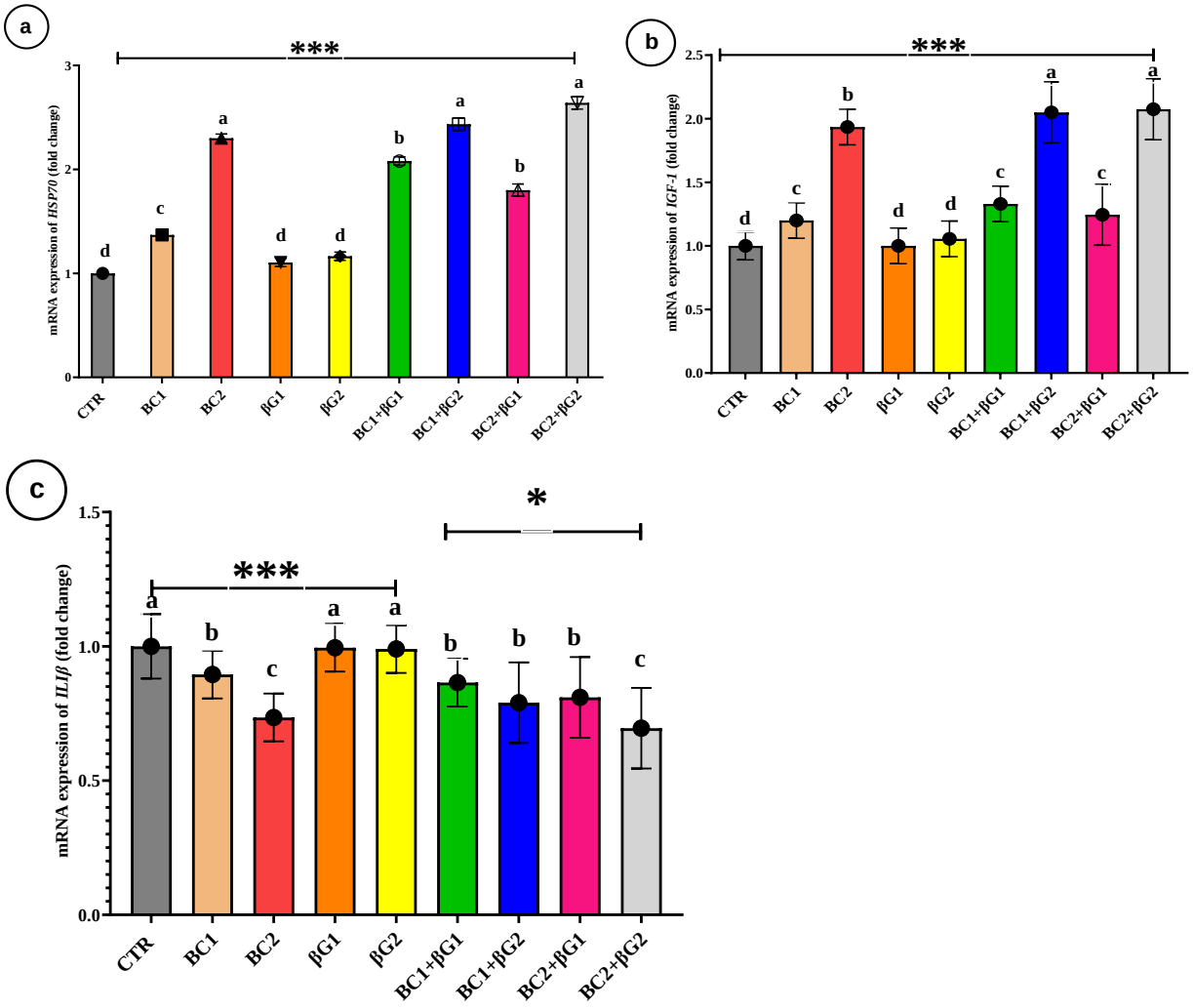

Fig. 1: *HSP70*: heat shock protein 70 (a); *IGF-1*: insulin-like growth factor-1 (b) and *IL-1β*: interleukin-1 beta (c) genes in livers of seabream fish (*S. aurata*) fed diets supplemented with *BC* (1 and 2 g kg^{-1}) and/or β glucans (0.5 and 1 g kg^{-1}) for 12 weeks. Bars show means ± S.E. ** or *** exhibited significant differences between groups ($P < 0.05$ or 0.001).

4. Discussion

The current study assessed how supplementing seabream fish (*S. aurata*) diets with *BC* (1 and 2 g kg^{-1}) and/or *β*G (0.5 and 1 g kg^{-1}) affected the fishes' growth performance (FBW, WG, RGR, SGR and mortality%), blood hematological parameters, serum biochemical measurements, antioxidants, stress indices, immune responses, and gene expression when they were exposed to Sub-optimal temperature temperatures. According to our study results, adding *BC* and *β*G to Seabream diets had a meliorative impact on the performance against adverse effects of sub-optimal temperature. Fish growth and feed efficiency are linked to the presence of BC and *β*G in the diet combination. Owing to **Soltani *et al*. (2019)**, findings the current experiment results indicated that the combination of BC and *β*G promotes seabream development because BC increases the diversity of beneficial bacteria in fish gastrointestinal tracts. Nutritional digestion and absorption through epithelial cells are facilitated by the growth of advantageous bacterial strains in the digestive system **(Kavitha *et al*., 2018) .**

Additionally, reducing the impact of pathogenic bacteria on intestinal immunity is achieved by dominating the beneficial microorganisms in the gut microbiota **(Burr *et al*., 2005)**. The immune system of the intestines is therefore linked to the immunological system of the entire body **(Dawood, 2021)**. The same outcomes are produced by prebiotics, most especially *β*-glucan, which inhibits pathogen colonization and reduces inflammation while promoting the growth and

activity of the ideal healthy intestinal microbial community **(Yan *et al.*, 2016)**. This hypothesis suggests that BC and *β*G had a synergistic effect on seabream performance. White blood cells called phagocytes are constantly produced from the bone marrow and are known to be in charge of eliminating germs and dead cells **(Secombes and Fletcher, 1992)**. Moreover, the cellular-immune system, which guards the fish body against infectious disorders, includes white blood cells **(Haghighi and Rohan, 2013)**.

Many studies **(Femi-Oloye *et al.*, 2020; Fath El-Bab *et al.*, 2022)** attribute elevated or decreased hematological and biochemical variables in fish relative to normal values mostly to feed additives. The results demonstrated that fish fed either BC or *β*G alone or in combination had normal biochemical levels, falling within what was thought to be the usual range for healthy fish. Fish fed *β*G /BC1 also showed higher amounts of globulin, and total protein than fish fed other treatments. Furthermore, it has been noted that the total protein content rises when beta-glucan and low levels of *Bacillus spp*. are mixed **(Azevedo *et al.*, 2016; De Souza *et al.*, 2020)**. Supplementing fish feed with probiotics or prebiotics promotes intestinal immunological responses against pathogens, including humoral and cell-mediated responses, resulting in an increment in immunoglobulin levels in the blood and an increase in total protein **(Hoseinifar *et al.*, 2018)**.

According to our data, concluded that the impact of BC alone or combined with BG supplemented diets boost cellular-immune activity. Major probiotic mechanisms of action involve epithelial barrier improvements, enhanced adherence to the intestinal mucosa, and associated inhibition of pathogen adhesion, competitive exclusion of pathogenic bacteria, generation of anti-microorganism molecules, and innate immune system regulation **(Bermudez-Brito *et al.*, 2012)**. In particular, strain-specific and dose-dependent release of different cytokines as well as the activation of natural killer (NK) cells, antigen-specific cytotoxic T-lymphocytes, and macrophages could all be features of cellular immune response induced by probiotic-based diets **(Ashraf and Shah, 2014)**.

It is commonly recognized that probiotics and prebiotics can shield fish against oxidative stress **(El-Shall *et al.*, 2019; Merrifield *et al.*, 2010)**. Under stressful situations, reactive oxygen species (ROS) may be produced in large quantities, damaging the cellular membrane by causing lipid peroxidation **(Slaninova *et al.*, 2009)**. By increasing the production of anti-oxidative enzymes like catalase (CAT) and reducing MDA activation, the cell biologically initiated numerous internal anti-oxidative responses to counteract the damaging effects of ROS on the cellular membrane **(Bandyopadhyay *et al.*, 1999)**. The present investigation shows higher CAT activity and lower MDA levels in fish-fed BG and BC, consistent with previous reports **(Cao *et al.*, 2019; Boonanuntanasarn *et al.*, 2016; Wongsasak *et al.*, 2015)**. **Kim *et al.* (2009)** reported higher CAT activities after being pre-treated with β glucan for 15 days in grass carp. The increased CAT activities and lower MDA levels might be attributed to the distinct activation of up-regulating antioxidant-related enzyme gene expression via the antioxidant properties of β-glucan **(Kim *et al.* (2009)**. Furthermore, fish administered BC had higher CAT, which the beneficial bacterial population may explain in boosting the overall immune response **(Adorian *et al.*, 2019).**

The transcription of certain growth, immunological, and stress-associated genes is frequently employed to understand the genetic foundation of the mechanism of action when researching the effects of functional feed additives on aquatic species **(Dawood *et al.*, 2018)**. High heat shock protein 70 *(HSP70*), which promotes protein integrity and lowers apoptosis, is secreted by fish cells in times of stress **(Jun *et al.*, 2015)**. According to the current findings, HSP70 was downregulated in seabream fish-fed diets supplemented with BC/*β*G combinations. This finding is related to the potential role that BC and *β*G may have in maintaining fish health **(Shi *et al.*, 2013)**. Furthermore, it is commonly recognized that growth hormone (GH) controls several important physiological processes in fish, including growth, metabolism, and mineral homeostasis **(Douros *et al.*, 2017)**. The findings of our study showed that the impacts of BC alone or in combination with BG resulted in increased GH expression levels in seabream. These findings were consistent with other previous reports **(Dawood *et al.*, 2020; Fath El-Bab *et al.*, 2022; Pilarski *et al.*, 2017)**. Probiotics significantly altered the expression of growth-related genes, demonstrating a desirable influence of these probiotics in overall fish metabolic activities **(Naiel *et al.*, 2022; Ibrahim, 2013).** Interleukin genes were estimated to maintain growth, differentiation, and activation during inflammatory and immunological responses **(McGeachy *et al.*, 2009)**.

Fish fed BC/*β*G had greater levels of interleukin-1 beta (*IL-Iβ*) according to the gene findings. The synergistic protective potential function of these mixtures in attracting and activating neutrophils in

inflammatory regions and promoting the immune system response function and overall health status of fish was confirmed by the activation of the interleukin genes in response to BC and *β*G -- supplemented diets **(Ai *et al*., 2007; Ali *et al*., 2015)**.

CONCLUSION

Finally, these findings revealed that dietary supplementation with *B. coagulans* alone or combined with *β*-glucan might improve the performance of *S. aurata* were exposed to suboptimal temperature levels. Furthermore, supplementation of feed additives (*B. coagulans* and *β*-glucan) might boost fish health by promoting immune responses, antioxidant capacity, and gene expression and altering some associated blood biochemical and hematological parameters.

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The authors declare no conflict of interest associated with the paper. The authors alone are responsible for the content and writing of this article.

AUTHORS CONTRIBUTION

Ahmed F. Fath El-Bab: General supervision, Conceptualization, Investigation, Methodology. Nayera M. S. El-dweny: Formal analysis, Investigation, Follow-up, Writing - original draft. Mohammed A. E. Naiel: Formal analysis, Supervision, Writing, Follow-up, Methodology, original draft.

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الملخص العربى

التأثير التآزري لبكتيريا الباسيلاس كوأجلانس و/أو البيتا جلوكان على أداء النمو والاستجابة المناعية لأسماك الدنيس المعرضة لدرجة حرارة دون المستوى الأمثل.

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كان الهدف من هذه التجربة هو تقييم تأثير مكملات *البكتريا العصوية* و/أو *البيتاجلوكان* الغذائية على النمو، والمؤشرات البيوكيميائية ومؤشرات الدم في الدم، ونشاط مضادات الأكسدة، والدلالات المناعية، والإنزيم الهضمي، والعوامل المناعية الخاصة بالتعبير الجيني لإصبعيات الدنيس تحت درجة حرارة دون المستوى الأمثل. تم توزيع إجمالي **٤٠٥** من إصبعيات الدنيس السليمة بمتوسط وزن **٩٥,** ٢٢ ± **٢, ١**جرام بالتساوي في ٢٧ (هابا)، بواقع **١٥** سمكة في كل هابا. تمت تغذية تسع مجموعات من الأسماك بعلائق متوازنة تحتوي على **٤٥ %** من البروتين الخام **٨,** **٤٨** ميجا جول/كجم من الطاقة الإجمالية، وتم استكمالها بمستويات مختبرة من *البكتريا العصوية* (**٠، ١ ، ٢**جرام/كجم من العليقة) أو *البيتاجلوكان* (**٠، ٥ ،١** جرام/كجم من العليقة)، وذلك لمدة٢ ١ أسبوعًا ثم تعرضوا لدرجة حرارة دون المستوى الأمثل لمدة أسبوعين. أظهرت النتائج أن المجموعة التي تغذت على نظام غذائي مكمل بـ ٢جرام من *البكتريا العصوية* و ١ جرام من *البيتاجلوكان* لكل كجم من العليقة أظهرت معدلات نمو نوعية ونسبية أعلى مقارنة بالمجموعة الكنترول. كما سجلت نفس المجموعات المعاملة أقل معدل وفيات. وفي الوقت نفسه، فإن المجموعات المكملة *بالبكتريا العصوية* وحده أو بالاشتراك مع *البيتاجلوكان* حسنت بشكل كبير جميع المؤشرات الكيميائية الحيوية والدموية المقدرة مقارنة بمجموعة الكنترول. وبالمثل، أظهرت المجموعات المكملة بـ*البكتريا العصوية* و/أو *البيتاجلوكان* تحسينات كبيرة في القياسات المناعية مثل الليزوزيم، ومستويات الجلوبيولين المناعي، ومؤشر البلعمة، ونشاط البلعمة. في نفس الاتجاه، أدت المعاملة إلى تحسين كبير في مؤشرات قدرة مضادات الأكسدة، وبروفايل الدهون (الدهون الثلاثية، والكوليسترول، والجلوكوز)، وتركيزات الكورتيزول، والتعبيرات الجينية. في الختام، تشير نتائج البحث إلى أن استخدام مكملات *البكتريا العصوية* و/أو *البيتاجلوكان* يعزز النمو ومقاومة إصبعيات الدنيس ضد التأثيرات السلبية لدرجات الحرارة دون المستوى الأمثل.