



The effects of probiotics supplementation on the growth performance, serum biochemical, and antioxidant activities in *Channa punctata* fingerlings

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Abstract

This research was done on *Channa punctata* to evaluate the effects of dietary *Bacillus subtilis* on some on the growth and some physiological activities. 120 healthy fish with 21.3 ± 2.5 g transferred into 12 aquaria and fed four diets: a control (0 g/kg) 3, 6, and 9 g/kg *B. subtilis* per kg feed for 90 days. The enhancements in growth parameters, with the highest final body weight was 104.4 ± 1.37 g and feed conversion ratio; 1.245 ± 0.0085 recorded in the 9 g/kg group. Lipid profiles also enhanced, with decreased triglycerides (101.2 ± 4.7 mg/dl, $p = 0.0009$) and improved high-density lipoprotein (HDL) (67.4 ± 2.33 mg/dl, $p = 0.0003$) in the 6 g/kg group. Digestive enzyme activity (lipase: 131.6 ± 3.57 U/L, $p < 0.0001$) and antioxidant responses (reduced catalase activity: 10.62 ± 0.2 U/mg, $p < 0.0001$) were similarly enhanced. This study concluded that *Bacillus subtilis* at 6–9 g/kg enhances growth, lipid metabolism, and oxidative stress resistance in *C. punctata*, regarded its use as a sustainable aquafeed additive.

Keywords: Antioxidant, *Bacillus subtilis*, *Channa punctata*, Growth performance, Lipid profile

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Introduction

The aquaculture industry encounters the increasing global demands due to the intensification sustainable protein essential for growth and health with the non-antibiotic involvements that ensure productivity and environmental integrity (Hamed *et al.*, 2024). The dietary supplements like probiotics are one of the natural approaches of incorporating into aquafeeds to improve growth and immunity in cultured fish (Brum *et al.*, 2025). The economically freshwater species *Channa punctata* (spotted snakehead) regarded as important freshwater fish species in Asia appreciated for its market demand and nutritional quality (Eissa *et al.*, 2024). The *Bacillus subtilis* in aquaculture holds significant promise to enhance growth performance in South and Southeast Asia by improving nutrient absorption and feed conversion efficiency (Dighiesh *et al.*, 2024).

However, *B. subtilis* as a potent probiotic in aquaculture regarded as, spore-forming bacterium gained important recognition due to its capability to tolerate severe gastrointestinal situations (Cheng *et al.*, 2023). The dietary *B. subtilis* also reported to progress in various teleosts with property of nutrient digestibility, control gut microbiota, and also stimulate non-specific immune responses, and rise fighting against pathogenic and ecological stressors (Albassam, Al-Doori and Shamkhi, 2021; Zaineldin *et al.*, 2021). Additionally, *B. subtilis* reported to improve in decreasing the serum cholesterol and triglyceride concentrations in maintaining the flesh

quality, fish health and also offer nutritional rewards for consumers (Youssuf *et al.*, 2025; Ahmed, 2023). Additionally, it also produces bioactive compounds with the exopolysaccharides, enzymes, and antioxidant molecules, which together respond oxidative damage encouraged by reactive oxygen species (Xue *et al.*, 2022). The antioxidative effects maintained by decreases in malondialdehyde which is demonstrated as lipid peroxidation in *Carassius auratus* and *Channa striata* (Liang *et al.*, 2024). Moreover, it also enhancing the activity of endogenous antioxidant enzymes to combat oxidative stress developing probiotic's potential (Wang *et al.*, 2024). Therefore, this study aims to evaluate dietary *B. subtilis* on the growth, biochemical, and antioxidant activities of *C. punctata*.

Materials and Methods

Fish Rearing

The research trial was done at the Saline Water Aquaculture Research Center (SWARC) in Muzaffargarh, Pakistan, under controlled laboratory conditions. 120 healthy *C. punctata* with a weight of 21.3 ± 2.5 g was used and acclimatized for 14 days in 1000-L fiberglass tanks and maintain the optimal water conditions through continuous aeration following standardized protocols (Swain *et al.*, 2022). After acclimatization, fingerlings were randomly circulated to 12 glass aquaria (120 L each) with a density ($80 \times 40 \times 38$ cm) with 10 fish per aquarium. Before the start of the experiment, the *C. punctata* were particular on the basis: (a) No visible skin damage, (b) Vigorous swimming behavior, (c) feeding response (Jiang *et al.*, 2025).

Experimental Diet Preparation and Feeding Protocol

Table 1 indicated the feed ingredients prepared for feeding *C. punctata*. The basal diet with *B. subtilis* at three levels (3, 6, and 9 g/kg), and a control (0 g/kg) thorough mixing using a twin-shell blender the diets were pelletized through a laboratory-scale feed extruder with 2 mm to yield uniform pellets for feeding by fingerlings. Post-pelletization, feeds were air-dried at 28±2°C for 48 hours to achieve <10% moisture content, then vacuum-sealed in oxygen-barrier bags and stored at -20°C to preserve probiotic viability. Probiotic stability was verified through serial dilution plating on nutrient

agar (HiMedia M001) at 37°C for 24 hours, with colony-forming units (CFU) counted using an automated colony counter (Scan® 500). Only batches maintaining ≥10⁸ CFU/g of viable *B. subtilis* throughout storage were used in the experiment (Gresse *et al.*, 2025). The 90-day feeding period with four treatments groups: Control (GP-1): Basal diet without supplementation GP-2: Basal diet + 3 g/kg *B. subtilis* GP-3: Basal diet + 6 g/kg *B. subtilis* GP-4: Basal diet + 9 g/kg *B. subtilis* Each treatment contained three replicates of 10 fish per aquarium (120 L). Fingerling feeds two time per day at 3% B.W. (Shafiq *et al.*, 2025).

Table 1: Ingredients of experimental feed for *C. punctata*.

Ingredients (g/kg)	Control (GP-0)	<i>B. subtilis</i> 3g/kg (GP-1)	<i>B. subtilis</i> 6g/kg (GP-2)	<i>B. subtilis</i> 9g/kg (GP-3)
Fish meal	19	19	19	19
Wheat bran	36	32.5	29.6	26.6
Wheat pollard	15	15	15	15
Maize germ	13	13	13	13
Cottonseed cake	11	11.5	11.5	11.5
Soybean meal	3	2.8	2.7	2.7
Soybean oil	1.8	2	2	2
Vitamin-mineral premix	1.2	1.2	1.2	1.2
* <i>B. subtilis</i>	0	3	6	9
<i>TOTAL</i>	100	100	100	100

**Bacillus subtilis* strain BS-ATCC 6633 was obtained from Aquaculture Probiotics Ltd. (Lahore, Pakistan) with guaranteed viability of 2×10⁹ CFU/g. Probiotic concentrations represent grams of active culture per kg of feed.

*Vitamin-mineral premix composition per kg diet: Vitamins: A (8,000 IU), D₃ (2,000 IU), E (150 mg), K₃ (10 mg), B₁ (15 mg), B₂ (20 mg), B₆ (25 mg), B₁₂ (0.05 mg), C (200 mg), niacin (100 mg), pantothenic acid (50 mg), folic acid (5 mg), biotin (1 mg) Minerals: Fe (100 mg), Zn (50 mg), Mn (20 mg), Cu (5 mg), I (1 mg), Se (0.5 mg),

Co (0.1 mg). The probiotic-supplemented diets maintained ≥10⁸ CFU/g viable *B. subtilis* throughout the 90-day feeding trial as confirmed by weekly plating on nutrient agar (37°C, 24h incubation).

Water Quality Monitoring and Maintenance

Water parameters were monitored following standardized aquaculture research protocols (Paul *et al.*, 2025). A multiparameter water quality probe (HI98194, Hanna Instruments) for pH and dissolved oxygen, and a portable spectrophotometer (DR3900, Hach).

Throughout the study period, water quality was maintained within the optimal ranges such as temperature $25.3 \pm 0.8^\circ\text{C}$, pH 7.5 ± 0.3 , dissolved oxygen 6.6 ± 0.3 mg/L (Kumar *et al.*, 2024).

Ethical Approval

The study accepted by the Animal Ethics Committee of the Institutional Committee, confirming agreement with ethical standards (Approval Code: APF-203; 2024).

Post-Trial Growth Evaluation

After the end of feeding period *C. punctata* undergo a 24-hour fasting period before growth parameters assessment. Growth performance parameters were evaluated. These parameters include initial weight, final weight (FW), Daily weight gain (DWG), Total weight gain (TWG), specific growth rate (SGR), relative growth rate (RGR), feed intake (FI), feed conversion ratio (FCR) was calculated according to (Owis *et al.*, 2024; Al Sulivany, Hassan and Mhammad, 2024; Omar and Al Sulivany, 2025; Zulfiqar *et al.*, 2025; Owais *et al.*, 2025; Yousefi *et al.*, 2025) by using the following established formulas.

$$\text{DWG (g/day)} = \frac{\text{Final weight} - \text{Initial weight}}{90}$$

$$\text{TWG (g)} = \text{FW} - \text{IW}$$

$$\text{SGR (\%)} = \frac{\ln(\text{FW}) - \ln(\text{IW})}{90} \times 100$$

$$\text{RGR, \%} = \frac{(\text{FW} - \text{IW})}{\text{Initial weight}} \times 100$$

$$\text{FI (g)} = \frac{\text{Total feed given}}{\text{Number of fish} - 90}$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{TWG (g)}}$$

Collection of the Blood

from each fingerlings the blood was collected by puncture the caudal vein with sterile syringe and then allowed the blood to clot at lab temperature. Centrifugation of the blood was done at 3000 rpm for 15 min to collect the serum, which was kept at -20°C until used to determine the lipid profile, digestive enzyme, and antioxidant activities.

Lipid Profile and Digestive Enzyme Parameters

Serum lipid profile test, Lipase (LIP), and amylase (AMYL) were measured by using of FUJIFILM (DRI_CHEM NX500-Czech Republic) according to the manufacturer's instructions of the slide reagent kit. The LDL and VLDL were calculated according to the formula as described by Friedewald, Levy and Fredrickson, 1972.

$$\text{LDL (mg/dl)} = \text{Cholestrol} - \text{HDL} - \frac{\text{TG}}{5}$$

$$\text{VLDL (mg/dl)} = \frac{\text{TG}}{5}$$

Antioxidant Enzyme Analyses

Catalase (CAT) was done following the method described by the Goth, 1991. Techniques by addition of 0.2 ml of serum to the 1 ml of (hydrogen peroxide (H₂O₂) 65 $\mu\text{mol/ ml}$ in the buffer sodium-potassium phosphate 60 mmol/L, with the pH 7.4) then incubating at 37°C for 1 min. Then, adding 1 ml of the ammonium molybdate reagent 32.4 mM, yellowish suspension, is a molybdate with H₂O₂ produced, the absorption was read by Jasco V-550 UV-vis spectrophotometer at 405 nm.

Superoxide Dismutase (SOD) was done depending on the capability of the enzyme to prevent pyrogallol autoxidation. The enzyme action is

measured as U/ml, which is described as the quantity of SOD enzyme to reduce 50% of pyrogallol. The protocol included calibrating the spectrophotometer to zero reads by using a Tris-EDTA. The absorptions were read at a 420 nm against Tris-EDTA buffer at zero time and after 1 min of the addition of pyrogallol (Campos-Shimada *et al.*, 2020).

Glutathione peroxidase (GPx) was done according to the Hafeman, Sunde and Hoekstra, 1974 Protocols. The procedure included by addition of 0.1 ml of serum to [5 mM of GSH/ 0.1 ml, 1.25 mM of H₂O₂/ 0.1 ml, 25 mM of NaN₃/0.1 ml, and 0.05 mM of phosphate buffer (pH 7)], 2.5 ml is the complete mixture volume.

Statistical Analysis

To conduct the statistical analysis, GraphPad Prism 9 from Finland. A one-way analysis of variance (ANOVA) followed by Duncan's Multiple comparisons (Tukey) was used to reveal significant changes between the treatments. A ($p < 0.05$) was used as the indicator for significance.

Results

The growth performance parameters of *C. punctata* fingerling were significantly enhanced by dietary supplementation with *B. subtilis* at varying concentrations (GP-1: 0 mg/kg, GP-2: 3 mg/kg, GP-3: 6 mg/kg, GP-4: 9 mg/kg) (Table 2 and figure 1; A, B, C, D, E, F, and G). The FBW exhibited a marked increase with higher probiotic levels, with values of 62.2 ± 0.66 g (GP-1), 80.6 ± 0.92 g (GP-2), 93.4 ± 0.92 g (GP-3), and 104.4 ± 1.37 g (GP-4) ($F = 318.8$, $p < 0.0001$). Similarly, DWG and TWG followed the same trend, reaching 0.91 ± 0.012 g/day and 82.6 ± 1.12 g in the GP-4 group ($p < 0.0001$ for both). On the other hand, the FIR improved significantly, with the highest value observed in GP-4 ($102.0 \pm 1.74\%$) compared to GP-1 ($62.0 \pm 0.86\%$) ($p < 0.001$). The FCR was most efficient in GP-4 (1.245 ± 0.0085) compared to GP-1 (1.54 ± 0.011) ($p < 0.0001$), while the SGR peaked in GP-4 ($1.758 \pm 0.02\%/day$) ($p < 0.0001$).

Table 2: Growth parameters in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals.

Growth Parameters	GP-1 (0mg/kg)	GP-2 (3mg/kg)	GP-3 (6 mg/kg)	GP-4 (9mg/kg)	P value
IBW (g)	22±0.7	21.4±0.5	21.4±0.5	21.4±0.5	0.8381
FBW (g)	62.2 ^a ±0.66	80.6 ^b ±0.92	93.4 ^c ±0.92	104 ^d ±1.37	<0.0001
DWG (g/day)	0.44±0.004	0.65±0.012	0.8±0.013	0.91±0.012	<0.0001
TWG (g)	40.2 ^a ±0.37	59.2 ^b ±1.07	72 ^c ±1.23	82.6 ^d ±1.12	<0.0001
FIR (%)	62 ^a ±0.86	85 ^b ±0.44	95.4 ^c ±1.8	102 ^d ±1.74	<0.001
FCR	1.54 ^a ±0.011	1.43 ^b ±0.0098	1.3 ^c ±0.007	1.245 ^d ±0.0085	<0.0001
SGR (%/day)	1.157 ^a ±0.02	1.474 ^b ±0.029	1.638 ^c ±0.032	1.758 ^d ±0.02	<0.0001

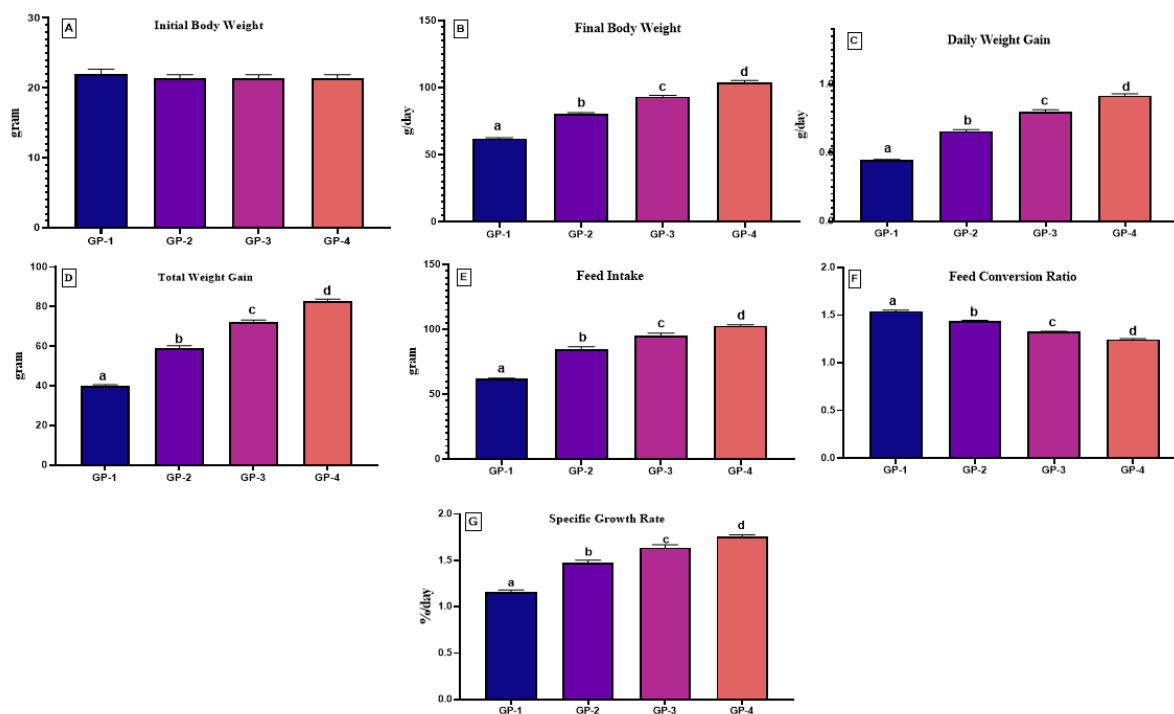


Figure 1: Growth performance parameters in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals. A: Initial body weight, B: Final body weight, C: Daily weight gain. D: Total weight gain. E: Feed intake. F: Feed conversion ratio. G: Specific growth rate.

The (Table 3 and figure 2; A, B, C, D, and E) characterize the lipid profile of *C. punctata* exhibited significant alterations with *B. subtilis* at different level (GP-0: 0, 3, 6, and 9 mg/kg). The level of TG decreased progressively with higher probiotic doses, reaching the lowest value in the GP-3 group (101.2 ± 4.7 mg/dl) compared to the control (123.0 ± 0.7 mg/dl) ($p = 0.0009$). Similarly, total cholesterol levels were significantly reduced in the GP-2 (133.0 ± 1.9 mg/dl) and GP-3 groups ($133.2 \pm$

2.3 mg/dl) relative to the control (143.4 ± 3.3 mg/dl) ($p = 0.018$). the HDL levels showed a notable increase in the GP-2 (65.4 ± 1.28 mg/dl) and GP-3 groups (67.4 ± 2.33 mg/dl) compared to the control (52.6 ± 2.6 mg/dl) ($p = 0.0003$). Conversely, the LDL and VLDL levels were significantly lower in the probiotic-supplemented groups, with the most pronounced reductions observed in the GP-3 group (LDL: 45.56 ± 2.2 mg/dl, VLDL: 20.24 ± 0.9 mg/dl) ($p < 0.0001$, for LDL; $p = 0.0009$, for VLDL).

Table 3: Lipid profile parameters in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals.

Lipid profile	GP-1 (0mg/kg)	GP-2 (3mg/kg)	GP-3 (6 mg/kg)	GP-4 (9mg/kg)	P value
TG (mg/dl)	$123^{a} \pm 0.7$	$119^{a} \pm 1.64$	$110.8^{ab} \pm 3.8$	$101.2^{b} \pm 4.7$	0.0009
Cholesterol (mg/dl)	$143.4^{a} \pm 3.3$	$143.6^{a} \pm 3.5$	$133^{b} \pm 1.9$	$133.2^{b} \pm 2.3$	0.018
HDL (mg/dl)	$52.6^{a} \pm 2.6$	$53.6^{a} \pm 2.71$	$65.4^{b} \pm 1.28$	$67.4^{b} \pm 2.33$	0.0003
LDL (mg/dl)	$66.2^{a} \pm 3.1$	$66.2^{a} \pm 2.45$	$45.44^{b} \pm 1.9$	$45.56^{b} \pm 2.2$	<0.0001
VLDL (mg/dl)	$24.6^{a} \pm 0.2$	$23.8^{a} \pm 0.32$	$22.16^{ab} \pm 0.7$	$20.24^{b} \pm 0.9$	0.0009

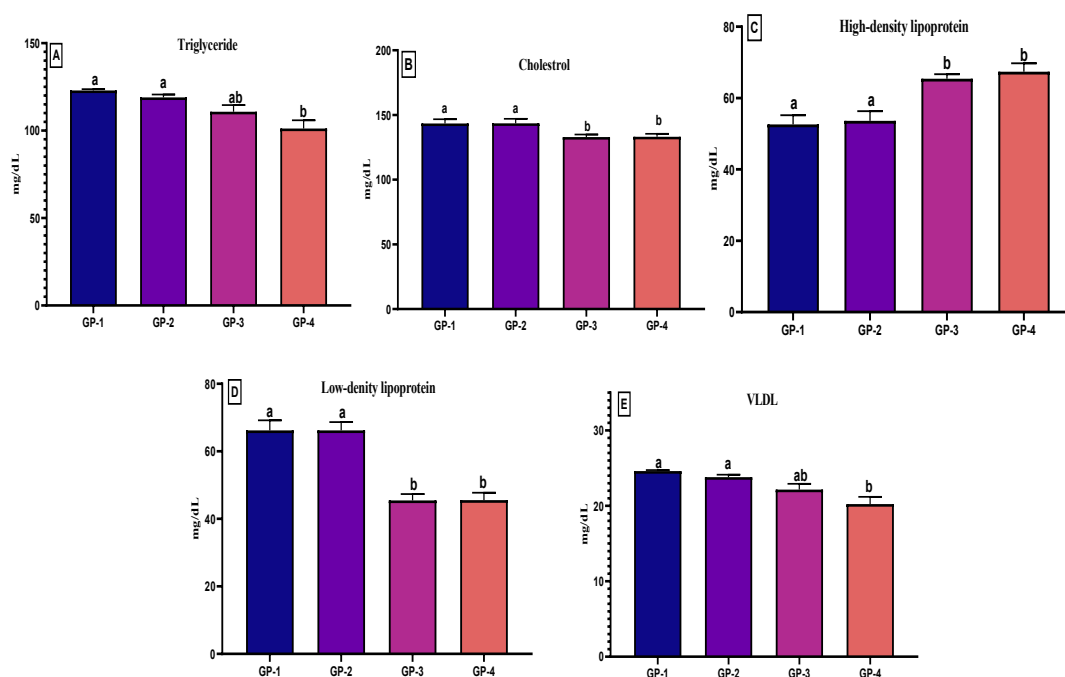


Figure 2: Lipid profile parameters in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals. A: Triglyceride, B: Cholesterol, C: High-density lipoprotein (HDL). D: Low-density lipoprotein (LDL). E: Very low-density lipoprotein (VLDL).

Adding *B. subtilis* to the diet significantly enhanced digestive enzyme activity in *C. punctata*. Lipase activity showed a progressive increase with higher probiotic concentrations, reaching 131.6 ± 3.57 U/L in the 9 mg/kg group (GP-4) compared to 88.6 ± 3.04 U/L in

the control (GP-1) ($p < 0.0001$). Similarly, amylase activity was significantly elevated in the 6 mg/kg (20 ± 0.44 U/L) and 9 mg/kg groups (22 ± 0.94 U/L) relative to the control (15.8 ± 0.8 U/L) ($p < 0.0001$) (Table 4 and figure 3; A and B).

Table 4: Digestive enzyme parameters in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals.

Enzyme Activity	GP-1 (0mg/kg)	GP-2 (3mg/kg)	GP-3 (6 mg/kg)	GP-4 (9mg/kg)	P value
Lipase (U/L)	$88.6^a \pm 3.04$	$96.8^b \pm 2.26$	$114.8^c \pm 5.74$	$131.6^d \pm 3.57$	<0.0001
Amylase (U/L)	$15.8^a \pm 0.8$	$16.6^a \pm 0.67$	$20^b \pm 0.44$	$22^{db} \pm 0.94$	<0.0001

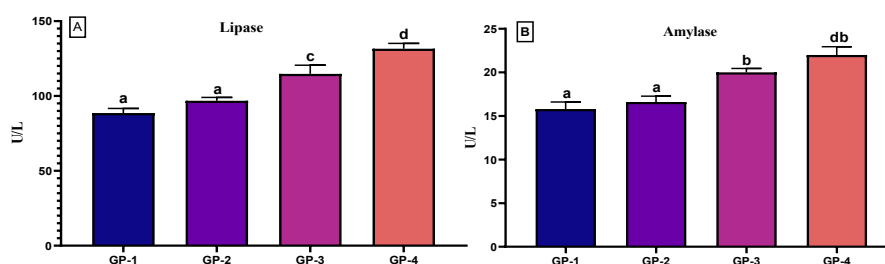


Figure 3: Digestive enzyme activities in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals. A: Lipase, B: Amylase.

Dietary supplementation with *B. subtilis* significantly influenced the antioxidant enzyme activity in *C. punctata* after 90 days of feeding (Table 5). Catalase activity exhibited a dose-dependent decrease, with the highest reduction observed in the 9 mg/kg group (10.62 ± 0.2 U/mg) compared to the control (14.26 ± 0.13 U/mg) (Figure 4; A). SOD levels also declined

significantly, with the lowest activity in the 9 mg/kg group (391 ± 6.14 U/mg) relative to the control (419.6 ± 3.37 U/mg) ($p = 0.0013$) (Figure 4; B). In contrast, GPX activity remained unaffected across all groups ($p = 0.7544$), with values ranging from 10.18 ± 0.38 U/mg (control) to 9.98 ± 0.67 U/mg (GP-3) (Figure 4; C).

Table 5: Antioxidant enzymes parameters in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals.

Antioxidant Enzymes	GP-1 (0mg/kg)	GP-2 (3mg/kg)	GP-3 (6 mg/kg)	GP-4(9mg/kg)	P value
CAT (U/mg)	$14.26^a \pm 0.13$	$13.34^b \pm 0.33$	$11.98^c \pm 0.16$	$10.62^d \pm 0.2$	<0.0001
SOD (U/mg)	$419.6^a \pm 3.37$	$412.8^a \pm 1.42$	$407.8^{ab} \pm 4.28$	$391^b \pm 6.14$	0.0013
GPX (U/mg)	10.18 ± 0.38	10.68 ± 0.3	10.42 ± 0.31	9.98 ± 0.67	0.7544

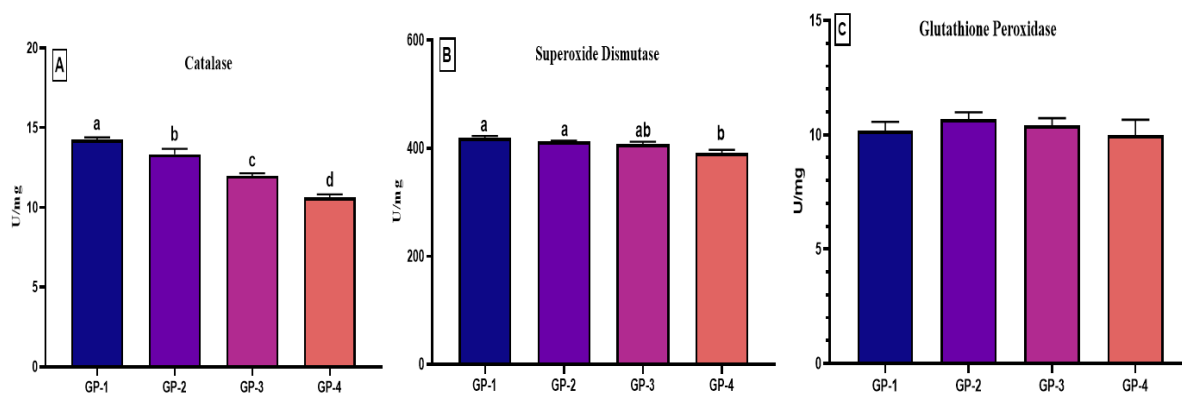


Figure 4: Antioxidant enzyme activities in *C. punctata* fingerling after feeding a diet supplemented with different concentrations of *B. subtilis* (0, 3, 6, and 9 mg/kg) for 90-day intervals. A: Catalase, B: Superoxide dismutase, C: Glutathione peroxidase.

Discussion

The research findings indicate that adding the probiotics (*B. subtilis*) to the diet of *C. punctata* led to an increase in their fish weight. Fish given the highest concentration of probiotics (9 mg/kg) grew faster, used their food more efficiently, and converted feed into body mass better than those given lower doses or no probiotics. Earlier findings on *Labeo rohita* and *tilapia* describes the similar benefits with *B. subtilis*. The *B. subtilis* produce enzymes that break food

particles, the enzymes proteases, cellulases, pectinases, subtilisin, and amylases regarded as absorbing nutrients easier (Al-Zahiri, Al-Dulaimy and Al-Bassam, 2024). Moreover, Cai *et al.*, (2022) described the probiotics regarded as sweet spot that benefits in growth performance. Eissa *et al.*, (2025) studied the spotted snakehead and evaluated the *B. subtilis* with the potential of probiotics that upregulation of lipolytic enzymes and fatty acid oxidation pathways and regarded as modulators of lipid

metabolism in aquaculture (Van Hai, 2015).

Dawood, Koshio and Esteban, (2018) conducted the study on Nile tilapia and described the significant decline in total cholesterol levels at high doses of probiotic. Begley, Hill and Gahan, (2006) also described that probiotic enhanced expression of apolipoprotein, enhanced the HDL levels which improved reverse cholesterol transport. Conversely, the decrease in LDL and VLDL levels revealed hepatic secretion of atherogenic lipoproteins, due to probiotic variation of bile acid absorption and intestinal cholesterol absorption (Chandrasekaran, Weiskirchen and Weiskirchen, 2024). These findings contrast with earlier reports in *Cyprinus carpio* (Hoseinifar *et al.*, 2018), indicating the lipid-lowering effects due to differences in probiotic strains. The hypolipidemic effects of *B. subtilis* also regarded as that involved in to yield short-chain fatty acids, hinder lipogenesis and promote β -oxidation (Danevčič *et al.*, 2021), its bile salt hydrolase activity, studied to decreases the cholesterol bioavailability (Uttlová *et al.*, 2016). The improvement in digestive enzyme activity with a diet with *B. subtilis* aligns with earlier studies indicating the probiotic's ability to control gut microbiota and progress nutrient absorption. *B. subtilis* conceals enzymes such as proteases, amylases, and lipases, directly improve host digestive effectiveness. Kumar *et al.*, (2006) conducted study on *Labeo rohita* and described that at higher probiotic concentrations (10^8 CFU/g) increase enzyme activity which also significantly upregulated digestive enzyme secretion. Liu *et al.*, (2025) also described that *B.*

subtilis involved in upregulation of intestinal brush border enzyme expression with the stabilization of gut mucosal integrity.

These findings contrast with previous studies on *Oreochromis niloticus*, wherever at the lesser probiotic concentrations (1–3 mg/kg) described the borderline properties, due to variations in host species or experimental period (Khormi *et al.*, 2026). The rise in lipase activity ascribed to *B. subtilis* which involved in the stimulation of bile salt secretion, which improves lipid emulsification (El-Naga *et al.*, 2025; Zou *et al.*, 2022), while the raise in amylase activities also recorded due to the probiotic with the variation of pancreatic enzyme secretion (Wang *et al.*, 2025; Jassim, 2022).

The variations in the antioxidant enzyme such as the decrease in CAT and SOD with the of *B. subtilis* lessen oxidative stress by reducing ROS production. These outcomes found similar with the findings by Jassim, Ati and Alhamd, (2020), who reported similar decreases in CAT and SOD activities in *Cyprinus carpio* with the probiotic diets, describing the improved digestability with reduced ROS generation. Furthermore, the lack of changes in glutathione peroxidase activity indicates that *B. subtilis* not effect this pathway directly, a result consistent with studies on *Cyprinus carpio* by De *et al.*, (2014), where GPX remained stable despite other antioxidant improvements. These findings differ from earlier work by Giri *et al.*, (2024), where probiotics improved SOD activity, due to variations in fish species, probiotic strains. The reduction in antioxidant

enzyme activities described that *B. subtilis* effectively lowers oxidative stress, dropping the reliance on enzymatic defenses.

Conclusion

The findings of this study demonstrate that the *B. subtilis* in the diet of *C. punctata* involved in marked improvements in growth, lipid metabolism, and antioxidant capacity, with higher concentrations showing more distinct benefits. It as a natural and sustainable feed additive that can enhance fish health and efficiency in aquaculture operations.

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