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RESEARCH PAPER

Enhancing Growth and Reproductive Performance of *Pangasianodon hypophthalmus* Brooders Through Multi-Strain Probiotic Supplementation in Induced Breeding

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Abstract

Due to the increasing global demand for protein, the commercial cultivation of striped catfish (*Pangasianodon hypophthalmus*) has significantly expanded, highlighting the need for sustainable growth and breeding strategies. In this context, the novelty of combining probiotics and hormones offers a promising approach to enhance both sustainability and productivity in aquaculture. This study aimed to evaluated the growth performance and induced breeding response of *P. hypophthalmus* brooders under the influence of multi-strain probiotics and hormonal treatments using pituitary gland (PG) extract and Ovaprim. Brooders were divided into four groups: T1 (basal diet 35% CP+*Lactobacillus rhamnosus*), T2 (basal diet 35% CP+*Bacillus subtilis*), T3 (basal diet 35% CP + *L. rhamnosus* + *B. subtilis*), and a control group fed only the basal diet of 35% CP. The culture was carried out in ponds with clayey, fertile soil, optimal pH (7.5–8.5), and proper drainage. Male and female brooders were cultured separately to avoid uncontrolled spawning. Growth performance was assessed via weight gain, feed utilization metrics, and reproductive parameters following hormonal induction.
Statistical analysis (ANOVA and Duncan's multiple range test) revealed that T3 showed the best overall performance. T3 brooders exhibited the highest mean net weight gain (136.31±10.34 g), specific growth rate (SGR: 0.333±0.01), protein efficiency ratio (PER: 6.88±0.04), and feed conversion efficiency (FCE: 206%), along with the lowest feed conversion ratio (FCR: 0.48±0.02). Reproductive outcomes were also superior in T3, with the highest fecundity (128.93), fertilization rate (73%), and hatching rate (62%) under Ovaprim and PG hormone application.
These findings highlight the effectiveness of multi-strain probiotics as a sustainable and viable alternative to conventional growth promoters in aquaculture. The integration of probiotics performance, offering a feasible strategy for large-scale catfish production.

Introduction

Aquaculture is one of the world's rapidly growing food industries, and it is essential to meet the growing need of animal protein. However, certain elements such as the spread of diseases, chemical pollution, environmental deterioration, and inefficient feed usage are significantly impeding the sector's ability to contribute to global food security (Little et al., 2016; Shah & Mraz, 2020).

Due to rising demand for protein, commercial production of striped catfish (*Pangasianodon hypophthalmus*) has expanded, driving the need for sustainable growth strategies (Islam et al., 2021). The white fillets of *P. Hypophthalmus* are purchased in over 130 countries globally and these fishes are stated as the third most critical group of freshwater fish (Zaidy & Yuniarti, 2021). This species has won interest due to its omnivorous consuming conduct, high stocking potential, robust market demand, and potential to evolve under environmental variations (Chowdhury et al., 2020; Jahan et al., 2019). It can also easily adapt to the artificial diet plan under controlled conditions. To grow profitability and satisfy the steadily increasing demand for fish, Pakistani fish producers have recently started raising this species in ponds (Bano et al., 2023).

However, the Juvenile stage of *P. Hypophthalmus* is vulnerable to high mortality. The fitness of the survivor fish is hard to sustain, and farmers regularly restock low-first-rate seeds, which causes the subculture to extend slowly. It has been pronounced that the primary nursery depicted an extremely low (40%–50%) survival rate than the second nursery segment (60–70%) (Haque et al., 2021; Hoa et al., 2021). Additionally, infected seeds from hatcheries or nurseries may also spread pathogens resulting in higher demise rate (Hasan et al., 2020). The delivery of fry that may be used to stock grow-out ponds in addition to farmers' earnings is significantly impacted by this lower survival (Hasan & Haque, 2020).

The undeveloped immune system, primarily renders the early embryonic phases making them susceptible to transmissible sicknesses and death (Chapute et al., 2020). Antibiotics are typically used as a preventative strategy to reduce *pangasius* fry mortality during the nursing period, however, their usage has been discouraged with the increase of antimicrobial resistance (Murk et al., 2016; Stephen et al., 2023). To grow healthy fish and enhance immune system, phytogenic health products (PHPs) are a good opportunity inclusive of probiotics, prebiotics, and different immune stimulants (Hernández-Contreras et al., 2023). Application of microbes like *Bacillus* species has been proven powerful to enhance fish development and survival by inhibiting Vibrio specie and other hazardous bacteria (Kuebutornye et al., 2020).

In aquaculture, the use of probiotics reduced farming operating costs and increased profit margin up to 20% approximately. Various gram-positive and gram-negative bacteria have been used as probiotics (Haque et al., 2021). Initially, probiotics were added to feed to boost growth and enhance health by boosting resistance to disease. Healthy fish intestines contain lactic acid bacteria, which are frequently employed in humans and other terrestrial animals. Likewise, *Bacillus* species are frequently employed to improve disease resistance, innate immune responses, and growth performance (Kuebutornye et al., 2019; Leistikow et al., 2022 Can et al., 2023). Although probiotics have been added to feed since the 1970s, less is known about their impact on pangasius fry and there is lack of knowledge on the usage with hormones.

Additionally, the cultured pituitary gland hormones have also been observed to be involved in ovulation and spawning induction of catfish (Oyeleye et al., 2016; Priyadarshi et al., 2021). Therefore, the current study aimed at assessing the growth performance of *P. hypophthalmus* broodstock. It strives to evaluate the effects of multi-strain probiotics and pituitary gland hormones on the growth and reproductive performance of *P. hypophthalmus* broodstock in a monoculture system. The research findings strive to enhance understanding of sustainable and advanced aquacultural practices, thereby improving the broodstock management as well as production efficacy of *P. hypophthalmus* farming.

Material and Methods

Preparation of Broodstock Ponds

The broodstock rearing ponds were selected based on their favourable conditions for brooders growth including clayey soil, around 7.5-8.5 pH, good drainage and productive pond soil (Ashashree and Kiran 2022). The ponds were dried and cleaned of wild aquatic weeds before brood stocking. Pond liming was performed that destroyed detrimental pathogens and also contributed as fertilizer and buffering agent. Moreover, predator attack was avoided following Hossain et al. (2016) and Ashashree and Kiran (2022). Physical properties such as pond area, water colour and depth as well as chemical properties including water temperature, dissolved oxygen (DO), pH, salinity and electrical conductivity were frequently monitored following the established procedure (Hossain et al. 2016; Ferosekhan et al. 2019). These parameters were kept uniform in all the ponds. (Table 1).

Collection and Maintenance of Brood Fish

Farm-raised, healthy and disease-free brooders of 2-2.5 year (virgin) and 3.4-3.5 kg average initial weight before feeding trail were selected for breeding. The sample was free of physical abnormalities. Male and female broods were kept in separate ponds avoid self-fertilisation size of a pond was 250m^2 with depth of 1.5m total volum of pond was 375m^3 with water exchange rate of 20%-30

Table 1. Water Quality parameters during breeding and hatching of *P. hypopthalamus*

Water Parameters	Min	Max
Temperature (ºC)	27	29
Dissolve oxygen (ppm)	5.8	6.0
pH	7.1	7.3
Hardness (ppm)	72	75
Alkalinity	71	76

per day for brooders. During the initial stage of brood care, they were kept stocking ponds. The broods were given food supplements including Multi Strain Probiotics Supplementation during trial from 04.01.2024, to 30.07. 2024 (four months before the breeding).

Feeding and Experimental Design

The brooders were divided randomly into four different groups, 1 control and 3 treatments (C, T1, T2 and T3), grown in separate ponds, each having three replicates. The control group was fed basic diet containing 35% crude protein (CP). Whereas, T1 was supplemented with 2 ×10^9 CFU/g *Lactobacillus rhamnosus, T*2 with 2 ×10^9 CFU/g, *Bacillus subtilis* and T3 contained both 2 ×10^9 CFU/g *Lactobacillus rhamnosus+Bacillus subtilis* (1:1) of the probiotic in the basic diet (Table 2). Each fish received approximately 2 × 10^{10} CFU/day of probiotics through feed, either from a single strain or combined (1 × 10^{10} CFU/g each of *L. rhamnosus* and *B. subtilis*). Probiotics were administered daily with feed for optimal gut health and immune support. In this study, commercial probiotic *Bacillus subtilis* (Creative Enzymes probiotics) and *Lactobacillus rhamnosus* (amBio co., Ltd) was used contained a concentration of 1×10^{10} billion colony forming unit (CFU). One gram of probiotic containing 1×10^{10} billion CFU was used to obtained, final concentrations of 2×10^9, 1×10^9 billion CFU of B. subtilis, and lactobacillus rhamnosus respectively. The volume of desired probiotics was calculated, using following formulae and then mix with sterile distilled water.

Volume of probiotic= Target CFU÷ Initial CFU× Initial volume

For confirmation of CFU in each concentration, plate count assay was performed using nutrient agar plate. Plates were incubated for 24 hours at 37°C and number of°olonies was counted afterwards by using a digital colony counter probiotics were stored at 4°C or lower in airtight, light-protected containers to preserve their viability throughout experiment.

Initially, the brooders were given diet 5 % of their body weights. The feed was effectively managed by providing once in the morning and evening. The experiment was started four months before the breeding season and the stocking density was 10 brooders, 5 male and 5 female per treatment along with control.

All broodfish were examined visually for clinical signs and tested using microbiological swabs to confirm disease-free status before hormone injection.

Growth Monitoring and Data Collection

The brooders were randomly chosen to assess growth performance every month. The parameters of growth such as feed conversion ratio (FCR), specific growth rate (SGR) and net weight gain (NWG) were observed by using the following formulas:

SGR = ln (Final wet body weight) - ln (Initial wet body weight) / no. of days x 100

Net weight gain = Average final weight – Average initial weight

Percent weight gain = Net weight gain / Final weight x 100

FCR = Feed intake / Net weight gain

Hormonal Administration, and Gamete Collection and Quality Assessment

The brooders were acclimatised in Fish Hatchery for 48 hours, 2 days before milt and egg collection. The brooders were weighed to determine the respective hormone dosage. Ovaprim hormone was used at standard injection rate of 0.6 ml kg^{-1} and 0.3 ml kg^{-1} for male and female fish respectively (Table 3). Whereas, PG hormone was used at standard injection rate of 0.009 mg/10 ml kg^{-1} BW in the first does and 0.048 mg/10 ml kg^{-1} BW in the second dose (Table 4) (Iqbal et al. 2021). Hormonal application was followed by brooders' reservation in different treatment tanks for 8 hours. The eggs and milt were collected from female and male fishes, respectively by gently massaging the abdomen. Milt was kept in cryovials placed in an ice box, while eggs were placed in plastic tubs.

Table 2. Nutritional composition of food for experimental groups of *P. hypophthalmus* brooders

Ingredients	Control	Treatment 1 *Lactobacillus rhamnosus* (2%)	Treatment 2 *Bacillus Subtilus* (2%)	Treatment 3 *Lactobacillus rhamnosus+Bacillus Subtilus* (2% Ratio 1:1)
Crude protein (%)	35	35	35	35
Lactobacillus rhamnosus (Probiotic, 2 × 10^7 C.F.U/g)	0.00	2× 10^9	0.00	1× 10^9
Bacillus Subtilus (Probiotic, 2 × 10^7 C.F.U/g)	0.00	0.00	2× 10^9	1× 10^9
Fish meal (g)	150	150	150	150
Rice polish (g)	350	350	350	350
Soy bean (g)	280	280	280	280
Corn gluten (g)	120	120	120	120
Vitamin mixture (g)	10	10	10	10
Premixes (g)	10	10	10	10
Molasses (g)	80	80	80	80
	1000g	1000g	1000g	1000g

Determination of Reproductive Performance

Stripping method was employed for eggs collection from female fish and fertilisation was allowed. Fertilisation rate was determined by estimating random number of eggs sampled in a graded 100 ml beaker. Sampled eggs were observed for post fertilisation (gastrula stage) and expressed as fertilisation percentage (Irawan et al. 2010). Fecundity and fertilisation rate were calculated by the following formula.

Fecundity Rate = Total Weight of eggs / No. of eggs g^{-1}(female body weight)

Relative Fecundity = Total No. of Eggs / Body weight of brooders ×100

Fertilisation Rate = Total No. of Fertilised Eggs /Total No. of fertilised and unfertilised eggs × 100

The hatching of fertilised eggs exposed the efficacy of probiotics and mineral diet for experiment. The hatching and survival rate of larvae were calculated and compared with the control. The following formulas were applied to determine hatching and survival rates (Irawan et al. 2010; Ezik et al. 2019):

Hatching Rate =Total no. of hatched larvae / total no. of sampled eggs ×100

Survival Rate% = No of survived fry in sample /total no of hatching in sample×100

Statistical Analysis

The research design was completely randomised. One way analysis of variance (ANOVA) was performed to analyse reproductive performance of brooders. Data normality was assessed using both Shapiro-Wilk and Kolmogorov-Smirnov tests. The data for growth rate and reproductive performance were normally distributed (P>0.05), validating the use of parametric tests. Mean and standard deviation (Mean±S.D.) were calculated for milt evaluation by using IBM SPSS Statistics 20 version (Charak et al. 2018). Moreover, Duncan's multiple range test was applied in post hoc analysis to check the significant differences in sperm count, spawning response, fecundity and fertilisation rate from each treatment.

Results

Growth Performance

Weight Gain and Growth Metrics

Observation recorded fortnightly for the weight gain showed that all the groups displayed improved growth over time. However, brooders of T3 that were exposed to 30% crude protein+*L. rhamnosus*+*B. subtilis* demonstrated highest growth than all the other treatments. T2 showed gradually increased growth but lower than T3, followed by T1 and control group. Thus, addition of multi strain probiotics significantly impacted the growth performance of *P. Hypopthalmus,* as illustrated in Figure 1.

The statistical outcome of mean net weight gain of *P. Hypopthalmus* brooders depicted that the control group demonstrated minimum mean net weight gain (91.54±6.73 g), followed by T1 (Basal Diet+*Lactobacillus rhamnosus*) and T2 (Basal Diet+*Bacillus subtilis*) which showed 116.11±7.25 g and 125.99±11.81 g of mean net weight gain, respectively (Table 5). The highest weight increase was observed in T3 that was treated with Basal Diet+*L. rhamnosus*+*B. subtilis,* that is, 136.31±10.34 g. This increasing trend in net weight gain reflects that the

Table 3. Doses of Ovaprim injected for induce breeding to female and male brooders of *P. hypopthalmus.*

Experimental Groups	Avarage body weight Male (g)	Avarage body weight Female (g)	1st dose (ovaprim) Hormone dose (0.3ml kg^{-1} BW)		2nd dose (ovaprim) Hormone dose (0.6ml kg^{-1} BW for female and 0.3ml kg^{-1})		p-Value
			male	female	male	female	
Control	2420.0±30.00	4360.6±11.0	-	1.210ml	0.65ml	2.42ml	<0.001
Treatment 1	2450.3±13.00	4675.6±5.13	-	1.220ml	0.65ml	2.44ml	<0.001
Treatment 2	2431.0±36.42	4686.0±5.29	-	1.220ml	0.65ml	2.44ml	<0.001
Treatment 3	2479.6±26.72	4808.6±8.08	-	1.228ml	0.65ml	2.45ml	<0.001

Table 4. Doses of PG injected for induce breeding to female and male brooders of *P. hypopthalmus*

Experimental Groups	Avarage body weight Male (g)	Avarage body weight Female (g)	1st dose (PG Hormone) Hormone dose (0.009mg/10 ml kg^{-1} BW)		2nd dose (PG Hormone) Hormone dose (0.048mg/10 ml kg^{-1} BW)		p-Value
			male	female	male	female	
Control	2455.3±35.0	4276.8±12.0	-	4.0 ml	1ml	2.42ml	<0.001
Treatment 1	2564.3±20.8	4682.3±10.5	-	4.6 ml	1ml	2.44ml	<0.001
Treatment 2	2586.3±13.0	4687.0±7.0	-	4.7ml	1ml	2.44ml	<0.001
Treatment 3	2604.0±20.5	5157.0±17.0	-	5.2 ml	1ml	2.45ml	<0.001

supplementation of multi strain probiotics significantly improved the growth performance of *P. Hypopthalmus* brooders.

Feed Utilisation

Though the survival rate of all the brooders exposed to different treatment groups was 100%, SGR, FCR, PER and FCE ratios varied across groups (Table 6). Specific growth Rate (SGR) was higher in T3 (0.333) followed by T2 (0.242), T1 (0.225) and control group (0.158). Similarly, Protein Efficacy Ratio (PER) was higher in T3 (6.88) followed by T2 (5.00), T1 (4.80) and control (3.22). Feed conversion efficacy (FCE) was also higher in T3 (206%) than all the other groups. T3 depicted lower Feed Conversion Ratio (FCR) (0.48) that was higher in control group (1.03). The growth performance and feed utilisation metrics showed that the group treated with a combination of probiotics continuously outperformed individual supplementation of each bacterium. Comparison of current study in Table 7.

Reproductive Performance

Induced Breeding Using Ovaprim

When grown on different diet treatments with Ovaprim hormone, brooders in T3 exhibited the highest reproductive performance compared to the others groups (Table 8). The total weight of eggs was highest in T3 (781.6g) and lowest in control (396.7g). Number of eggs kg^{-1} body weight of brooders was highest in T3 (162 g) and minimum in control (92.2 g). The highest fecundity rate was 125.25 in T3, followed by 95.55 in T1. Similarly, fertilisation rate was higher in T3 (73%) followed by T1 (69%), T2 (68%) and control (62%). Highest hatching rate was also shown by T3 brooders that was 62% and control group has lowest rate of 53%.

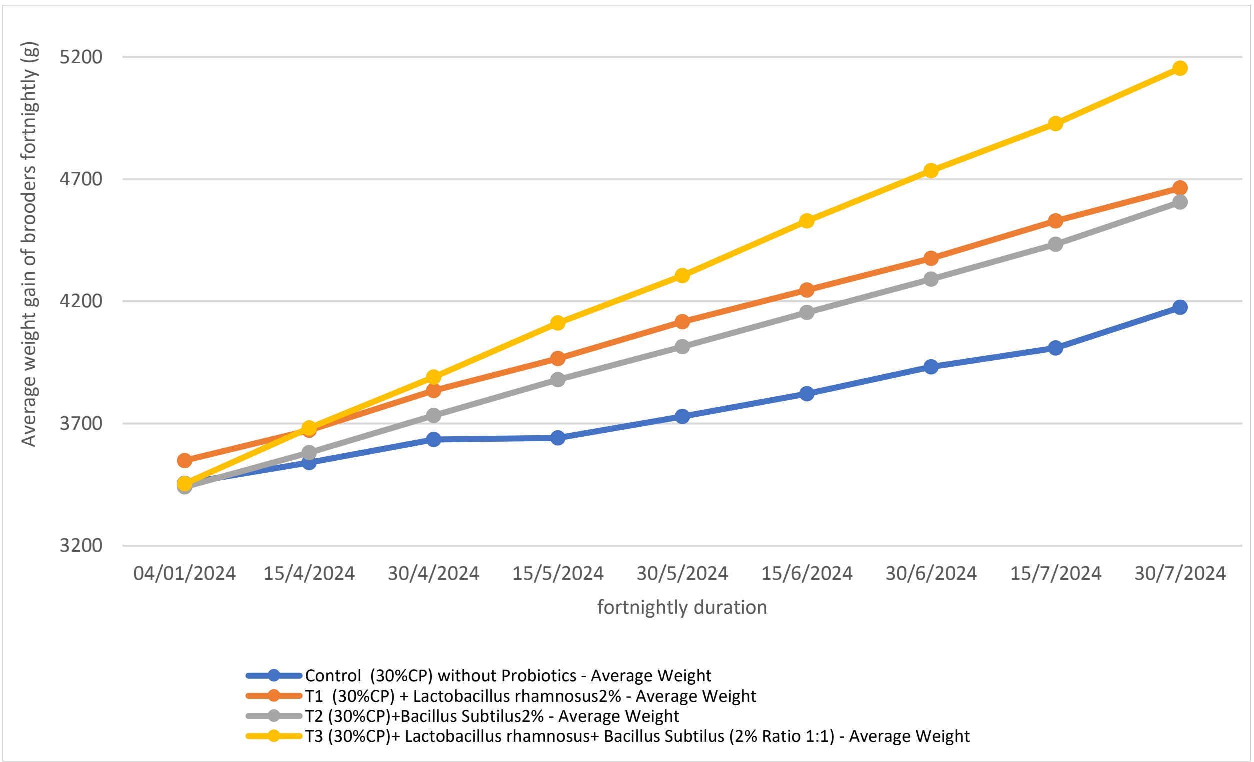

Figure 1. Fortnightly average weight gain (g) of *P. Hypopthalmus* broodstock in all treatments. Each treatment group (Control, T1, T2, and T3) is represented, showing significant differences in net weight gain. The data indicate that T3 brooders achieved the maximum growth performance, suggesting a synergistic effect of probiotics.

Table 5. ANOVA result of net weight gain.

Treatment	No.of replicates	Net weight gain (g) Mean±St.deviation
Control (Basal Diet)	8	91.54±6.73[a]
T1 (Basal Diet+*Lactobacillus rhamnosus*)	8	116.11±7.25[b]
T2 (Basal Diet+*Bacillus subtilis*)	8	125.99±11.81[c]
T3 (Basal Diet+*L. rhamnosus*+*B. subtilis*)	8	136.31±10.34[d]

*Different letters represent the significance level of each group. A statistically significant difference is observed, with the highest weight increase in T3, followed by T2, T1, and the control group

Therefore, enhanced reproductive features such as higher fecundity rate, fertilisation rate and hatching rate designate a mutual significant impact of multi strain probiotics, thereby advocating their application for *P. hypopthalamus*.

Induced Breeding Using PG Hormone

Similar to induced breeding outcome under Ovaprim application, brooders supplemented with multi strain probiotics depicted most reproductive features under the application of PG hormone. Total weight of eggs (g) by total BW of brooders was highest in T3 (864.9g) and lowest in control (456.3g). Likewise, fecundity rate (128.93), egg eyed survival rate (83%), fertilisation rate (73%), and egg hatching rate (62%) was highest in T3 and lowest in the control group (Table 9). Thus, multi strain probiotics positively influenced the gamete quality under the application of PG hormone.

Comparison of Reproductive Performance Under Pg and Ovaprim Application

Though the application of both the hormones improved the reproductive performance of all the groups of brooders, PG hormone outperformed Ovaprim.

Table 6. Growth and survival in *P. hypopthalamus* brooders under varying treatment groups during summer (April-August)

Parameters	Control	T_1	T_2	T_3	p-Value
No. of fish stocked (10)	24	24	24	24	-
Initial Weight (g)	3455±9.2	3548.6±18.0	3440.6±18.3	3453.67±6.02	-
Final Weight (g)	4175±7.5	4663.6±5.6	4606±8.5	5154±9.8	<0.001
Net weight gain (g)	720±2.6	1117±2.7	1166± 2.9	1701±3.8	<0.001
SGR	0.158±0.01	0.225±0.01	0.242± 0..02	0.333±0.01	<0.001
FCR (g)	1.03±0.02	0.69±0.01	0.66± 0.01	0.48±0.02	<0.001
PER	3.22±0.03	4.80±0.02	5.00± 0.03	6.88±0.04	<0.001
FCE (%)	96.9	144	150	206	<0.001
Survival rate (%)	100	100	100	100	-

Based on observed means. * The mean difference is significant at the .05 level. b. Dunnett t-tests treat one group as a control, and compare all other groups against it The table summarizes key growth performance metrics, including Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Feed Conversion Efficiency (FCE) across all treatment groups. Brooders in the T3 group exhibited superior feed utilization efficiency, lower FCR, and higher SGR, indicating enhanced nutrient absorption due to probiotic supplementation.

Table 7. Comparison of current findings with published probiotic studies in pangasius and related species

Study	Probiotic(s)	Growth Performance	FCR	Survival Rate	Notes
Current Study (2025)	*L. rhamnosus*, *B. subtilis* (1:1, 2%)	↑ SGR by ~18% in T3	↓ to 1.0	↑ to 94%	Strongest performance in combined probiotic group
Ahmed et al. (2021)	*L. rhamnosus* (2%)	↑ Weight gain 12–14%	↓ to 1.5	↑ to 90%	Mono-strain probiotic, good immune response
Linh et al. (2023)	*L. rhamnosus + B. subtilis* (1:1)	↑ SGR by 15%	↓ to 1.4	↑ to 92%	Synergistic effects noted, similar probiotic inclusion
Haque et al. (2020)	*B. subtilis* alone	↑ Growth ~10%	~1.6	~88%	Spore-former, stable but less effective alone
Farooq et al. (2022)	Commercial multistrain probiotics	↑ Growth 11%	~1.4–1.5	90–91%	Used 3 strains including *Bacillus* spp.

Table 8. Induce breeding performance of *P. hypopthalamus* female brooders grown on different diet treatments using Ovaprim hormone

No. Obs.	Parameters	Brooders treated with Ovaprim (Control)	Brooders treated with Ovaprim (T1)	Brooders treated with Ovaprim (T2)	Brooders treated with Ovaprim(T3)
1	Total Weight of eggs (g) by total BW of brooders	396.6±2.27	571.4±2.62	567.6±7.02	781.6±3.0
2	No. of eggs g / kg BW of brooders	92.2	124	123	162
3	Total No of Eggs	305153±41.58	439538±56.7	436384±65	601230±42.67
4	Fecundity rate	70.96	95.55	94.86	125.25
5	No of egg survived	216658±54.44	347235±51.9	342561±80	493008±92.66
6	Egg eyed survival Rate	71	79	78.5	82
7	Fertilized egg	189194±58.10	303281±36.3	303286±38.15	438897±79
8	Fertilisation Rate %age	62	69	68	73
9	No of eggs Hatched	161731±78.31	254932±58.2	257445±50.23	372762±80.87
10	Hatching rate %age	53	58	59	62

The table presents reproductive performance metrics. T3 brooders exhibited the highest fecundity and fertilization rates, highlighting the positive influence of multi-strain probiotics on reproductive success.

Brooders of T3 (35% CP+*L. rhamnosus*+*B. subtilis*) showed highest number of eggs produced, number of eggs survived, number of fertrilised eggs and number of eggs hatched than all the other treatments when exposed to Ovaprim. This performance was further improved when PG hormone was applied. The results of these reproductive measures are illustrated in Figure 2, 3, 4 and 5.

Discussion

Growth Performance

Probiotics are being applied worldwide in aquaculture to increase fish health and resistance to infections. Different compositions of probiotics are used to meet the needs of different species. For instance, probiotics such as *Lactobacillus* and *Bacillus* species are regularly used in tilapia farming to enhance the stability of intestine microbiota, boost immunity, and inhibit infections (Shao et al., 2020; Zhang et al., 2021). *Bacillus* and *Enterococcus* have been used in carp farming to increase digestive performance and decrease the number of harmful bacteria (Hossain et al., 2022). Tariq et al. (2023) confirmed that *Bacillus subtilis* is effective in improving characteristics and resistance to sickness in both carp and tilapia. Additionally, the combination of *Bacillus* and *Lactobacillus* spp was used in diverse species including salmonids and sea bass. These probiotics are used to improve the microbiome, enhance the conversion of feed, and immune system. However, the performance of probiotics might vary substantially among species. For example, *Bacillus* lines are successful in enhancing water and lowering natural waste, however their impact on disease resistance in fish can be different from in cold-water fish (Kılıç & Gültekin, 2024). Therefore, it is important to remember the individual needs of each species when chosing probiotic compositions. This is because equal strains may not yield same outcomes due to differences in their microbial habitats and metabolic reactions.

Table 9. Induce breeding performance of *P. hypopthalamus* female brooders grown on different diet treatments using PG hormone

No. Obs.	Parameters	Brooders treated with PG Hormone(Cotrol)	Brooders treated with PG Hormone Treatment 1	Brooders treated with PG Hormone Treatment 2	Brooders treated with PG Hormone Treatment 3
1	Total Weight of eggs (g) by total Bw of brooders	456.3±15	675.8±5	699.5±2.6	864.9±6.8
2	No. of eggs/kg Bw of brooders	111	146.9	152.1	173
3	Total No of Eggs	351000±93.57	519846±56.50	538076±49.51	665307±85.60
4	Fecundity rate	84.33	111.31	116.59	128.93
5	No of egg survived	256230±47.14	405479±57.03	425080±67.26	552204±71.75
6	Egg eyed survival Rate	73	78	79	83
7	Fertilized egg	214110±56	348296±88.50	365891±78.47	485674±60.09
8	Fertilisation Rate %age	61	67	68	73
9	No of eggs Hatched	182520±81.01	296312±62	312085±40.84	412490±71.33
10	Hatching rate %age	52	57	58	62

Brooders in the T3 group produced the highest number of fertilized eggs and had the highest hatching rates. The data suggest that probiotic supplementation enhances gamete quality and reproductive efficiency under PG hormone application.

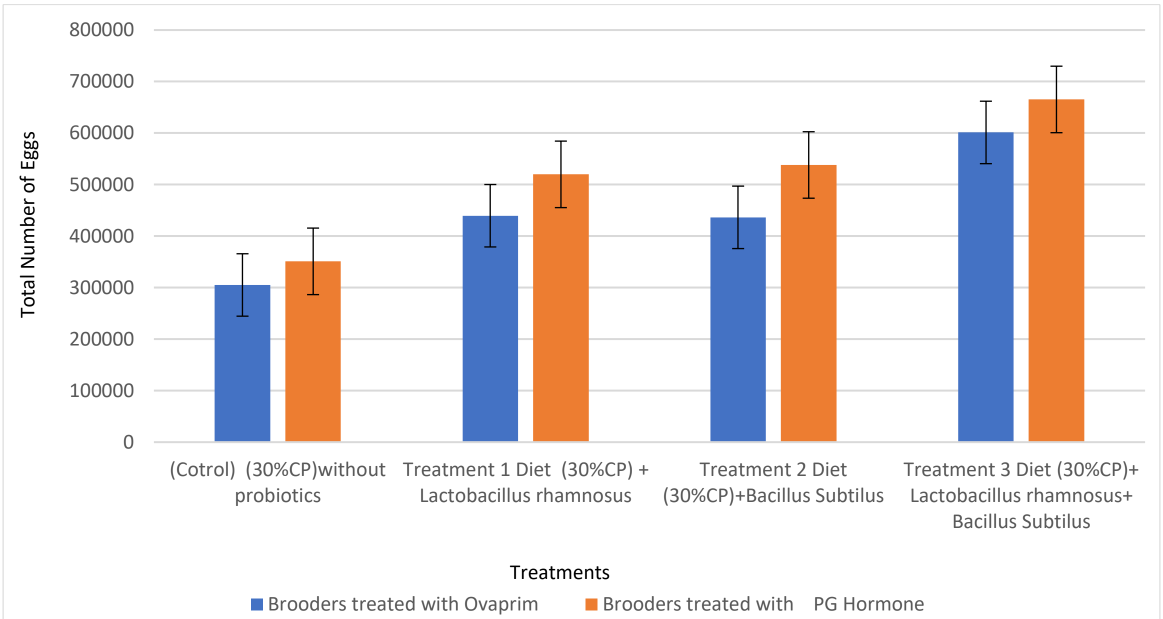

Figure 2. Total Number of Eggs produced under PG and Ovaprim application. The figure illustrates that T3 brooders consistently exhibited the highest egg production, emphasizing the role of probiotic supplementation in enhancing reproductive output.

The current research proved that striped catfish displayed an increase in their digestive enzymes, which is the cause of the substantial weight gain determined in fish given probiotic supplements. According to earlier studies on freshwater fish which includes Nile tilapia, grass carp, and African catfish (Xue et al., 2020), *B. Subtilis* can enhance nutrient digestion in gut providing more energy for fish growth. They improve the performance of digestion enzymes producing a huge form of exo-enzymes (Giebichenstein et al., 2022; Seo & Park, 2022). El-Saadony et al. (2021) have shown that nutritional supplements containing *Lactobacillus* sp. Increase dietary digestibility. The capacity of fish to take in nutrients from food is more advantageous due to the correlation among functioning of digestive enzymes and the gastrointestinal function of the fish (Amenyogbe, 2023).

The inclusion of beneficial microbes within the multi-strain probiotics may also have contributed to the group's weight benefit by improving absorption, digestion, and intake of nutrients throug the introduction of a most appropriate intestinal surroundings (Khatun et al., 2022). Compared to the control group, multi-stress probiotics (*Bacillus subtilis*

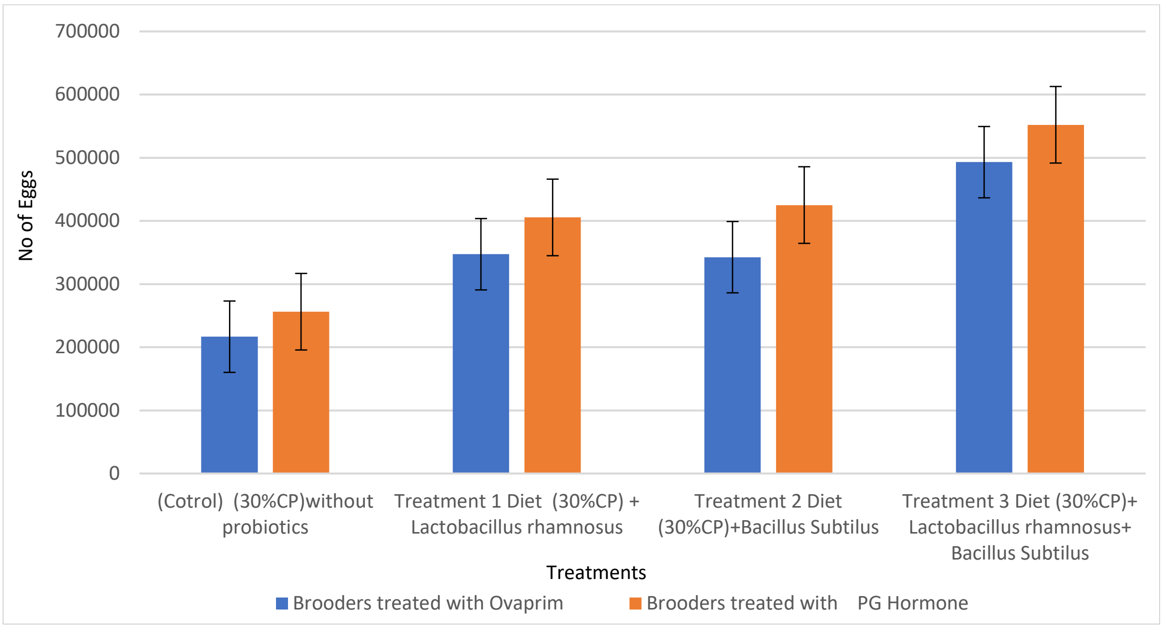

Figure 3. Total number of eggs survived under PG and Ovaprim application. The data suggests a significant improvement in egg survival in T3 compared to the control, highlighting the protective effects of multi-strain probiotics on embryo viability.

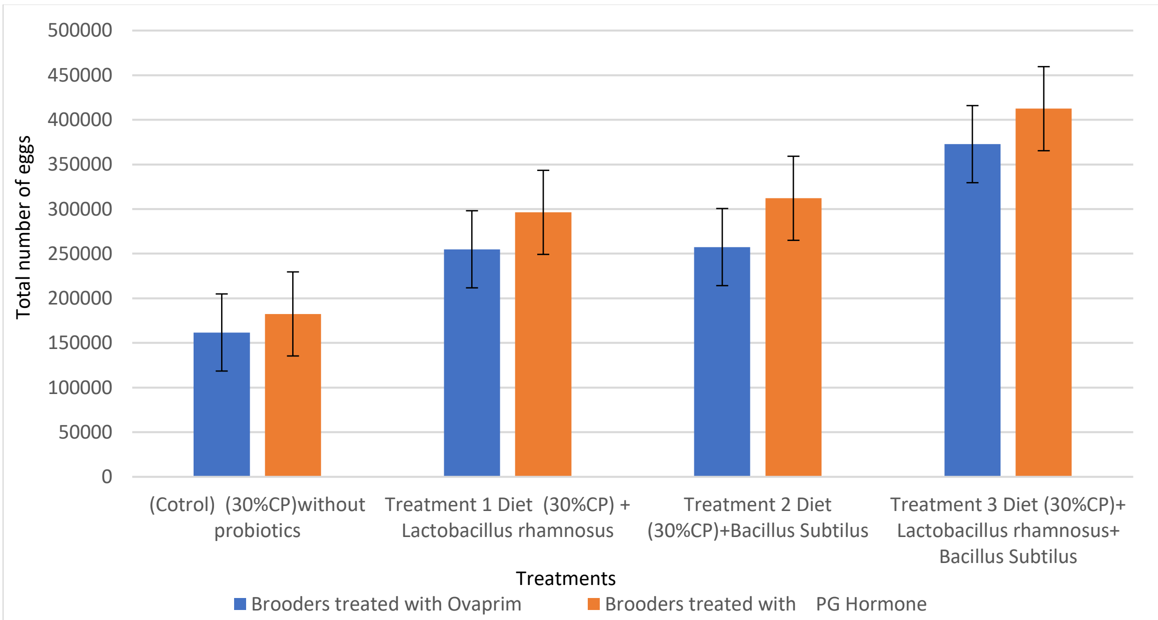

Figure 4. Total number of eggs fertilised under PG and Ovaprim application. The higher fertilization rates in T3 brooders indicate that probiotic supplementation significantly enhanced gamete quality and reproductive efficiency.

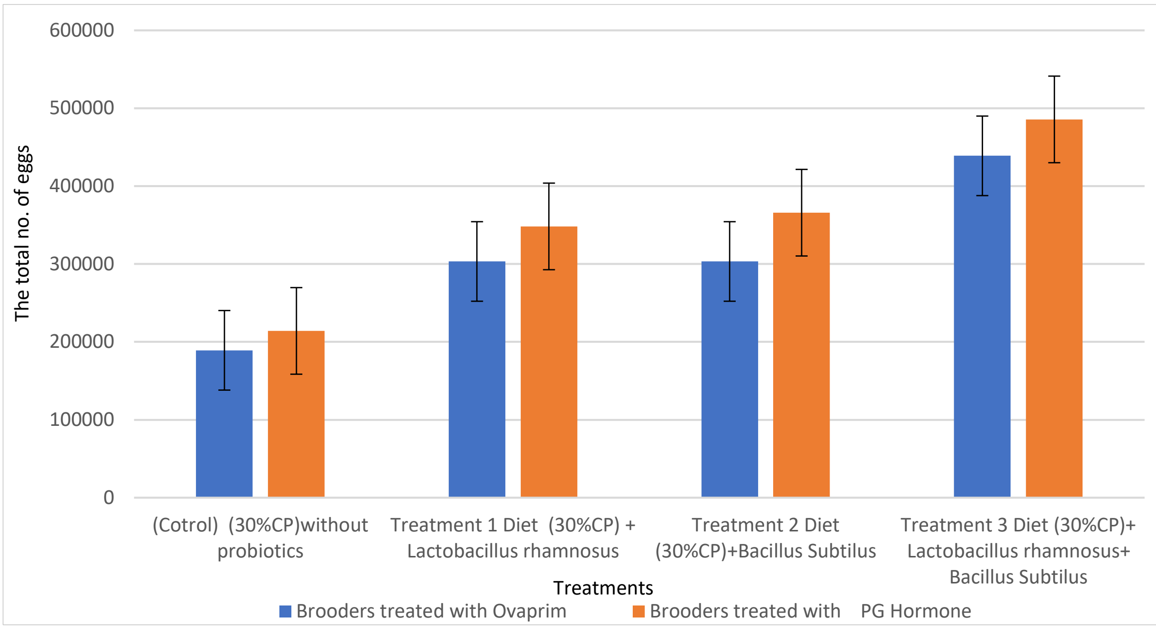

Figure 5. The total variety of eggs hatched underneath PG and Ovaprim utility. This figure depicts the hatching achievement rate underneath unique treatment conditions. Brooders within the T3 group exhibited the highest hatching charges, reinforcing the beneficial impact of probiotics on reproductive performance.

and *Lactobacillus acidophilus*) showed higher weight gain (136.31±10.34 g). Consistent with our findings, latest research indicated that including probiotics together with Basal Diet, *Lactobacillus spp.,* and *Bacillus spp.* notably improved body weight (Dumitru et al., 2021; Wang et al., 2024). Shekarabi et al. (2022) concluded that broiler farm animals' increase and health of the intestine have been improved by supplementing their diets with multi-enzymes (xylanase, amylase, cellulose, pectinase, and glucanase) and multi-stress probiotics (*Lactobacillus species* and *Bacillus species*).

Feed utilization results demonstrated a higher SGR (0.333), PER (6.88) and FCE (206%) in the group supplemented with multi-strain probiotics. These findings are congruent with preceding studies showing that supplementing with probiotic improves SGR, PER, BWG, FI, and FCR in broiler chicks while compared to antibiotic remedy (Mirsalami & Mirsalami, 2024). However, administration of suitable dosage must be considerd to get best results (Zommiti et al., 2020).

The multi-strain probiotic fed group also depictd a lower FCR (0.48) than others. This outcome aligns with the assumptions of Deng et al. (2022) and Chitura (2024) who investigated the effects of nutritional probiotics on feed conversion ratio. The studies connoted that animals given probiotics had greater improvements in consumption of feed, and feed conversion as compared to the ones within the antibiotic organization. Probiotics promote nutrient absorption in the intestinal tract. This, in turn, leads to better usage of vitamins, improved performance, and higher food cosumption (Mondal et al., 2024). Aquaculture experts, who need to maximise productiveness while preserving their operations economically, should take into account the cost-benefit ratio of adopting multi-strain probiotics in comparison to traditional aquaculture techniques. Multi-strain probiotics offer widespread benefits than traditional remedies. They can enhance fish health by balancing gut microbiota, improving immunity, and raising resistance to infections. All of these factors contribute to advanced growth costs and fewer epidemics of ailments (Zhang et al., 2021). According to research (Puvanasundram et al., 2021), multi-strain probiotics are efficient than single-strain probiotics because they provide a much wider variety of microbial sanctifications. Additionally, such compositions further enhance feed conversion ratios, lower the demand remedies with chemicals, and improve water by avoiding pathogens and the accumulation of organic waste (Ahmad et al., 2024). Though multi-strain probiotics are costly than traditional techniques at the start, research has tested that the long-term advantages result in a fair compensation of investment (Shao et al., 2020). Furthermore, worldwide demand for probiotic-based techniques is rapidly increasing in aquaculture replacing antibiotics due to antibiotics resistance. The use of multi-strain probiotics is not equally in step with regulatory patterns, however, it provides a sustainable and low-cost alternative to traditional methods. (Kılıç & Gültekin, 2024).

Probiotics, when administered effectively, offer an exciting alternative to traditional antibiotics, which are associated with the increase of antibiotic-resistant bacteria and damaging environmental implications (Kılıç & Gültekin, 2024). Multi-strain probiotics help make fish farming less complicated to maintain by improving fish health, increasing feed conversion, and minimising the need for chemical remedies, which lowers the ecological impact (Shao et al., 2020). On the other hand, it is also essential to consider the ecological responsibility of probiotics manufacturing. Producing probiotics requires fermentation through morganismsanism with significant water consumption and the supply of raw components (Mondal et al., 2022).

Recent studies suggest that combining *Lactobacillus rhamnosus* and *Bacillus subtilis* in aquaculture diets offers complementary benefits for fish health. *L. rhamnosus* enhances gut integrity and inhibits pathogens through antimicrobial compounds, while *B. subtilis* contributes digestive enzymes and withstands feed processing. Together, they boost nutrient absorption, immune responses (e.g., lysozyme activity, phagocytosis), and overall performance in *Pangasius hypophthalmus*, leading to better growth and disease resistance than when used alone (Ahmed *et al.,* 2021; Linh et al., 2023).

Reproductive Outcomes

Moreover, reproductive performance was also augmented under the combined application of probiotics. Under ovaprim influence, multi-strain probiotics fed group showed higher total weight of eggs (781.6g) Number of eggs kg^{-1} body weight of brooders (162 g), fecundity rate (125.25), fertilisation rate (73%) and hatching rate (62%).

Previous research (Ameer et al., 2021; Otoh et al., 2024) also observed that the hormone administered group of *C. Gariepinus* had significantly greater hatching, fertilization rate. Assan et al. (2022) also asserted that artificial hormones are effective. The researchers observed that injecting clariid catfish (H*eterobranchus bidorsalis*) with the artificial hormone ovaprim led to an extra quantity of fertilized eggs, as well as capability to hatch, and survival rates. Das and No (2022) also observed that the artificial hormone Ovaprim effectively stimulated mating in *Hemibagrusnemurus* species of river catfish.

Whereas, application of PG hormone surpassed the yield of Ovaprim and resulted in even higher total weight of eggs (864.9g), fecundity rate (128.93), egg eyed survival rate (83%), fertilisation rate (73%), and egg hatching rate (62%) in T3 group.

PG (Pituitary Gland extract) often outperforms Ovaprim in inducing spawning in some fish species due to higher receptor affinity and natural hormone complexity. PG contains natural gonadotropins (GtH-I and GtH-II), which closely mimic endogenous fish hormones and bind effectively to pituitary and gonadal receptors, triggering a more physiologically synchronized ovulation. In contrast, Ovaprim a synthetic mix of salmon GnRH analog and dopamine antagonist may have reduced stability and slightly delayed action in species less responsive to salmonid hormones (Mishra et al., 2017).

These results are supported by previous research which also comfired that brooders' potential to breed is advanced by supplementing with multi-strain probiotics, specially while handled with PG hormone treatment (Gurram et al., 2022). Probiotics aid in restoring intestine microbiota harmony, stress reduction, and immune response enhancement, all of which cause progressed fertility and eggs quality (Rani et al., 2023). Eissa et al. (2024) found that addition of B. subtilis+B. licheniformis (0.03 g/m3 water) enhanced reproductive performance in Red Tilapia by up-regulating reproductive genes and improving health profile.

Environmental Impact

Therefore, it is crucial to analyze environmentally friendly manufacturing techniques. These methods encompass the native materials for fermentation, optimising production methods to the power consumption of electricity, and minimising waste by reusing by-products. Additionally, the evolution of probiotics from clearly occurring strains or the use of recyclable streams from special sectors for probiotic media contributes to lowering the bad environmental outcomes related to their production (Ghosh et al., 2021).

The reseach outcome emphasizes the potential of using multi-strain probiotics in aquaculture positioning them as an effective substitute to traditional growth promoters. They can significantly enhance productivity while avoiding dependence on manufactured hormones, thereby complying with environmentally friendly and economical practices. The study presents a fasible and sustainable approach for commercial-scale production of catfish.

Conclusion

On the basis of the data collected every two weeks for the weight gain, it was found that all of the groups had shown greater development over the course of time. From all the treatments, T3 supplemented with 35% crude protein+*L. rhamnosus*+*B. subtilis* brooders depicted the highest growth and reproductive performance. The findings indicate that the T3 group performed better than the T2 and T1 groups, as well as the control group, in terms of the Specific Growth Rate (SGR), the Protein Efficacy Ratio (PER), the Feed conversion efficacy (FCE), fecundity rate, fertilization rate and hatching rate. Therefore, supplementing broodstock of *P. hypophthalmus* with dietary multi-strain probiotics can improve function of immune system, intestinal fitness, and absorption of nutrients, which in turn improves growth, usage of feed, and quality of gonad. Probiotics may also result in increased fecundity, greater quality of egg, and hatchability, as well as improved feed conversion prices and decreased waste. These benefits are related to immunological regulation, extended digestive interest of enzymes, and a balanced gut microbe. Future researches should concentrate on enhancing probiotic traces, dosages, and shipping systems whilst examining sustainability and long-time benefits in aquaculture. Probiotics are promising to elevate aquaculture output and broodstock health. The study does not cover other vital nutrients and their ratios, such as fatty acids and micronutrients, even if all diets include 35% protein. PG hormone surpassed ovaprim, however, the underlaying phenomena needs further exploration. Future research should examine the link between hormones and probiotics, since probiotics may improve hormone treatment in aquaculture, leading to better fish health and reproduction. This method would clarify the inequalities' sources and help create more effective aquaculture management approaches.

Ethical Statement

Not applicable.

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Author Contribution

IA and HR conceptualized and planned experiments. IA conducted the experiment, analyzed results, and drafted the original manuscript; HR supervised and aided experiment implementation; FA, AJ, and MN reviewed and edited the manuscript.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

Ahmad, U., Sultan, A., Shuaib, M., Atif, M., Alqhtani, A. H., Pokoo-Aikins, A., ... & Siddiqui, S. A. (2024). The effects of poultry by-product meal and multi-strain probiotics on production performance, immune response, gut health and nutrient utilisation of broilers. *Italian Journal of Animal Science*, *23*(1), 1250-1257. https://doi.org/10.1080/1828051X.2024.2391084

Ahmed, H. M. M., Fathy, S. A., & El-Gendy, M. M. A. (2021). *Effect of dietary probiotic combinations on growth, feed efficiency, and immunity of Nile tilapia (Oreochromis niloticus).* Aquaculture Reports, 20, 100739. https://doi.org/10.1016/j.aqrep.2021.100739

Ameer, M. W., Jabeen, F., Asad, M., Kaukab, G., Bashir, A., Rasheed, M., ... & Akram, M. (2021). Comparative efficacy of Ovaprim and hMG (menotropin) to induce breeding in African catfish (*Clarias gariepinus*). *Fish Physiology and Biochemistry*, *47*(5), 1559-1564. https://doi.org/10.1007/s10695-021-01003-x

Amenyogbe, E. (2023). Application of probiotics for sustainable and environment-friendly aquaculture management-A review. *Cogent Food & Agriculture*, *9*(1), 2226425. https://doi.org/10.1080/23311932.2023.2226425

Ashashree, H. M., & Kiran, B. R. (2022). A Review on post breeding Brood Stock management of Indian Major Carps, *Catla catla* and *Labeo rohita* at Bhadra Fish Farm, Karnataka. *Applied Aquatic and Terrestrial Eco-Biology*, 122.

Assan, D., Anane, K., Abarike, E. D., Alhassan, E. H., & Ampofo-Yeboah, A. (2022). Evaluation of induced breeding of catfish (*Clarias gariepinus*), using different doses of normal saline diluted ovaprim. *Journal of Applied Aquaculture*, *34*(2), 456-468. https://doi.org/10.1080/10454438.2020.1866142

Bano, S., Khan, N., Arslan, M., Fatima, M., Khalique, A., & Wan, A. H. (2023). Impact of various dietary protein levels on the growth performance, nutrient profile, and digestive enzymes activities to provide an effective diet for Striped Catfish (Pangasius hypophthalmus). *Turkish Journal of Fisheries and Aquatic Sciences*, *23*(7). https://doi.org/10.4194/TRJFAS22204

Can, E., Austin, B., Steinberg, C.E.W., Carboni, S., Sağlam, N., Thompson, K., Yiğit, M, Seyhaneyildiz Can, S., Ergün, S. (2023). Best practices for fish biosecurity, well-being and sustainable aquaculture. Sustainable Aquatic Research,2(3), 221-267.

Chaput, D. L., Bass, D., Alam, M. M., Al Hasan, N., Stentiford, G. D., van Aerle, R., ... & Tyler, C. R. (2020). The segment matters: Probable reassortment of tilapia lake virus (TiLV) complicates phylogenetic analysis and inference of geographical origin of new isolate from Bangladesh. *Viruses*, *12*(3), 258. https://doi.org/10.3390/v12030258

Charak, S. R., Agarwal, N. K., & Shah, T. K. (2018). An Approach for determination of semen quality of golden mahseer (Tor putitora) from central Himalaya. *The Pharma Innovation Journal*, *7*(1), 83-86. ISSN (P): 2349-8242

Chitura, T. (2024). Growth performance and meat attributes of poultry in response to dietary probiotic supplementation: A review. *Advances in Animal and Veterinary Sciences*, *12*(5),977-985. https://dx.doi.org/10.17582/journal.aavs/2024/12.5.977.985

Chowdhury, M. A., Roy, N. C., & Chowdhury, A. (2020). Growth, yield and economic returns of striped catfish (*Pangasianodon hypophthalmus*) at different stocking densities under floodplain cage culture system. *The Egyptian Journal of Aquatic Research*, *46*(1), 91-95. https://doi.org/10.1016/j.ejar.2019.11.010

Das, S. S., & No, A. (2022). *Effect of diluted synthetic hormone on spawning performance of Indian major carps* (Doctoral dissertation, Department of Aquaculture, OUAT, Berhampur).

Deng, Y., Verdegem, M. C., Eding, E., & Kokou, F. (2022). Effect of rearing systems and dietary probiotic supplementation on the growth and gut microbiota of Nile tilapia (*Oreochromis niloticus*) larvae. *Aquaculture*, *546*, 737297. https://doi.org/10.1016/j.aquaculture.2021.737297

Dumitru, M., Hăbeanu, M., Sorescu, I., & Tabuc, C. (2021). Effects of *Bacillus spp*. as a supplemental probiotic in diets for weaned piglets. *South African Journal of Animal Science*, *51*(5), 578-586. http://dx.doi.org/10.4314/sajas.v51i5.4

Eissa, E. S. H., El-Sayed, A. F. M., Hendam, B. M., Ghanem, S. F., Abd Elnabi, H. E., Abd El-Aziz, Y. M., ... & Dighiesh, H. S. (2024). The regulatory effects of water probiotic supplementation on the blood physiology, reproductive performance, and its related genes in Red Tilapia (*Oreochromis niloticus X O. mossambicus*). BMC Veterinary Research, 20(1), 351. https://doi.org/10.1186/s12917-024-04190-w

El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., ... & Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. *Fish & shellfish immunology*, *117*, 36-52. https://doi.org/10.1016/j.fsi.2021.07.007

Ezike, C. O., Echor, F. O., Agbo, A. N., & Uwadiegwu, N. C. (2019). Effects of Storage periods on viability, hatchability, survival and motility of cryopreserved C. gariepinus semen. *World Journal of Innovative Research*, *7*(3), 40-45. ISSN: 2454-8236

Ferosekhan, S., Sahoo, S. K., Giri, S. S., Das, B. K., Pillai, B. R., & Das, P. C. (2019). Broodstock development, captive breeding and seed production of bagrid catfish, Mahanadi rita, Rita chrysea (Day, 1877). *Aquaculture*, *503*, 339-346. https://doi.org/10.1016/j.aquaculture.2019.01.028

Ghosh, K., Mukherjee, A., Dutta, D., Banerjee, S., Breines, E. M., Hareide, E., & Ringø, E. (2021). Endosymbiotic pathogen-inhibitory gut bacteria in three Indian Major Carps under polyculture system: A step toward making a probiotics consortium. *Aquaculture and fisheries*, *6*(2), 192-204. https://doi.org/10.1016/j.aaf.2020.03.009

Giebichenstein, J., Giebichenstein, J., Hasler, M., Schulz, C., & Ueberschär, B. (2022). Comparing the performance of four commercial microdiets in an early weaning protocol for European seabass larvae (*Dicentrarchus labrax*). *Aquaculture Research*, *53*(2), 544-558. https://doi.org/10.1111/are.15598

Gurram, S., Chinni Preetam, V., Vijaya Lakshmi, K., Raju, M. V. L. N., Venkateswarlu, M., & Bora, S. (2022). Synergistic effect of probiotic, chicory root powder and coriander seed powder on growth performance, antioxidant activity and gut health of broiler chickens. *PloS one*, *17*(6), e0270231. https://doi.org/10.1371/journal.pone.0270231

Haque, M. M., Hasan, N. A., Eltholth, M. M., Saha, P., Mely, S. S., Rahman, T., & Murray, F. J. (2021). Assessing the impacts of in-feed probiotic on the growth performance and health condition of pangasius (*Pangasianodon hypophthalmus*) in a farm trial. *Aquaculture reports*, *20*, 100699. https://doi.org/10.1016/j.aqrep.2021.100699

Hasan, N. A., & Haque, M. M. (2020). Dataset of white spot disease affected shrimp farmers disaggregated by the variables of farm site, environment, disease history, operational practices, and saline zones. *Data in Brief*, *31*, 105936. https://doi.org/10.1016/j.dib.2020.105936

Hasan, N. A., Haque, M. M., Hinchliffe, S. J., & Guilder, J. (2020). A sequential assessment of WSD risk factors of shrimp farming in Bangladesh: Looking for a sustainable farming system. *Aquaculture*, *526*, 735348. https://doi.org/10.1016/j.aquaculture.2020.735348

Hernández-Contreras, Á., Teles, A., Salas-Leiva, J. S., Chaves-Pozo, E., & Tovar-Ramírez, D. (2023). Feed Additives in Aquaculture. In *Sustainable Use of Feed Additives in Livestock: Novel Ways for Animal Production* (pp. 811-846). Cham: Springer International Publishing. https://link.springer.com/chapter/10.1007/978-3-031-42855-5_28

Hoa, T. T. T., Boerlage, A. S., Duyen, T. T. M., Thy, D. T. M., Hang, N. T. T., Humphry, R. W., & Phuong, N. T. (2021). Nursing stages of striped catfish (*Pangasianodon hypophthalmus*) in Vietnam: Pathogens, diseases and husbandry practices. *Aquaculture*, *533*, 736114. https://doi.org/10.1016/j.aquaculture.2020.736114

Hossain, M. K., Hossain, M. M., Mim, Z. T., Khatun, H., Hossain, M. T., & Shahjahan, M. (2022). Multi-species probiotics improve growth, intestinal microbiota and morphology of Indian major carp mrigal Cirrhinus cirrhosus. *Saudi Journal of Biological Sciences*, *29*(9), 103399. https://doi.org/10.1016/j.sjbs.2022.103399

Hossain, M. T., Alam, M. S., Rahman, M. H., Al Asif, A., & Rahmatullah, S. M. (2016). Present status of Indian major carp broodstock management at the hatcheries in Jessore region of Bangladesh. *Asian-Australasian Journal of Bioscience and Biotechnology*, *1*(2), 362-370. https://doi.org/10.3329/aajbb.v1i2.61604

Iqbal, S., Atique, U., Abbas, F., Ahmad, S., & Haider, M. S. (2021). Impact of Temperature Variations on Breeding Behavior of Cirrhinus mrigala during Induced Spawning. *Pakistan Journal of Zoology*, vol. 54(4), pp 1877-1882, 2022 https://dx.doi.org/10.17582/journal.pjz/20200510140530

Irawan H, Vuthiphandchai V, & Nimrat S. (2010). The effect of extenders, cryoprotectants and cryopreservation methods on common carp (*Cyprinus carpio*) sperm. *Animal Reproduction Science*. *122* (3-4): 236-243. https://doi.org/10.1016/j.anireprosci.2010.08.017

Islam, M. M., Ferdous, Z., Mamun, M. M. U., Akhter, F., & Zahangir, M. M. (2021). Amelioration of growth, blood physiology and water quality by exogenous dietary supplementation of pepsin in striped catfish, *Pangasianodon hypophthalmus*. *Aquaculture*, *530*, 735840. https://doi.org/10.1016/j.aquaculture.2020.735840

Jahan, A., Nipa, T. T., Islam, S. M., Uddin, M. H., Islam, M. S., & Shahjahan, M. (2019). Striped catfish (*Pangasianodon hypophthalmus*) could be suitable for coastal aquaculture. *Journal of Applied Ichthyology*, *35*(4), 994-1003. https://doi.org/10.1111/jai.13918

Khatun, A., Chowdhury, S., Roy, B., Gani, S., Ray, B., & Ahmed, T. (2022). Effects of feeding multi-strain probiotics and multi-enzymes to broilers on growth performance, intestinal morphology and cost effectiveness of production. *Advances in Animal and Veterinary Sciences*, *10*(2), 389-396. http://dx.doi.org/10.17582/journal.aavs-/2022/10.2.389.396

Kılıç, N., & Gültekin, G. (2024). Sustainable Approaches in Aquaculture: Pharmacological and Natural Alternatives to Antibiotics. *Marine Science and Technology Bulletin*, *13*(3), 239-250. https://doi.org/10.33714/masteb.1488998

Kuebutornye, F. K., Abarike, E. D., & Lu, Y. (2019). A review on the application of Bacillus as probiotics in aquaculture. *Fish & shellfish immunology*, *87*, 820-828. https://doi.org/10.1016/j.fsi.2019.02.010

Kuebutornye, F. K., Abarike, E. D., Lu, Y., Hlordzi, V., Sakyi, M. E., Afriyie, G., ... & Xie, C. X. (2020). Mechanisms and the role of probiotic Bacillus in mitigating fish pathogens in aquaculture. *Fish physiology and biochemistry*, *46*, 819-841. https://link.springer.com/article/10.1007/s10695-019-00754-y

Leistikow, K. R., Beattie, R. E., & Hristova, K. R. (2022). Probiotics beyond the farm: Benefits, costs, and considerations of using antibiotic alternatives in livestock. *Frontiers in Antibiotics*, *1*, 1003912. https://doi.org/10.3389/frabi.2022.1003912

Linh, N. Q., Binh, D. V., & Hoa, N. T. (2023). *Synergistic effects of Lactobacillus rhamnosus and Bacillus subtilis in feed on growth performance and immune responses in striped catfish (Pangasianodon hypophthalmus).* Aquaculture and Fisheries, In Press. https://doi.org/10.1016/j.aaf.2023.03.012

Little, D. C., Newton, R. W., & Beveridge, M. C. M. (2016). Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. *Proceedings of the Nutrition Society*, *75*(3), 274-286. https://doi.org/10.1017/S0029665116000665

Mirsalami, S. M., & Mirsalami, M. (2024). Effects of duo-strain probiotics on growth, digestion, and gut health in broiler chickens. *Veterinary and Animal Science*, *24*, 100343. https://doi.org/10.1016/j.vas.2024.100343

Mishra, A. K., & Pal, A. K. (2017). Comparative efficacy of synthetic hormone and PG in inducing breeding in Indian major carps. Aquaculture Reports, 6, 24–29. https://doi.org/10.1016/j.aqrep.2017.01.002

Mondal, K. C., Samanta, S., Mondal, S., Mondal, S. P., Mondal, K., & Halder, S. K. (2024). Navigating the frontiers of mineral absorption in the human body: Exploring the impact of probiotic innovations: Impact of probiotics in mineral absorption by human body. *Indian Journal of Experimental Biology (IJEB)*, *62*(07), 475-483. https://doi.org/10.56042/ijeb.v62i07.12071

Mondal, S., Mondal, D., Mondal, T., & Malik, J. (2022). Application of probiotic bacteria for the management of fish health in aquaculture. In *Bacterial Fish Diseases* (pp. 351-378). Academic Press. https://doi.org/10.1016/B978-0-323-85624-9.00024-5

Murk, A. J., Rietjens, I. M., & Bush, S. R. (2018). Perceived versus real toxicological safety of pangasius catfish: a review modifying market perspectives. *Reviews in Aquaculture*, *10*(1), 123-134. https://doi.org/10.1111/raq.12151

Otoh, A., Ekanem, I., Okoko, A., Asangusung, P., & George, U. (2024). Comparative study of inducing broodstock with natural and artificial hormones on reproductive performances of Clarias gariepinus. *Asian Journal of Fisheries and Aquatic Research*, *26*(3), 31-38. https://doi.org/10.4314/tfb.v31i1.7

Oyeleye, O. O., Ola, S. I., & Omitogun, O. G. (2016). Ovulation induced in African catfish (Clarias gariepinus, Burchell 1822) by hormones produced in the primary culture of pituitary cells. *International Journal of Fisheries and Aquaculture*, *8*(7), 67-73. https://doi.org/10.5897/IJFA2015.0523

Priyadarshi, H., Das, R., Singh, A. A., Patel, A. B., & Pandey, P. K. (2021). Hormone manipulation to overcome a major barrier in male catfish spawning: The role of oxytocin augmentation in inducing voluntary captive spawning. *Aquaculture Research*, *52*(1), 51-64. https://doi.org/10.1111/are.14869

Puvanasundram, P., Chong, C. M., Sabri, S., Yusoff, M. S., & Karim, M. (2021). Multi-strain probiotics: Functions, effectiveness and formulations for aquaculture applications. *Aquaculture Reports*, *21*, 100905. https://doi.org/10.1016/j.aqrep.2021.100905

Rani, K., Kaur, G., & Ali, S. A. (2023). Probiotic-prebiotic therapeutic potential: a new horizon of microbial biotherapy to reduce female reproductive complications. *PharmaNutrition*, *24*, 100342. https://doi.org/10.1016/j.phanu.2023.100342

Seo, J., & Park, J. (2022). Does stocking density affect growth performance and hematological parameters of juvenile olive flounder Paralichthys olivaceus in a recirculating aquaculture system? *Animals*, *13*(1), 44. https://doi.org/10.3390/ani13010044

Shah, B. R., & Mraz, J. (2020). Advances in nanotechnology for sustainable aquaculture and fisheries. *Reviews in Aquaculture*, *12*(2), 925-942. https://doi.org/10.1111/raq.12356

Shao, X., Fang, K., Medina, D., Wan, J., Lee, J. L., & Hong, S. H. (2020). The probiotic, Leuconostoc mesenteroides, inhibits Listeria monocytogenes biofilm formation. *Journal of Food Safety*, *40*(2), e12750. https://doi.org/10.1111/jfs.12750

Shekarabi, S. P. H., Ghodrati, M., Dawood, M. A., Masouleh, A. S., & Roudbaraki, A. F. (2022). The multi-enzymes and probiotics mixture improves the growth performance, digestibility, intestinal health, and immune response of Siberian sturgeon. *Annals of Animal Science*, *22*(3), 1063-1072. https://doi.org/10.2478/aoas-2022-0006

Stephen, J., Mukherjee, S., Lekshmi, M., & Kumar, S. H. (2023). Diseases and Antimicrobial Use in Aquaculture. In *Handbook on Antimicrobial Resistance: Current Status, Trends in Detection and Mitigation Measures* (pp. 263-285). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-9279-7_15

Tariq, A., Salman, M., Mustafa, G., Tawab, A., Naheed, S., Naz, H., ... & Ali, H. (2023). Agonistic antibacterial potential of Loigolactobacillus coryniformis BCH-4 metabolites against selected human pathogenic bacteria: An in vitro and in silico approach. *PloS one*, *18*(8), e0289723. https://doi.org/10.1371/journal.pone.0289723

Wang, Z., Wang, X., Zhu, C., Xiong, Y., Yan, K., & He, S. (2024). Effects of Bacillus subtilis and Lactobacillus on growth performance, serum biochemistry, nutrient apparent digestibility, and cecum flora in heat-stressed broilers. *International Journal of Biometeorology*, 1-9. https://doi.org/10.1007/s00484-024-02780-9

Xue, J., Shen, K., Hu, Y., Hu, Y., Kumar, V., Yang, G., & Wen, C. (2020). Effects of dietary Bacillus cereus, B. subtilis, Paracoccus marcusii, and Lactobacillus plantarum supplementation on the growth, immune response, antioxidant capacity, and intestinal health of juvenile grass carp (Ctenopharyngodon idellus). *Aquaculture Reports*, *17*, 100387. https://doi.org/10.1016/j.aqrep.2020.100387

Zaidy, A. B., & Yuniarti, T. (2021). Effects of water exchange and feed quality on carcass composition, ratio, color and growth performance of striped catfish (Pangasianodon hypophthalmus), 14. http://www.bioflux.com.ro/aacl

Zhang, L., Han, H., Li, X., Chen, C., Xie, X., Su, G., ... & Lai, T. (2021). Probiotics use is associated with improved clinical outcomes among hospitalized patients with COVID-19. *Therapeutic advances in gastroenterology*, *14*, https://doi.org/10.1177/17562848211035670

Zommiti, M., Chikindas, M. L., & Ferchichi, M. (2020). Probiotics—live biotherapeutics: a story of success, limitations, and future prospects—not only for humans. *Probiotics and antimicrobial proteins*, *12*, 1266-1289. https://doi.org/10.1007/s12602-019-09570-5