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PROBIOTICS IMPROVING ZOOTECHNICAL PERFORMANCE AND HEALTH STATUS OF COMMON CARP (*CYPRINUS CARPIO*) DURING BIOFLOC BASED NURSERY PHASE

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SUMMARY

The current work was conducted to evaluate the impacts of Bioflocs (BFT) based system and probiotic additions in nursery phase of juvenile common carps (*Cyprinus carpio*). Effects of a probiotic PondToss™ containing a mixture of different *Bacillus subtilis*, *B. amyloliquefaciens*, *B. pumilus* and *B. Bacillus licheniformis*), 5.9×10^7 CFU/ gram was tested for growth measurements and health status. In a completely randomized design, four experimental treatments were replicated and subjected to be studied as T1: fish held in clear water nursery tanks (CWNS), received no probiotic PondToss™ (the control group). The T2, CWNS tanks supplemented with probiotic PondToss™ at 2.0 billion CFU/ gram at a dose of 1 g/ kg of diet. T3: fish held in BFT tanks without supplementing probiotics whereas the T4 BFT Tanks supplemented with probiotic PondToss™ at 2.0 billion CFU/ gram at a dose of 1 g/ kg. Fish were fed the experimental diet for 12 weeks and the results at the end of this work demonstrated that, the highest (worst) levels of nitrogenous compounds were recorded in T1 however, the corresponding improved values were in favor of T4 (BFNS+ Probiotic PondToss™). The fish group in T4 biofloc nursery system (BFNS) supplemented with probiotic PondToss™) showed a significant (P<0.05) increase in final live body weiht gain, ADG and specific growth rate (SGR, %/day) compared to other groups. survival rates were improved in BFNS (98.33 %) and BFNS-probiotic enriched group (99.58%) compared to the control 92.5%. the biochemical parameters were significantly improved in biofloc based nursery system (BFNS) and were in favor where probiotic was added. A continuous intake of mixture probiotic *Bacillus subtilis, B. amyloliquefaciens, B. pumilus and B. licheniformis*, 5.9×10^7 CFU/ gram at a dose of 1 g/ kg of feed in Biofloc based nursery of carps is recommended due to the improved performance, survival and helth status.

***Keywords:** Common carp, feed additives, probiotics, Bacillus sp., growth, biochemical parameters.*

INTRODUCTION

Aquaculture encompasses the cultivation of aquatic organisms such as fish, crustaceans, mollusks, and aquatic floras and plants. Continuously, this practice necessitates active and dynamic intervention in the rearing procedures to boost and maximizing production, which can include measures like modern culturing and stocking techniques, fertilization for enriching natural food, feeding, habitat modification, and predator control. The common carp (*Cyprinus carpio*) ranks as the fourth part most widely farmed species in the Globe, with substantial production concentrated in Europe and Asia.

Consequently, considerable research efforts have been dedicated to developing breeding programs aimed at enhancing the performance of common carp in aquaculture (Xu *et al.*, 2014). The emergence of bacterial strains resistant to antibiotics has led to an increasing interest in investigating other methods to supply distinct functional characteristics. Dietary supplements such as probiotics, prebiotics, synbiotics (applying mixing probiotics and prebiotics), and immunostimulants have been shown to manipulating performance and health status (Ebrahimi *et al.*, 20212 , Dawood *et al.*, 2019, Tahoun, 2022 and El-Naga *et al.*,2024). These micro-organisms are commonly found in the gastrointestinal tracts of host animals (Newaj-Fyzul *et al.*, 2014). Probiotics have recently developed recognition as valuable beneficial bacteria in cultivated organisms. Outstandingly, probiotics have seen widely and effective use in both human and veterinary medicine, with remarkable examples including *Lactobacillus sp*. and yeast, Saccharomyces (Fuller, 1989). Correspondingly, lactic acid bacteria (LAB) are extensively and commonly used in carp farming (Giri *et al.*, 2013). Although numerous studies have examined the effects of probiotics on fish and other aquatic organisms, there is limited research on the potential interactions and combined possessions of probiotics within biofloc based systems, commonly applied in fish farming.

Common carp (*Cyprinus carpio*), a highly economically important freshwater species, is extensively cultured worldwide. However, the intensification of aquaculture practices and the subsequent deterioration and expected declines in water quality have which imperatively led to more stress and frequent disease outbreaks in common carp farming in recent years. While various studies have demonstrated the biological benefits of probiotics on fish health, limited research has focused specifically on common carps reared under conditions of biofloc established nursery.

Nevertheless, the aquaculture sector plays a crucial role in meeting the global demand for animal protein, making it a vital source of food production, especially in light of the growing global population. Asia accounted for 70% of global aquatic animal production, followed by Europe, Latin America, and the Caribbean at 9%, Africa at 7%, North America at 3%, and Oceania at 1%. China continued to be the largest producer allover the world, contributing 36% of global production, after that India (8%), Indonesia (7%), Vietnam (5%), and finally Peru (3%) (FAO, 2024).

So, the need for more sustainable alternatives intensive systems like zero water exchange system known as biofloc systems Heterotrophic bacterial growth and organic carbon buildup are minimized by the quick removal of particulates from the environment and a certain amount of water exchange (Khanjani *et al.*, 2024). In order to maximize crop intensification while consuming the least amount of water, innovative strategies have recently been devised. An innovative technique that functions intensively with very narow to zero water exchange is called BFT. Moreover, recent years have seen a surge in the use of technology in aquaculture, highlighting the significance of resourceful systems that boost output and enhance crop hygienic conditions while lowering reliance on natural resources.

Hlordzi *et al*. (2020) stated that although the great capacity of *Bacillus sp.* bacteria in manipulating and bioremediation Further and more research on the mechanisms used by Bacillus species is crucial to understand and realized to get an optimization for these mechanisms. Finally, the authors emphasized that current studies ignored *Bacillus* species in water quality managing despite their potential proficiencies. The lights on the full potential of *Bacillus* species in the bioremediation of aquaculture water shed was recommended.

Therefore, the aim of the present study is to explore the effects of supplementing probiotic *Bacillus* on water quality, growth traits, and some biochemical measurements of juvenile common carp at nursery in a biofloc system.

MATERIALS AND METHODS

Carp juvenile husbandry:

The present experimental work was conducted at a private commercial fish farm, located in Al-Hamoul province, Kafrelsheikh Governorate. The investigation followed with the Guide for the Care and Use of Laboratory Animals published by Faculty of Agriculture, Kafrelsheikh University, Egypt. A stock of juvenile common carp (*Cyprinus carpio*) of visually uniform size was obtained from the privately earthen nursery ponds located in Balteem province, Kafrelsheikh. The fish were then stocked in cement tanks with a capacity of 6 m^3, equipped with continuous aeration, and were adapted for new tank environment for 10 days (acclimatization period), the carps were fed the experimental diet, and half of water volume in the 6 m^3 adaptation tank was daily exchanged with renewed water. Following adaptation period, fish with almost the same initial weights (5.06 to 5.08 g) were randomly and carefully chosen and disseminated across the twelve tanks and stocked at a density of 80 fish/ m^3, each with a capacity of 2 m^3. The 12 experimental tank system were arranged into 4 groups with three replicates per group.

Experimental fish and feeding protocol:

The nursing trial was conducted in varying 4 experimental treatment groups, in an experimental and totally randomized scheme, and every group were completed in 3 replicates per treatment. Feeding protocol was performed as the following: common carp were daily fed by hand at two meals frequencies

(9:00 and 14:00 hours), respectively. The 4 treatments were tested as follows: The fish in first group (T1), were held in clear water nursery system (CWNS) represent the control group received no supplemented probiotic. The T2: CWNS were supplemented with probiotic (PondToss™ at 2.0 billion CFU/g produced by Keeton Industries Co.) at a dose of 1 g/ kg of diet. The T3: fish were held in Biofloc nursery system (BFNS) tanks received no probiotic whereas the last fish group T4 represent BFNS tanks supplemented with PondToss™ at 2.0 billion CFU/g at a dose of 1 g/ kg of diet. The fish were fed the experimental diets for 12 weeks. Feeding was stopped fortnightly for twenty-four hours prior water sampling and in the same day fish were weighed to exhibit their growth performance. In Biofloc-managed nursery tanks (BFNS), no water exchange (zero water exchange) was made except for the compensation for evaporation occurred. Starch as carbon source was added to maintain the optimum C: N ratio (>15) to activate heterotrophic conditions. The carbon source was mixed in a plastic mug with tank water sample and spread over tank surface noontime ((Avnimelech, 1999 and Azim and Little, 2008).

Diet preparation and experimental treatments:

The experimental fish feed (basal diet) was conveyed by thoroughly collaborating all ingredients, including fish and soybean meals, corn grain, wheat bran, rice polishing, and premix of vitamins and minerals (Table 1).

Table (1): Experimental treatments design.

Item	Treatments			
	T1	T2	T3	T4
Nursery system	Clear water nursery system		Biofloc nursery system	
Probiotic PondToss™	(0 g/ kg diet)	(1 g/ kg diet)	(0 g/ kg diet)	(1 g/ kg diet)

The experimental diet was prepared by supplementing the powdered ingredients with oil and water for forming the dough, which was extruded into 1-2 mm pellets using a pelletizer at Al-Amanah Factory for Fish Feed processing located in Motobas, at northern of Kafr El-Sheikh Governorate, Egypt. The prepared feed was then air-dried at room temperature and stored refrigerated at 4 °C for the period of the experimental duration. The daily amounts were calculated based on a feeding level of 1.5 % of the total fish biomass. Daily feed amounts were distributed at (09:00 am, 2:00 pm, The feed amount was adjusted every two weeks after fish sampling. Fish were fed according to the study protocol, and the quantities of consumed feeds for each replicate was noted and accurately recorded daily to calculate total feed intake per fish. The fish in each tank were bulk-weighed fortnightly to monitor weight gain and adjust feeding rate and feed intake accordingly. This allowed for exact scheming of calculations required for determine feed and protein utilization i.e feed intake and the FCR (feed conversion ratio). The proximate composition for diet and carcass were analysed following the standard procedures outlined by the (AOAC, 2012). To prepare the experimental diets, the commercial pellet was crushed, mixed with water and the supplements added to obtain diet. Finally, diets were again made into pellets, allowed to dry, and stored at 4 C until use (Table 2).

Water quality analysis:

On a bi-weekly base, water samples were attained from each experimental tank by using a portable colorimeter produced by Hanna instrument company to determine total ammonia (HANNA, HI97715, and, nitrite HI97708 and nitrate (Hanna HI781) and pH (using a digital PH meter, (Milwaukee, MW102)). The TDS (total dissolved salts) and the TSS (total suspended solids) were measured by TDS and Lovibond, TB211 IR), respectively. The examination included values of the water DO (dissolved oxygen) (by using HANNA, HI9146-04 DO meter) and water temperature which were tested and recorded daily. Water Alkalinity was measured following methods of APHA, (1998). Floc volume was measured on a bi-weekly base by the Imhoff cone is used to measure suspended flocs in the BFT tanks. the floc volume on the bottom of the Imhoff cone was detected after 15-20 minutes of sedimentation (Avnimelech, 1999 and 2007).

Sample preparation:

After the whole experiment course which lasted for 12 weeks, all fishes were fasted for 24 hours prior to the final sampling. Fish from each tank were collected and placed in separate polypropylene containers, where they were anesthetized using clove oil (50 µl/L, Merck, Germany). Subsequently, the fish (n= 9) from each tank were individually weighed and counted to assess growth performance, feed efficiency, and survival rates. Additionally, at the conclusion of the feeding trial, a representative sample of fish (n= from each tank were randomly selected and stored at −18 °C for proximate whole-body composition assessments.

Fish growth performance and feed utilization:

Before starting the experiment, common carp juveniles were acclimatized to the new tank environments for one day. On the first day of the experiment, the initial body weight (IBW) of the fish was measured using an electronic precision balance. The daily feed intake (FI) of the fish was also recorded. On the end of the experimental period (84 days), the fish were seined using a nylon net, placed in a single separate plastic bucket container filled with prober volume of water, and subjected to a solution of clove oil (Merck, Germany) @ of 50 µl/L to be anesthetized. The fish from each experimental unit (tank) were individually weighed and counted to assess zootechnical measurements, feed utilization efficiency and survival percent. The following equations were used for these calculations:

Body weight gain = FBW - IBW.
Weight gain, WG = (FBW-IBW) ×100/IBW.
Specific growth rate (SGR% g/ day) = 100 ((Ln FBW-Ln IBW)/T).
Feed conversion ratio (FCR) = feed intake /weight gain,
Where the FBW denoting final body weight (g), the IBW indicating initial body weight (g), the T representing period in days and FI symbolizing feed intake in grams.
Survival rate (SR %) = total number of fish at the end of the experiment × 100 / total number of fish at the start of the experiment.
- Protein Efficiency Ratio (PER) = Weight Gain / Protein Intake;
- Protein Productive Value (PPV, %) =100 [Retained Protein / Protein Intake];
- Energy Utilization (EU, %) = 100 [Retained Energy / Energy Intake];

Table (2): Ingredients and composition of the basal diet.

Ingredients	%
Fishmeal (65%CP)	50
Soybean meal (44% CP)	350
Corn gluten meal	200
wheat middling's	300
Corn oil	26.45
Di-calcium phosphate	43.26
Calcium carbonate	18.83
Vit. and Min. Premix[1]	2
DL-methionine 99%	6.67
L-lysine 95%	2.29
Amecozym X[2]	0.5
Total	**1000**
Proximate composition	
DM %	92.50
CP %	35.00
EE %	9.00
CF %	5.20
Ash %	13.27
NFE %	37.53

[1]Each 1 kg contains: Vitamin. A 4.8 I.U.; Vitamin. D2 0.8 I. U; Vitamin E, 4.0 g; Vitamin. K, 0.8 gm; Vitamin B, 0.49, Vitamin. B2, 1.6 gm; Vitamin. B6, 0.6 gm.; VitaminB 12, 4 mg; Vitamin Nicotincacid 8 gm; Vitamin Pantothenic acid 49 gm.; Vitamin Folicacid, 400 mg; Biotin, 20 mg; Zinc 22 gm; Copper, 4.0 gm; Iodine,0.4 gm.; Iron, 12 mg; Manganese, 22 gm.; Choline chloride, 200 mg. and Selenium 0.04 gm.
[2] The commercial enzyme complex used AmecoZyme 2X® is commercial complex enzymes produced by AMECO-BIO&CO (339 W.LEMON AVE. ARCADIA, CA 91007 U.S.A) and composed of mainly spore-type fermentation enzymes that are recognized as heat-resistible and contain a mixture of different enzymes: 5.500,000 IU Amy lease, 500,000 IU Xylanase, 2,000,000 IU Protease, 15,000 IU Cellulose, 150,000 IU Lipase, 40,000 IU Phytase, 30,000 IU B-Glucanase and Alphagalactosidase

Biochemical analysis:

Representative fish samples (n=9) from each tank were collected and placed in separate polypropylene containers to assess some biochemical parameters. Commercial test kits (Bio-Diagnostic Company, Cairo, Egypt) were used to measure the total protein (TP), albumin (ALB), and globulin (GLO). The amounts of ALB and TP were quantified using Doumas *et al.* (1971) procedures of ALB were subtracted from TP to determine the amount of GLO. globulin and A/G ratio determined according to Henry (1964). Urea measured according to Fawcett and Scott (1960), and creatinine measured calorimetrically as described by Murray (1984).

Statistical analysis:

The experimental data were analyzed using one-way ANOVA in SPSS version 22 (SPSS® Inc., IL, USA). Differences between group means were assessed at a 5% significance level. The post-hoc comparisons among treatments conducted using Duncan's multiple range test (Duncan, 1955) to identify significant differences.

RESULTS AND DISCUSSION

Water quality:

The impact of the probiotic PondToss^{TM} on water quality assessments is existing in Table (3). The results revealed that there were no significant (P > 0.05) effects for different systems, clear water nursery system (CWNS) or biofloc system and probiotic levels (0 or 1 g/ kg diet) on water temperature, DO and pH levels. However, the highest (worst) levels of nitrogenous compounds were recorded in T1 (CWNS without probiotics) the corresponding improved values were in favor of biofloc nursery system supplemented with probiotic (T4). On the contrary, the monitored lowest values for total alkalinity, Floc volume and (TSS) were observed in T1.

Table (3): Effect of nursery system and varying levels of probiotic PondToss^{TM} on water quality criteria

Item	Treatments			
	T1	**T2**	**T3**	**T4**
Nursery system	**Clear water nursery system**		**Biofloc nursery system**	
Probiotic PondToss^{TM}	(0 g/ kg diet)	(1 g/ kg diet)	(0 g/ kg diet)	(1 g/ kg diet)
Temp (ºC)	28.379±0.28 a	28.39±0.3 a	28.73±0.28 a	28.54±0.28 a
DO (mg/L)	5.03±0.01 a	5.03±0.01 a	5.02±0.01 a	5.02±0.01 a
pH	9.93±1.02 a	7.93±1.07 a	7.96±1.02 a	7.99±1.02 a
NH_3-N (mg/L)	0.35±0.01 a	0.28±0.01 b	0.28±0.01 b	0.26±0.01 b
NO_2-N (mg/L)	0.59±0.02 a	0.48±0.02 b	0.42±0.02bc	0.39±0.02 c
NO_3-N (mg/L)	35.92±1.09a	31.34±1.15ab	30.50±1.09b	28.50±1.09 b
Total Alkalinity	193.26±14.23b	213.52±15.0 ab	229.10±14.23ab	258.90±14.23 a
FV	1.25±1.192b	1.61±1.26 b	10.90±1.19 a	11.65±1.19 a
TSS	120.96±11.41b	135.89±12.03b	226.50±11.41a	231.20±11.41 a

Means in the same row having varying letters are significantly different (P≤0.05).
Treatments; T1 clear water nursery system (CWNS) without probiotic (Control group), T2: CWNS+ PondToss^{TM} (1g/ kg diet). T3: Biofloc nursery system (BFNS) without probiotic. T4: BFNS+ PondToss^{TM} (1g/ kg diet).

The accumulation of inorganic nitrogen in rearing water is a key characteristic of intensive aquaculture systems, demanding routine checking and assessment of specified parameters of culturing water quality. In open-culture aquaculture systems, the incoming water serves as a primary source of organisms that can induce various fish diseases. In contrast, semi-closed or water reuse or recirculating water based-systems provide significant benefits over open systems i.e. ponds and pen or cage systems, as they allow for the treatment of inlet water to totally prevent or mitigate the entry of undesired pathogens, thereby reducing associated risks. aquafeeds typically have high protein content, ranging from twenty % to forty-five%. One important feature of rearing aquatic animals in intensive aquaculture systems is the continuous accumulation of inorganic nitrogen in water, which requiring regular monitoring and assessment of particular culturing water quality indices. In flow-through system or open-aquaculture systems, the renewing entering water may transmit major sources of pathogen organisms that can cause a variety of fish diseases (Ogello *et al.*, 2021). However, compared to open systems like ponds and pen or cage systems, the semi-closed, water reuse or recirculating water-based systems and even closed culturing systems offer important advantages because they enable the treatment of inlet water to completely avoid or alleviate the entry of unwanted pathogens related infections, thus depressing associated diseases or epidemic risks. During the metabolism and because of the protein catabolism processes and conversion to amino acids which considering the main substructure of relatively high dietary protein concentrations in aquafeeds which usually fluctuating, from 20 to 45%, Nearly,70 to 75% of the nitrogen in dietary protein is lost, wasted and so released into the water environment, either as wasted metabolites or by-products, or as uneaten feed particles that are later broken down by microbial community. The water's total ammonia nitrogen (TAN) is subsequently created from this wasted nitrogen. There is a known equilibrium between an ionized less toxic ammonium (NH^{+4}) fraction and the other fraction as un-ionized very toxic ammonia (NH_3). This equilibrium is known to be impacted by some main factors especially water temperature, water salinity, and water pH. Even at low concentrations, nitrogen in the form of ammonia can delay the growth of cultured organisms and is extremely toxic to the majority of aquatic life. Maintaining its concentration at low levels is therefore essential to the welfare, health, and general well-being of the cultured species (Saeedi *et al*., 2024).

From this standpoint, interest in sustainable innovative and non-traditional intensive systems in aquaculture like Biofloc based systems is now growing due to their great benefits, such as the very promising biofloc system, which is now spreading worldwide. Based on this, the current work was designed to try to benefit from its advantages in the nursery and rearing of common cultured fishes. The continuous aeration in experimental units (CWNS and BFNS) tanks was responsible for the adequate e amounts of dissolved oxygen found in this study, which were within the permissible ranges required for shrimp survival and growth. The concentrations of water DO, and estimated values of different water dissolved forms of nitrogen were within recommended limits for Pacific carps. Many studies evaluating water quality in BFT revealed reduced water ammonia and nitrite levels in zero water exchange-biofloc scheme which confirmed the present results which absolutely in favor of in BFNS compared to CWNS (Mabroke *et al.*, 2021; Ogello *et al.*, 2021; Tahoun, 2022; Hassan *et al.*, 2022a; Omran *et al.*, 2024 and El-Naga eat al, 2024). The much better water quality in all biofloc treatments compared to the CWNS group in this experiment confirms heterotrophic bacteria`s absorption of nitrogen, with the majority of the ammonia in the culture system being absorbed. Concerning the nitrogenous constituents in BFT groups, it should be noted that all were within acceptable concentrations (Avnimelech, 2007; Hassan *et al.*, 2022b) and were suitable for shrimp culture (Cardona *et al.*, 2016). The observed FV and TSS values in our study were also within the recommended range and consistent with the findings of Tahoun (2022) who investigated the effects of probiotics in different systems (CW and BFT). PondToss™ probiotic have shown promising effects in sustaining good water quality for shrimp pond farming. They can efficiently minimize nitrogen wastes, resulting in a cleaner aquatic habitat for shrimp. Probiotics help to decompose organic waste, maintain the ecosystem's balance in the pond, and regulate the nitrogen cycle. Several studies (Hai, 2015) have found that supplementing shrimp feed with *Lactobacillus* probiotics can eliminate harmful nitrogenous effluents which influence the culture water environment. Comparable results were also confirmed by of Hlordzi *et al*., 2020 who highlighted the capacity of microorganisms is linked to their genetic makeup. Therefore, to enhance the bioremediation efficiency of Bacillus species, a better understanding at the genetic level and the development of new genetic tools are highly recommended. Different strains of Bacillus species may have diverse optimal conditions to fulfill their role as water quality regulators; however, a set of optimal conditions is required to maximize the efficiency of Bacillus species in water quality management.

Growth and nutrient utilization efficiency:

The final body weight, weight gain, ADG, specific growth rate (SGR) and survival rates reared under different nursery protocols and probiotics levels are summarized in Table 6). Results indicating that carps were significantly affected by nursery system and varying levels of probiotics. The fish group in T4 (BFNS+ probiotic Pondtoss®) showed a significant increased final gain in body weight, ADG and specific growth rate (P<0.05) compared to other groups.

Table (4): Growth and survival of common carps (*Cyprinus carpio*) held in different nursery systems at varying levels of dietary probiotics.

Item	Treatments

	T1	T2	T3	T4
Nursery system	**Clear water nursery system**		**Biofloc nursery system**	
Probiotic PondToss™	(0 g/ kg diet)	(1 g/ kg diet)	(0 g/ kg diet)	(1 g/ kg diet)
IBW (g)	5.06 ±0.01a	5.08 ±0.01a	5.06 ±0.023a	5.07 ±0.02a
FBW (g)	109.67±2.03c	123.67±2.33b	119.52 ±1.8b	136.33±0.88a
WG (g)	104.60±2.03c	118.59±2.34b	114.47±1.78b	131.26±0.88a
ADG	1.49 ± 0.029c	1.69 ± 0.035b	1.63 ± 0.024b	1.88 ± 0.012a
SGR (% /day)	4.39 ± 0.026c	4.56 ± 0.029b	4.52 ± 0.017b	4.70 ± 0.018a
SR (%)	92.50± 1.44b	96.67± .83 a	98.33± .42 a	99.58± .41 a

Means in the same row with varying letters are significantly different (P≤0.05).

Treatments; T1 clear water nursery system (CWNS) without probiotic (Control group), T2: CWNS+ PondToss™ (1g/ kg diet). T3: Biofloc nursery system (BFNS) without probiotic. T4: BFNS+ PondToss™ (1 g/ kg diet).

Growth and nutrient utilization efficiency:

Numerous studies have highlighted the critical importance of nutritional and feeding strategies, as these factors can significantly influence fish performance (Ringø *et al.*, 2012). Thus, there is a persuasive need to develop and implement alternative feeding plans and strategies to improve fish health, prevent disease outbreaks, and address related challenges. The "natural probiotic" effects of biofloc technology present a promising alternative to conventional methods of disease control, which typically involve antibiotics, antifungals, probiotics, and prebiotics. The "natural probiotic" capabilities of BFT can operate both internally and externally, providing protection against pathogens such as Vibrio species and ectoparasites. This beneficial effect is largely facilitated by a diverse array of microorganisms, primarily bacteria, which serve as the first trophic level within the BFT (Emerenciano *et al.*, 2013). In the aquatic environment, biofloc microorganisms exert their protective effects against pathogens primarily through competition for space, substrates, and nutrients. Essential nutrients, mainly nitrogen, that requisite by both hetero-trophic bacterial community and bacteria like Vibrio species, thereby limiting the growth of the latter. Additionally, the proliferation of pathogens can be inhibited by various compounds excreted by microorganisms within the biofloc system, as well as by environmental factors such as the intensity of light and the type of carbohydrates utilized as a carbon source (Emerenciano *et al.*, 2013). Emerenciano *et al.* (2009) demonstrated that after a 60-day period, tilapia (initial weight 0.98 g) cultured in a biofloc system exhibited significantly fewer ectoparasites in their gills and mucous membranes compared to those reared in a conventional clear water system (CW). This suggests that the biofloc system not only enhances fish health but also contributes to the overall welfare of the cultured organisms by mitigating the presence of ectoparasitic infections. Biofloc based systems are considered brilliant and an emerging technology, which assurances intensive production with waste recycling and water saving. This system beliefs many advantages such as minimizing of water use, increased productivity per unit area, wastewater reuse, and elimination harmful aquaculture effluents and consequent pollution (Hlordzi *et al.*, 2020, Yu *et al.*, 2023 and Saeedi *et al.*, 2024).

Concerning feed and protein utilization efficiency (Table 5), the FCR was improved in probiotic supplemented group and the improved (P≤0.05) values was observed in fish T4 (BFNS+ probiotic pondtoss™@ 1 g/kg diet. The best protein efficiency ratio (PER) was also documented significant difference and. There are no noteworthy alterations amongst fish groups in terms of both and PPV% and EU% Table (5). Reported positive effects of probiotic supplements on feed intake found increased feed intake due to increased improves water quality and increases competition for resources, which improves nutrient digestibility and in turn more efficient utilization nutritional input and resources (Tahoun et al, 2011, Dawood et and Koshio 2016 and Dawood et al., 2019, Saeedi et al., 2024. The improved feed conversion due to probiotic supplementation in the present study is supported by the findings of (Tahoun *et al.*,2022 and El-Naga *et al.*, 2024). Moreover, the manipulation in biofloc probiotic supplemented BFNS tanks and due to the contribution of bacterial community genera *Bacillus sp*. addition w increased mineralization of organic particulate matter, augmenting primary productivity, and keeping and managing more stable water and rearing conditions throughout regulating both nitrification and denitrification dynamics (Saeedi *et al.*, 2024).

Table (5): Protein and feed utilization of water quality in tanks of carp (*Cyprinus carpio*) held in different nursery system at varying levels of probiotics. (mean ± S.E).

Item	Treatments			
	T1	T2	T3	T4
Nursery system	**Clear water nursery system**		**Biofloc nursery system**	

Probiotic PondToss™	(0 g/ kg diet)	(1 g/ kg diet)	(0 g/ kg diet)	(1 g/ kg diet)
WG (g)	104.60±2.03c	118.59 ± 2.34b	114.47±1.78b	131.26 ±0.88a
FI g/fish /84 days	214.67± 2.60b	226.0 ± 3.06b	226.0 ± 5.51b	248.33±2.03a
FCR	2.053± 0.03a	1.907 ±0.01b	1.97 ± 0.08ab	1.90 ± 0.018b
PER	1.55± 0.02b	1.66±0.01ab	1.61± 0.06ab	1.68 ± 0.02a
PPV (%)	33.90±1.07a	36.37 ±0.2a	35.31± 1.53a	36.77± 0.48a
EU (%)	21.93 ± 0.6a	22.81±0.16a	22.39± 0.93a	2.29±0.34a

Means within the same row have varying letters are significantly different (P≤0.05).
Treatments; T1 clear water nursery system (CWNS) without probiotic (Control group), T2: CWNS+ PondToss™ (g/ kg diet). T3: Biofloc nursery system (BFNS) without probiotic. T4: BFNS+ PondToss™ (g/ kg diet).

Whole-body proximate analysis:

Body chemical composition of common carp (*Cyprinus carpio*) reared in different nursery system (CWNS or BFNS) supplemented with different doses of probiotic pondtoss™ are presented in Table (6). Results showed no significant differences were recorded among different groups in terms of body DM, CP and ash percentages. Conversely, T1 recorded the highest lipid percentage, compared to other groups. No significant differences were recorded among different groups in terms of body DM, CP and ash percentages. Conversely, T1 recorded the highest lipid percentage, compared to other groups.

Biochemical analyses:

The data on biochemical parameters as affected by the experimental nursery system nursery and probiotics are shown in table 7. The MCV, Hemoglobin, RBC, Alb, Glob. And total protein values were improved and the highest recorded levels were in favor of T4 (BFNS plus probiotics) compared with other experimental treatments which exhibited lower values. The WBC exhibited contradictory trend and the lowest (improved values) were in favor of T4 (BFNS plus probiotics). The corresponding declined values were recorded for T1 clear water nursery system (CWNS).

Table (6): Body proximate composition of carp (Cyprinus carpio) stocked in different nursery system and fed varying levels of probiotics. (% on dry matter basis).

Item	At the end of experiment (% on DM basis)			
	T1	T2	T3	T4
Nursery system	**Clear water nursery system**		**Biofloc nursery system**	
Probiotic PondToss™	(0 g/ kg diet)	(1 g/ kg diet)	(0 g/ kg diet)	(1 g/ kg diet)
DM%	30.00 ± 0.58a	30.33 ± 0.33a	30.67±0.333a	29.33 ± 0.882a
CP%	69.67 ± 1.20a	69.83 ± 0.73a	69.97 ± 0.26a	70.33 ± 0.33a
EE%	18.73 ± 0.12a	17.00 ± 0.29b	17.69 ± 0.16b	17.69 ± 0.41b
Ash%	14.50 ±0.10a	14.30 ± 0.06a	14.33 ± 0.03a	14.37 ± 0.07a

Means within the same row having varying letters are significantly different (P≤0.05).
Treatments; T1 clear water nursery system (CWNS) without probiotic (Control group), T2: CWNS+ PondToss™ (g/ kg diet). *T3: Biofloc nursery system (BFNS) without probiotic. T4: BFNS+* PondToss™ (g/ kg diet).

Table (7): Biochemical analyses of common carp (*Cyprinus carpio*) as affected by nursery systems and varying levels of probiotics.

Item	Treatments			
	T1	T2	T3	T4
Nursery system	**Clear water nursery system**		**Biofloc nursery system**	
Probiotic PondTossTM	(0 g/ kg diet)	(1 g/ kg diet)	(0 g/ kg diet)	(1 g/ kg diet)
MCV	22.33±0.33c	24.00±0.58b	24.33±0.33b	26.00±0.58 a
Hemoglobin	7.17±0.17c	8.13±0.19b	8.17±0.17b	9.17±0.44 a
RBC	1.07±0.03a	1.03±0.04ab	0.99±0.01ab	0.96±0.01 b
WBC	6.43±0.03a	6.35±0.07a	5.87±0.03b	5.40±0.12 c
ALB	1.08±0.02c	1.10±0bc	1.13±0.01ab	1.16±0.01 a
GLOB	0.49±0.03c	0.60±0.06bc	0.82±0.02 b	1.11±0.13 a
Total Protein	1.57±0.03c	1.70±0.06c	1.95±0.03 b	2.27±0.12 a

Means in the same row with varying letters are significantly different (P≤0.05).
Treatments; T1 clear water nursery system (CWNS) without probiotic (Control group), T2: CWNS+ PondToss™ (g/ kg diet). *T3: Biofloc nursery system (BFNS) without probiotic. T4: BFNS+* PondToss™ (g/ kg diet).

Hematological and Biochemical and parameters:

Numerous reports highlight the valuable and impacts of *Bacillus* species in various species of cultured fish and shrimps. Some mechanisms were proposed for the actions resulting in benefits attained from probiotic supplementing practices include the following scenarios i.e. producing of inhibitory complexes, exclusion and competition with potential pathogens, suppression and suppression of virulence gene expression, augmentation of the immune response, development and enhancement of intestinal morphology and functions, and in turn facilitation and enhancement of digestive enzyme activity (Das *et al.*, 2013; Giri *et al.*, 2013). Consequently, the application of probiotics can lead to enhanced health status, improved disease resistance, better growth performance, optimized body composition, and balanced microbial communities (Khanjani *et al.*, 2024, Omran, *et al.*, 2024 and El-Naga et al, 2024). A well-known natural immunostimulants include probiotics, prebiotics, and symbiotic. However, the physiological responses including immune responses triggered by the oral administration of these immune-stimulants in targeted tissues, particularly the digestive tract, have not been extensively investigated until recent years. The present study indicated the potential effects of biofloc technology (BFT) conjugated with probiotic additives on fish biochemical and hematological status. The findings lined up with earlier investigations indicating that the application of probiotics enhances water quality, nutrient utilization, and the zootechnical performance (Chen *et al.*, 2022; Tahoun, 2022; Amiin *et al.*, 2023, Omran *et al.*, 2024, El-Naga, 2024). The incorporation of probiotics notably enhanced the growth performance metrics of carps, revealing improved weight gain and nutrient utilization in l groups (T2, T3, T4) compared to the control (T1) after an 84-day trial. Notably, enhanced growth performance and health status were observed in Nile tilapia (Tahoun, 2022). Dietary probiotics are believed to enhance fish growth rates by improving feed utilization, as evidenced by the improved feed conversion ratio (FCR) observed in this study. No significant differences were recorded among different experimental groups in terms of protein content while the highest carcass lipid content was found in the fish in CWNS and much lower values were recorded for fishes in other treatments (fish in CWNS fed a diet containing 1 g/kg probiotic; BFNS tanks and finally fish in BFNS fed a diet containing 1 g/kg probiotic PondToss™ contained *Bacillus sp*. Hematological parameters and plasma total protein concentration were also significantly higher ($p < 0.05$) in the fingerlings held in BFNS and BFNS those held in BFNS and fed diets containing 1 g/kg probiotic in relation to control. Many reports Therefore, symbiotics (applications for both probiotics and prebiotics in the same time prompting the immune-modulatory activity advancing the well-being health benefits in different aquatic organisms. Many studies highlighted the benefits and importance of probiotics and prebiotics (Dawood and Koshio, 2016, Tahoun, 2022, Amiin *et al.*, 2023, El-Naga, 2024). It is posited that these effects may depend on several factors, including the concentration and solubility of prebiotics in the diet, the fish species, water temperature, and the time of the feeding interval. It is further hypothesized that growth-enhancing effects arise from probiotics encouraging a confined intestinal immune response, which consequently reinforces resistance against pathogens that could interrupt and delay weight gain and possibly lead to disease outbreaks.

CONCLUSION

The results of this study indicate that the implementation biofloc nursery system (BFNS) and BFNS supplemented with dietary probiotic PondToss™ at 2.0 billion CFU/g of diet, significantly enhanced the growth rates and zootechnical parameters of common carp juveniles. Furthermore, the dietary inclusion of probiotics enhanced biochemical parameters and health of carps, consequently, the common carp exhibited improved survival.

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تحسين أداء النمو والحالة الصحية لأسماك المبروك العادي (*Cyprinus carpio*) باستخدام البروبيوتيك خلال مرحلة الحضانة في نظام البيوفلوك

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تم إجراء هذه الدراسة لتقييم تأثير تطبيق نظام البيوفلوك (BFT) وإضافة البروبيوتيك خلال مرحلة الحضانة لصغار أسماك المبروك العادي (Cyprinus carpio). تم اختبار تأثير بروبيوتيك تجاري PondToss™ يحتوي على خليط من عدة سلالات من بكتريا الباسيلس *Bacillus subtilis, B. amyloliquefaciens, B. pumilus* و *B. llicheniformis* على قياسات النمو والحالة الصحية للأسماك.
تمت التجربة باستخدام تصميم عشوائي كامل، حيث تم تقسيم الأسماك إلى أربع معاملات تجريبية بثلاث مكررات لكل معاملة كما يلي:
المعاملة الاولي:T1 تحضين صغارأسماك المبروك في أحواض بمياه رائقة (CWNS) بدون إضافة بروبيوتيك PondToss™ (المجموعة الضابطة).
المعاملة الثانية:T2 تحضين صغارأسماك المبروك في أحواض CW مع إضافة البروبيوتيك PondToss™ بجرعة 1 جم/كجم علف.
المعاملة الثالثة :T3 تحضين صغارأسماك المبروك في أحواض BFNS بدون إضافة بروبيوتيك PondToss™.
المعاملة الرابعة :T4 تحضين صغارأسماك المبروك في احواض BFNSمع إضافة بروبيوتيك PondToss™ بجرعة 1 جم/كجم علف
تم تغذية الأسماك على العلف التجريبي لمدة 12 أسبوعًا. سجلت المجموعة الضابطة T1أعلى قيم مستويات من المركبات النيتروجينية (أي الأسوأ)، بينما أظهرت المعاملة الرابعة (BFNS+ مع اضافة البروبيوتيك PondToss™)تحسينات معنوية واضحة في معايير جودة المياه.
أظهرت مجموعة الاسماك في المعاملة الرابعة T4 زيادة معنوية (P<0.05) في مقاييس الوزن النهائي، معدل الزيادة اليومية في الوزن (ADG)، ومعدل النمو النوعي (SGR) مقارنة بالمجموعات الأخرى. كما تحسنت معدلات البقاء بشكل كبير في معاملتي البيوفلوك BFNS، حيث بلغت 98.3%بينما ارتفعت الي 99.6 % في BFT المعاملة الرابعة (BFT +بروبيوتيك PondToss™) مقارنة بمجموعة التحكم التي سجلت .92.5% فقط بالإضافة إلى ذلك، تحسنت المعايير البيوكيميائية بشكل ملحوظ في نظام الحضانة المعتمد على البيوفلوك، وكانت النتائج أفضل في المجموعات التي أضيف إليها البروبيوتيك.
بناءً على هذه النتائج، يوصى بالاستخدام المستمر للبروبيوتيك المحتوي على مزيج *Bacillus subtilis, B. amyloliquefaciens, B. pumilus, B. licheniformis* بتركيز 2 مليار وحدة تكوين مستعمرات/CFU لكل جرام بمعدل اضافة 1 جم /كجم من العلف في أحواض البيوفلوك بمرحلة التحضين لأسماك المبروك، وذلك نظراً لتحسين الأداء الإنتاجي، معدل البقاء، والحالة الصحية للأسماك.