ADDITION OF COMMERCIAL PROBIOTIC (*LACTOBACILLUS DELBRUEKII* AND *L. FERMENTUM*) IN RED TILAPIA BROODSTOCK DIET IN DIFFERENT REARING SYSTEMS. I- EFFECTS ON REPRODUCTIVE PERFORMANCE AND LARVAL QUALITY

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SUMMARY

This experiment was carried-out to evaluate the combined effects of dietary commercial probiotic Lactéol Fort® supplementation and different rearing systems on reproductive performance of red tilapia broodstock. In completely randomized, 2X2 factorial design, 2 different tank systems, clear water (CW) and biofloc combined with two different levels (0 and 1 g kg^{-1}) of dietary probiotic Lactéol Fort® attaining 4 experimental treatments; T1: CW without probiotic), T2: CW + probiotic, T3: biofloc without probiotic) and T4: biofloc + probiotic with three replicates for each treatment compromising twelve tanks (each 2 m^3). Red tilapia broodstock reproductive performance was compared during the reproductive period. The probiotic Lactéol Fort® containing *Lactobacillus delbruekii* (10^{13} CFU: colony forming unit) g^{-1} and *L. fermentum* (10^{13} CFU) g^{-1} was administered as a feed additive to broodstock diet. For the reproductive performance assay, 216 females and 72 males of red tilapia *Oreochromis mossambicus* × *O. niloticus* at density of 24 (18 females: 6 males) tank^{-1} equivalent to stocking density of 9: females: 3 males m^{-3} with a sex ratio of 3 females to 1 male. Average body weight of female and male tilapia broodstock were 127.5 and 148 g, respectively). Reproductive variables (spawned female %, absolute and relative fecundity) were estimated in four subsequent seed clutches. No mortalities were found in male and female tilapia broodstock as affected by biofloc and probiotic levels. The results revealed that broodstock fed the commercial probiotic in biofloc tanks (T4: biofloc + probiotic), had the highest values for reproductive performance followed by T3 (biofloc without probiotic addition), T2 (CW + probiotic), and finally T1 (CW without probiotic). The results recommend continuous intake of probiotic (Lactéol Fort® containing *Lactobacillus delbruekii* (10^{13}) CFU g^{-1} and *L. fermentum*, 10^{13} CFU (colony forming unit) g^{-1} at a dose of 1 g kg^{-1} of broodstock feed in biofloc tanks during the spawning season of red tilapia, due to the higher reproductive performance and fry survival.

***Keywords**: Red tilapia biofloc, clear water, Probiotic, reproductive performance, larval survival*

INTRODUCTION

Tilapia ranks the world's second most popular important cultured fish, after carps. This species is getting more and more popular farmed fish due to its advantage of rapid growth, resistance and advantageous adaptability to high stocking densities, disease resistance, adaptable feeding habits, and tolerance to poor quality (Prabu *et al*., 2019). There were many studies dedicated with different effects of different rearing system (clear or biofloc) and the combination effect of the probiotics on reproductive performance of different fish and crustaceans species (Xu and Pan, 2013, Xu and Pan, 2014, Ekasari *et al*., 2014, Kim *et al*., 2014, Kim *et al*., 2015, Kumar *et al*., 2017, Long *et al*., 2015, Cardona *et al*., 2016, de Souza *et al*., 2016, Verma *et al*., 2016, Carnevali *et al*., 2017, Rodiles *et al*., 2018, Abdel-Tawwab *et al*., 2021 Eid *et al*., 2021 and Ogello *et al*., 2021). On the other *hand*, there was lack of information and studies concerning the effect of different rearing system on red tilapia broodstock reproduction, therefore the aim of this study was to evaluate the effect of adding commercial probiotics to red tilapia broodstock diet in different rearing system.

Probiotics have many positive effects in maintaining water quality, growth performance, the innate immune system and reproduction (Dias *et al*., 2012a; Newaj-Fyzul and Austin, 2015; Peredo *et al*., 2015;

Soto, 2017, Abo-State and Tahoun, 2017). The positive contribution of probiotic application to improving reproductive performance of broodstock and fecundity has been studied by Mehrim *et al*. (2015). The improvement of the usage of feed components together with symbiotic (probiotics, prebiotics, and phytochemicals), may be a feasible opportunity for reinforcing boom performance (Sutili, *et al*., 2018, Abdel-Tawwab *et al*., 2021 and 2022), optimizing resistance to infectious diseases (Nayak, 2010; El-Ezabi *et al*., 2011; Sutili *et al*., 2018) and lowering the portions of antibiotic carried out in fish farming (Sutili *et al*., 2018). Live microorganisms make up probiotics, which may also have effects on the health status of the host via modulating the intestine microbiota. Probiotics use has been a powerful opportunity to antimicrobial boom promoters within the farm animals' industry, and they can be microorganism or non-bacterial species or lines. However, there was a growing approach in administering multi strain probiotics in current years. Multistrain probiotics contain many species of vital microorganisms as a consortium that is useful to the administered animal.

Biofloc technology (BFT) plays three major roles including (i) water quality maintenance through nitrogen compounds uptake, resulting in in situ microbial protein; (ii) nutrition, increasing culture feasibility by reducing feed costs and lowering freed conversion ratio (FCR); and (iii) pathogen competition. Because of a complex interaction between particulate organic matter, physical substrates, and a wide range of microorganisms. The biofloc technology is described as the "blue revolution" era in aquaculture and has been widely applied. BFT is based on in situ microorganism production (Emerenciano *et al*., 2017).

The objective of this work was to evaluate the effects of probiotic Lactéol Fort® containing *Lactobacillus delbruekii* (10^{13} CFU g^{-1}) and *L. fermentum* (10^{13} CFU g^{-1}) as a feed additive in red tilapia broodstock diet held in biofloc and clear water tank system.

MATERIALS AND METHODS

Experimental design:

The experiment was carried out at a commercial tilapia hatchery located in Kafr Al-Shaikh Governorate, northern Egypt. The experimental design was completely randomized with four treatment groups: 4 experimental treatments; T1: CW; without probiotic, T2: CW + probiotic, T3: biofloc without probiotic and T4: biofloc + probiotic with three replicates for each treatment. The experimental concrete tank system was supplied with brackish groundwater with a salinity of 6000 ppm and a good aeration system to maintain continuous aeration and strong mixing by using air distributors and PVC pipelines connected to an air blower.

Experimental diet:

The utilized experimental basal diet contained 35% crude protein (CP), 7% ether extract (EE), 4.75% crude fiber (CF), 11% ash. A lyophilized powder of commercial probiotic that posed in the local market under the trade mark (Lactéol Fort®) produced by Rameda, Giza Governorate, (Egypt), each gram of probiotic contains: *Lactobacillus delbruekii* (10^{13} CFU g^{-1}) and *L. fermentum* (10^{13} CFU g^{-1}). The probiotic was homogenized in vegetable oil, sprayed on the feed. The diet was offered twice a day as 2% of the total biomass readjusted every two weeks. In biofloc tanks, organic carbon was added to stimulate the biofloc formation and growth. Biofloc helps to improve water quality, reduces the need for water exchange and may serve as additional natural feed source. Corn starch was added daily into the biofloc tanks to adjust the C/N ratio to equal 10.

Experimental broodstock:

Red tilapia (*Oreochromis mossambicus* × *O. niloticus*) broodstock was obtained from a commercial tilapia hatchery located in Kafr El-Shaikh Governorate. Male and female broodstock were netted from broodstock holding tanks, manually selected, and transferred to conditioning tanks, where they were held and kept separately for 15 days for acclimatization to the new environment until starting the experiment, which lasted for 12 weeks. A total number of 216 females and 72 males of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) were distributed at density of 24 (18 females: 6 males) $tank^{-1}$ equivalent to stocking density of 9: females: 3 males m^{-3} with a sex ratio of 3 females to 1 male. Average body weight of female and male tilapia broodstock were 127.5 and 148 g, respectively). Female and male red tilapia broodstock average bodyweights were recorded at the beginning, during successive spawning, and at the end of the experiment, which lasted for 12 weeks. Natural mating (mouth brooding) was practiced by placing the broodstock in the experimental breeding tanks (each 2 m^3). The selected males and females of red tilapia broodstock were well-conditioned before mating and given better space and feed, to improve and increase sexual maturation. Every tank was inspected once every week for seed. Seed outputs were recorded and representative sample swim-up fry from the 4^{th} clutch was collected from each tank to determine the larval survival and quality via salinity challenge test.

Table (1): Composition and proximate analysis of the broodstock basal diet.

Ingredient	**%**
Fish meal	25
Corn gluten	10
Soy bean meal	30
Corn grain	27.5
Vegetable Oil	5
Di-Calcium Phosphate	2
Antiaflatoxin	0.1
Vitamin C	0.1
Min./ Vit. Mix [1]	0.3
Proximate composition	
Dry matter (%)	90
Crude protein (%)	35
Ether extract (%)	7.25
Crude Fiber (%)	4.75
Ash (%)	11
Nitrogen free extract (%)[2]	42

Each kg contains: retinyl acetate, 3000 IU; cholecalciferol, 2400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49 % ascorbic acid), 120 mg; cyanocobalamin, 0.024 mg; choline chloride, 1200 mg; d-biotin, 0.168 mg; niacin, 12 mg; folic acid, 1.2 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3077 mg; ferrous sulfate ($FeSO_4·7H_2O$, 20 Fe), 65 mg; manganese sulfate ($MnSO_4$, 36 % Mn), 89 mg; zinc sulfate ($ZnSO_4·7H_2O$, 40 % Zn), 150 mg; copper sulfate ($CuSO_4. 5H_2O$, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), 11 mg; Celite AW 521 (acid-washed diatomaceous earth silica), 1000 mg Agri-Vet Co., Cairo, Egypt.

1) *NFE (nitrogen free extract = 100 – (CP + EE +CF + Ash)*

Analytical methods:

Samples of the fish were randomly taken at the beginning and at the end of the experiments. At the end of the trial fish from experimental tanks were captured, weighed and finally frozen at -18^o C for the final body analysis. Fish samples were killed and kept frozen until performing the body chemical analysis. Samples of the experimental fish diet were taken, grounded, and stored in a deep freezer at -18 ^{o}C until proximate analysis. All chemical analyses of fish and fish diet were determined according to the AOAC (1995), where fish were dried at 105^o C overnight in a forced air oven to determine dry matter content. Crude protein was measured by the Micro-Kjeldahl method (N X 6.25) after acid digestion. Ether extract was determined by extraction in Sohxelt apparatus using petroleum ether. Ash content was determined in a muffle furnace at 550^o C for 10 hours. Nitrogen free extract (NFE) was calculated by differences. Proximate analysis of fish diet was done using the previous methods, which has been used in fish analysis, in addition, the basal diet content of crude fiber was determined by digesting the fat-free samples in acid and alkali. Initial analyses were carried out on a pooled sample of fish, which were weighed and frozen prior to the experiments.

Water quality parameters:

Water quality criteria (pH, temperature, dissolved oxygen, ammonia nitrogen, nitrite, nitrate, alkalinity (expressed as Ca CO_3), floc volume and total suspended solids were periodically determined. Water samples from each experimental tank were taken to assess parameters for water pH, temperature and dissolved oxygen (DO) and were monitored once a day, whereas ammonia nitrogen (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), alkalinity, floc volume (FV) and total suspended solids (TSS) biweekly. Water pH was measured using Milwaukee- PH600 pH meter, while water temperature measured using digital thermometer. The water DO, NH_3-N, NO_2-N, NO_3-N, and TSS were determined using a photometer and multi-test kit. Floc volume (VF) measured by using Imhoff cone and the volume of floc on the bottom of the cone was observed after 15 min of sedimentation (as described by Avnimelech, 2007). Water Alkalinity was measured by titration with sulfuric acid till pH point of 4.5 (APHA, 1998).

Spawning performance and seed output:

Average female weights = (average final weight + average initial weight)/ 2

Absolute fecundity (total seed number) = summation of seeds for four clutches.

Relative fecundity (seeds/g female) = Total seed number for each female/mean weight (g).

Seeds/female/day= Total seed number/days of the experiment (84 days).

Salinity Stress test:

A salinity stress test was carried out on late larval tilapia. About two hundreds red tilapia larvae were collected from each broodstock spawning tank to assess the maternal effects of using probiotics and application of biofloc on larval quality. The larvae were graded based on size, and one hundred larvae of similar size were chosen from each batch for use in the salinity challenge test. The average body weight of the larvae from the control and biofloc tanks were 0.13± 0 and 0.12± 0 mg, respectively. The salinity stress test was carried out at a concentration of 35 g/L NaCl, which had previously been determined as the lethal tolerant (LT50) for red tilapia larvae after 50 minutes in a preliminary experiment as described by Ekasari *et al*. (2015a). Twelve plastic tanks (two liters each) were filled with 1.5 liters of 35 g/L saline water. A hundred larvae from the experimental broodstock tanks were distributed at a density of 20 fish/ plastic tank. After 1 hour of immersion in saline water, the larvae were transferred to another tank containing freshwater with aeration, and the number of survivors was determined 1 hour later. The survival of the larvae in the freshwater tanks was also determined 24 hours after transfer.

Statistical analysis:

The experimental data were analyzed with portable SAS software, version 9.1.3 (SAS Stat 9.1, SAS Institute Incorporation), and values of probability ($P<0.05$) were considered significant. Data were statistically analyzed in factorial ANOVA. The mean of treatments was compared by Duncan's (1955) multiple range tests.

RESULTS AND DISCUSSION

Water quality criteria:

The water quality measurements of the experimental spawning tanks as affected by biofloc application and dietary probiotic administration during the experiment are shown in Table (2).

All water quality criteria including, water pH, temperature, DO, NH_3-N, NO_2-N, NO_3-N, alkalinity, FV and TSS were in acceptable range for tilapia (Sipaúba-Tavareset *et al*., 2010, Emerenciano *et al*., 2017, Khanjani *et al*., 2017 and Khanjani *et al*., 2021). There were no significant differences among different experimental treatments in water temperature, pH, and DO, while water ammonia, nitrite, nitrate, total alkalinity and FV and TSS were significantly influenced by different rearing system and probiotic levels. Among BFT groups, the BFTS treatments showed the lower values of total ammonia nitrogen and NO2 -nitrogen and the highest NO_3 -Nitrogen concentration among varying experimental tank groups, which may point toward a higher population of NH_3 and NO_2 oxidizing bacterial community rather than other treatments, which was consistent with the results of other researchers (Correia *et al*., 2014 and Khanjani *et al*., 2021). The explanation of increased TSS in biofloc treatments could be contributed to the additional of carbon source to the experimental tanks, which has generated encouraging conditions for the growth of floc- associated organisms and these findings are in concordance with those of Khanjnai *et al*. (2017).

Table (2): Water quality criteria of spawning tanks as affected by different rearing systems and probiotic Lactéol Fort® levels.

Time	Treatment	T1	T2	T3	T4
	System	Clear		Biofloc	
	Probiotic	0 g kg^{-1}	1g kg^{-1}	0 g kg^{-1}	1g kg^{-1}
At start	pH	7.87 ± 0.03^{a}	7.93 ± 0.03^{a}	7.83 ± 0.03^{a}	7.94 ± 0.02^{a}
	Temp.	27.97 ± 0.03^{a}	28.00 ± 0^{a}	27.87 ± 0.03^{a}	27.97 ± 0.03^{a}
	DO	5.02 ± 0.017^{a}	5.00 ± 0^{a}	5.00 ± 0^{a}	5.02 ± 0.02^{a}
	NH_3-N	0.34 ± 0.024^{a}	0.43 ± 0.04^{a}	0.41 ± 0.01^{a}	0.28 ± 0.03^{b}
	NO_2-N	0.63 ± 0.07^{a}	0.61 ± 0.03^{ab}	0.52 ± 0.05^{ab}	0.47 ± 0.0^{a}
	NO_3-N	40.67 ± 1.45^{a}	37.67 ± 1.86^{ab}	32.00 ± 0.58^{a}	31.67 ± 3.84^{b}
	Alkalinity	131.67 ± 11.0^{a}	130.67 ± 2.85^{a}	158.67 ± 9.91^{a}	147.67 ± 6.84^{a}
	FV	0^{c}	1.17 ± 0.17^{ac}	4.33 ± 0.33^{b}	8.33 ± 0.88^{a}
	TSS	94.67 ± 2.60^{b}	75.67 ± 15.39^{b}	96.67 ± 3.33^{b}	140.00 ± 10.0^{a}
Day 15	PH	7.967 ± 0.033^{a}	7.93 ± 0.03^{a}	8.00 ± 0.06^{a}	7.98 ± 0.01^{a}
	Temp.	27.90 ± 0.06^{a}	27.97 ± 0.09^{a}	27.83 ± 0.03^{a}	28.03 ± 0.03^{a}
	DO	5.00 ± 0.03^{a}	5.01 ± 0.0^{a}	4.98 ± 0.02^{a}	5.00 ± 0.03^{a}
	NH_3-N	0.40 ± 0.01^{a}	0.37 ± 0.01^{ab}	0.32 ± 0^{b}	0.27 ± 0.03^{bc}
	NO_2-N	0.63 ± 0.07^{a}	0.61 ± 0.03^{a}	0.57 ± 0.05^{a}	0.52 ± 0.06^{a}
	NO_3-N	40.67 ± 1.45^{a}	37.67 ± 1.86^{ab}	32.00 ± 0.58^{b}	31.67 ± 3.84^{b}
	Alkalinity	232.7 ± 12.0^{a}	231 ± 2.52^{a}	258.7 ± 9.91^{a}	252.7 ± 10.17^{a}
	FV	0	2.08 ± 0.300^{c}	9.33 ± 0.67^{b}	11.00 ± 0.58^{a}
	TSS	94.6 ± 2.60^{b}	104.00 ± 2.0^{b}	126.67 ± 3.33^{b}	168.00 ± 24.27^{a}
Day 30	PH	8.00 ± 0^{a}	7.93 ± 0.03^{a}	8.00 ± 0.06^{a}	6.99 ± 0.97^{b}
	Temp.	29.90 ± 0.06^{a}	30.00 ± 0^{a}	29.87 ± 0.06^{a}	20.09 ± 9.91^{a}
	DO	5.01 ± 0.02^{a}	5.01 ± 0.01^{a}	5.03 ± 0.02a	3.50 ± 1.50^{a}
	NH_3-N	0.39 ± 0.01^{a}	0.36 ± 0.012^{a}	0.31 ± 0.01b	0.26 ± 96.17^{b}
	NO_2-N	0.71 ± 0.01^{a}	0.65 ± 0.03^{a}	0.56 ± 0.05^{b}	0.27 ± 0.03^{c}
	NO_3-N	37.33 ± 1.20^{a}	36.00 ± 1.53^{a}	32.67 ± 0.88^{b}	29.00 ± 4.73^{b}
	Alkalinity	269.7 ± 0.9^{a}	268.7 ± 2.3^{a}	272.7 ± 4.37^{a}	273.0 ± 7.0^{a}
	FV	0	2.33 ± 0.88^{b}	14.33 ± 1.45^{a}	16.00 ± 0.58^{a}
	TSS	115.6 ± 2.33^{d}	137.00 ± 11.9^{c}	224.67 ± 39.3^{b}	313.00 ± 13.45^{a}
Day 45	PH	8.00 ± 0^{a}	7.93 ± 0.03^{a}	8.00 ± 0.05^{a}	8.00 ± 0.01^{a}
	Temp.	29.90 ± 0.06^{a}	30.00 ± 0^{a}	29.86 ± 0.07^{a}	30.00 ± 0^{a}
	DO	5.00 ± 0.03^{a}	5.013 ± 0.01^{a}	5.167 ± 0.17^{a}	5.00 ± 0.03^{a}
	NH_3-N	0.39 ± 0.0^{a}	0.36 ± 0.01^{a}	0.31 ± 0.01^{b}	0.29 ± 0.01^{b}
	NO_2-N	0.70 ± 0.06^{a}	0.65 ± 0.03^{a}	0.5 ± 0.05b	0.50 ± 0.06^{b}
	NO_3-N	37.33 ± 1.20^{a}	36.00 ± 1.53^{a}	32.67 ± 0.88^{b}	31.8 ± 3.23^{b}
	Alkalinity	269.6 ± 0.88^{b}	268.6 ± 2.33^{b}	272.66 ± 4.37^{b}	282.67 ± 4.10^{a}
	FV	0	1.67 ± 0.33^{c}	22.00 ± 0.58^{b}	26.00 ± 1.00^{a}
	TSS	122.3 ± 1.45^{c}	140.67 ± 9.2^{c}	233.33 ± 37.12^{b}	282.33 ± 11.78^{a}
Day 60	PH	7.93 ± 0.03^{a}	7.93 ± 0.03a	7.87 ± 0.09a	7.67 ± 0.03^{a}
	Temp.	27.00 ± 0.10^{a}	27.00 ± 0.03^{a}	26.93 ± 0.03^{a}	26.90 ± 0^{a}
	DO	5.12 ± 0.04^{a}	5.03 ± 0.02^{a}	5.00 ± 0.03^{a}	5.02 ± 0.02^{a}
	NH3-N	0.32 ± 0.02^{a}	0.27 ± 0.01^{ab}	0.24 ± 0.02^{b}	0.19 ± 0.05^{b}
	NO2-N	0.50 ± 0.01^{a}	0.45 ± 0.03^{a}	0.35 ± 0.05^{b}	0.29 ± 0.06^{c}
	NO3-N	33.67 ± 2.33^{a}	31.00 ± 2.52^{ab}	28.00 ± 3.06^{b}	26.00 ± 1.53^{b}
	Alkalinity	263.00 ± 4.16^{a}	264.67 ± 7.22^{a}	287.67 ± 83.37^{a}	286.33 ± 8.11^{a}
	FV	0	3.00 ± 1.00^{b}	22.00 ± 2.08^{a}	23.67 ± 1.33^{a}
	TSS	132.67 ± 9.02^{c}	130.67 ± 2.85^{c}	267.33 ± 22.17^{b}	306.00 ± 7.37^{a}

a, b and c mean in the same row with different superscripts are significantly ($p \leq 0.05$) different.
SE=standard error.

One of the major advantages of biofloc as an intensive system is the ability to manage the aquatic environment and critical water quality parameters i.e ammonia and nitrite and in turn manipulate and optimize the environment in favor for fish health and growth (Crab *et al*., 2009 and Crab 2010, Singh *et al*., 2020). In BFT, the microbial conversion of nutrient waste in rearing systems (mainly ammonia-nitrogen) into microbial biomass which can be utilized back by the cultured aquatic animals i. e fish and shrimps as a food source, (Avnimelech, 2009, Crab *et al*., 2012, and Emerenciano *et al*., 2017 and Prabu *et al*., 2019). Thus, the nutrient by-product and wastes that are possibly toxic for the cultured organisms can be maintained and kept on low levels and the feeding efficiency can be enhanced. Moreover, the affirmative in influence on the immunity (Ekasari *et al*., 2014; kim *et al*., 2014 and Cardona *et al*., 2016) and the reproductive outputs of the cultured organisms (Emerenciano *et al*., 2014, Ekasari *et al*., 2015a, Braga *et al*., 2015, Cardona *et al*., 2016 and Ekasari *et al*., 2016).

Reproductive performance:

As shown in Table (3), the spawned female percent from the treatment 4 (reared in biofloc and administered dietary probiotics were significantly higher compared to other experimental treatments during the four seed clutches.

Table (3): Number and percent of spawned females as affected by different rearing systems and probiotic Lactéol Fort® levels.

System	Clear				Biofloc			
Probiotic (g kg^{-1})	0	1	0	1	0	1	0	1
Treatment	T1		T2		T3		T4	
Spawned females	Number	(%)	Number	(%)	Number	(%)	Number	(%)
1st clutch	16.00	88.89^{b}	18.00	100.00^{a}	18.00	100.00^{a}	18.00	100.0^{a}
2nd clutch	15.00	83.33^{c}	15.00	83.33^{b}	16.00	88.89^{ab}	17.00	94.44^{a}
3rd clutch	14.00	77.78^{c}	15.00	83.33^{b}	15.67	87.04^{ab}	16.67	92.59^{a}
4th clutch	15.00	83.33^{b}	17.33	96.30^{a}	17.67	98.15^{a}	17.33	96.30^{a}

a, b and c mean in the same row with different superscripts are significantly (p≤0.05) different.
SE=standard error

The highest percent values for spawned females at different rearing systems were in favor of T4 (Biofloc + probiotic), where the first, the second, the third and the fourth subsequent seed clutches (100, 94.44, 92.59 and 96.30%, respectively. The lowest corresponding percent of spawned female were found in treatment (T1: CW + probiotic). The values were 88.89, 83.33, 77.78 and 83.33% for the first, the second, the third and the fourth collections, respectively. In this study, the spawned females % are very satisfactory (ranged between 77.78 to 100 %) are much higher than those found in the study of Dias *et al*., (2020) examined continuous intake of a dietary probiotic at a dose of 0.5 g kg^{-1} of feed (10^{10} CFU g^{-1}) during the breeding season of Nile tilapia of probiotic *Bacillus subtilis* bacteria which were applied as an additive in the diet of Nile tilapia broodstock compared to control (without probiotic) or even alternate probiotic intake at a same dose. Increasing values for spawning female number, collected surviving fry were found in the probiotic groups.

The data on reproductive performance including in the present study (absolute and relative fecundity and system productivity (Table 4) showed that no significant difference among different broodstock groups in the 1st seed clutch, while in the 2nd, 3rd and the 4th seed clutches, the highest mean seed production was recorded for T4 (biofloc + probiotic), while the lowest mean seed production noticed at T1 (CW without probiotic).

Concerning the absolute fecundity "total seed number), the results declared that the highest significant differences (P<0.05) was found at treatment T4 (966.31 seeds) compared with the other three treatments T1 (828.31), T2 (829.42) and T3 (919.69 seeds), respectively with significant (P<0.05) increase in T3 as compared to T1 and T2. The same trend was observed at all the other reproductive performance parameters of female broodstock (Absolute and relative fecundity, seeds/female/day and seeds/tank/day. The highest seeds/g female (7.46), seeds/female/day (11.55) and seed/tank/day (46.20) were found in T4.

There were significant (P<0.05) differences between the other three treatments and the corresponding values were 7.16, 10.95 and 43.79 (T3) and 6.59, 9.65 and 38.95 (T1) and 6.59, 9.86 and 39.44 (T 2). There were no significant differences between T1 and T2 in terms of (Absolute, relative fecundity, seeds/female/day, and seeds/tank/day). From the above-mentioned results concerning the affirmative impact of biofloc on reproduction in tilapia fish, which may consider a fish of choice for the reason that it is perfectly fit to biofloc systems consuming bioflocs, as well as its tolerance to high temperatures and suspended solids concentrations in culture waters (Avnimelech, 2011; Poli *et al*., 2019 and Dilmi *et al*., 2021). Tilapias are getting more and more popular farmed fish due to their adaptability. Emerenciano *et al*. (2012) pointed-out that although BFT has been effectively applied in shrimp farming, little information are known about its role on spawning performance. Therefore, further research is needed to optimize many factors i.e broodstock size and age, stocking density, sex ratio and nutrient requirements which can affect the reproduction of tilapia under biofloc (zero water exchange system).

Table (4): Reproductive performance of red tilapia broodstock as affected by different rearing systems and probiotic Lactéol Fort® levels.

Treatment	T1	T2	T3	T4
System	Clear		Biofloc	
Probiotic levels	0 g kg^{-1}	1 g kg^{-1}	0 g kg^{-1}	1 g kg^{-1}
Female average weight (g)	125.92± 0.58^{a}	125.83±1.67^{a}	128.58±1.62^{a}	129.50±1.53^{a}
1st clutch (seeds $female^{-1}$)	199.94±12.86^{a}	188.63±2.91^{a}	195.70±3.21^{a}	197.04±1.45^{a}
2nd clutch (seeds $female^{-1}$)	200.69±1.15^{b}	216.44±5.13^{a}	226.93±5.23^{a}	231.85±5.96^{a}
3rd clutch (seeds $female^{-1}$)	169.22±15.49^{b}	191.11±4.70^{ab}	205.94±22.21^{ab}	227.03±4.49^{ab}
4th clutch (seeds $female^{-1}$)	259.57±12.96^{b}	232.13±3.13^{b}	291.11±4.63^{a}	310.40±10.43^{a}
Absolute fecundity (seeds/female)	829.42±11.02^{c}	828.31±3.82^{c}	919.69±17.35^{b}	966.31± 15.85^{a}
Relative fecundity seeds/ g female	6.59±0.09^{b}	6.59±0.11^{b}	7.16±0.22^{a}	7.46 ±0.13^{a}
Seeds/ female/ day	9.65±0.18 ^{c}	9.86±0.05^{b}	10.95±0.21^{b}	11.55±0.23^{a}
Seeds/ /Tank/ day	38.59±0.72^{c}	39.44± 0.18^{b}	43.79±0.8^{b}	46.20±0.9^{a}

a, b and c mean in the same row with different superscripts are significantly (p≤0.05) different. SE= standard error

BFT is useful for mass production of live food resources, which are required for successful hatchery larvae culture. Bioflocs improve fish reproduction by enhancing gonad formation and ovary development in fish broodstock. Bioflocs are natural biosecurity agents that reduce the use of antibiotics, which have a variety of environmental consequences in the aquaculture environment (Ogello *et al*., 2021). Moreover, previous studies on biofloc systems validated improved feed efficiency and reduced nutrient waste in culture systems, moreover, contributing positive influence on the immunity (Ekasari *et al*., 2014; kim *et al*., 2014 and Cardona *et al*., 2016) and the reproductive outputs of the cultured organisms (Emerenciano *et al*., 2014, Ekasari *et al*., 2015a, Braga *et al*., 2015, Cardona *et al*., 2016 and Ekasari *et al*., 2016). Emerenciano *et al*. (2012) pointed-out that although BFT has been effectively applied in shrimp farming, little information are known about its role on spawning performance. Moreover, the consumption of bio floc by the cultured aquatic animals might release specific microbial components as immune stimulating substance such as β-1,3 glucan lipopolysaccharides and peptidoglycan (Ekasari *et al*., 2014). This has been supported with the aid of using the developing portions of proof indicating the immune-stimulatory outcomes of biofloc structures on the classy aquatic organisms (Xu and Pan, 2013, Xu and Pan, 2014, Ekasari *et al*., 2014, Kim *et al*., 2014, Kim *et al*., 2015, Kumar *et al*., 2017, Long *et al*., 2015, Cardona *et al*., 2016, de Souza *et al*., 2016, Verma *et al*., 2016 and Ogello *et al*., 2021).

In current decades, there was a great attention paid for using probiotics in fish aquaculture. Working on probiotic potential effect on reproductive performance, Mehrim *et al*. (2015) assessed the impact of supplementation different dietary graded levels of Hydroyeast® consist of of probiotic bacteria (*Lactobacillus acidophilus*, *Bifedobacterium longhum* and *Bifedobacterium thermophylu*, and *Streptococcus faecium* 22.5 X 10^{8} CFU kg^{-1} plus oligosaccharides (50,000 mg Kg^{-1}); enzymes mixture including (amylase 3.7 X 10^{6} , proteases X 10^{5} , cellulose 2 X 10^{5} , phytase 3 X 10^{3} units kg^{-1} , pectinase (1 X 10^{5}) , xylanase (1 X 10^{4}) live yeast (5 X 10^{12} colony forming units (CFU) kg^{-1}; on hematological and biochemical parameters, serum intercourse hormones, and the spawning performance of the breeder tilapia *Oreochromis niloticus*. Similar results on the effect of probiotic *Saccharomyces cerevisiae* to aquafeed and substantial beneficial effects on seed production of broodstock Nile tilapia, enhanced growth of fry and improved nutrient utilization. However, there is no effect on chemical body composition were observed (Abo-State and Tahoun, 2017). Recently, efforts to preserve environmentally pleasant aquaculture have endorsed using useful bacterial, along with probiotics, as feed enrichment additives, increasing the bioavailability of key nutrients, i.e important components such fatty acids and amino acids (in egg formation, maturation as well as embryo development and larval and fry growth) and may modulate the genes expression involved in lipid metabolism (Carnevali *et al*., 2017; Dias *et al*., 2012b; Rodiles *et al*., 2018, Abdel-Tawwab *et al*., 2021 and Abdel-Tawwab *et al*., 2022).

Salinity stress test:

Data on Survival rates of offspring as affected by different rearing systems and probiotic levels are shown in Table (5). The dietary probiotic supplementation and application of biofloc technology had a substantial impact on the larval quality in terms of growth and quality and robustness, a much more pronounced effect on survival rates.

Table (5): Survival rates of offspring as affected by different rearing systems and probiotic levels.

Treatment	T1	T2	T3	T4
System	Clear		Biofloc	
Probiotic	0 g kg^{-1}	1 g kg^{-1}	0 g kg^{-1}	1 g kg^{-1}
Survival rate (%) (after 2 hours)	75.00±2.89^{b}	83.00 ± 0.04^{b}	83.67± 3.18^{a}	90.67±2.33^{a}
Survival rate (%) (after 24 hours)	51.67±4.41^{b}	65.00 ± 2.88^{a}	68.33±01.67^{a}	73.00±1.53^{a}

After carrying-out the challenge of salinity stress test. It is observed that, the offspring survival rates (after 2 and 24 hours) were significantly differed among the experimental groups (Table 5). The highest survival rates (90.67% and 73%) were in favor of broodstock group of T4 (Biofloc + probiotic) compared to T1(CW without probiotic), which recorded the lowest values for offspring survival rates (75 and 51.67% after 2 and 24 hours, respectively.) This result highlights the importance to consider both biofloc based hatchery systems and addition of probiotics on red tilapia broodstock. Future studies should be performed to investigate whether or not probiotics interacting with microbial community in bioflocs. However, the choice of the most suitable diet for each species broodstock is a major challenge in fish farming, requiring reproductive and work planning strategies. Precise definition of techniques that will provide higher reproductive efficiency is necessary to ensure viable progeny (Merrifield *et al*., 2010). In the recent past scientists have described BFT as self-sustaining technology, capable of recycling culture wastewater and generating natural food simultaneously. Based on previous literature evidence, the great potential of BFT towards improving yield, safety, and economic sustainability for tilapia farming (Ogello *et al*, 2014, Ekasari *et al*., 2015 a,b Long *et al*., 2015, Mabroke *et al*., 2019, Eid *et al*., 2021, Dilmi *et al*., 2021 and Mabroke *et al.*, 2021).

CONCLUSION

Red tilapia broodstock fish held in biofloc tank conditions and fed diet supplemented with probiotic Lactéol Fort® at dose of 1 g kg^{-1} diet had significant effects on reproductive performance. The sustainable biofloc technology was also confirmed for higher fry production, larval quality, and survival.

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إضافة البروبيوتيك التجاري لاكتيول فورت® لعلائق قطعان التفريخ لأسماك البلطي الأحمر المرباه في نظامي المياه الرائقة والبيوفلوك.1- التأثيرات على الأداء التناسلي وجودة الزريعة الناتجة

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أجريت هذه التجربة لتقييم التأثيرات المشتركة للمكملات الغذائية للبروبيوتيك الغذائي التجاري لاكتيول فورت® مع نظامين مختلفين للتربية (المياه الرائقة والبيوفلوك) على الأداء التناسلي لقطيع البلطي الأحمر. في تصميم تجريبي عشوائي، تم اختبار نظامين (المياه الرائقة أو نظام البيوفلوك جنبًا إلى جنب مع مستويين مختلفين (صفر و 1 جم بروبيوتيك لاكتيول فورت®/ كجم من العلف) تحقق أربعة معاملات تجريبية تم تكرارها في ثلاث مكررات لكل معاملة من المعاملات الأربعة في تجربة عاملية بنظام تجريبي مكون من 12 تانك (حجم التانك الواحد منها 2 متر مكعب). و تمت مقارنة الأداء التناسلي لقطيع البلطي الأحمر خلال فترة التكاثر. تم تقييم استخدام مستوي البروبيوتيك لاكتيول فورت® والذي يحتوي على كل من بكتريا اللاكتوباسيلس ديلبروكي بتركيز 10^{13} / وحدة تشكيل مستعمرة جرام و بكتريا اللاكتوباسيلس فيرمنتوم بتركيز 10^{13} وحدة تشكيل مستعمرة /جرام كمضاف غذائي في علائق قطعان التفريخ لأسماك البلطي الأحمر .تم استخدام 216 أنثى و 72 ذكر من ذكور البلطي الأحمر *Oreochromis mossambicus* × *O. niloticus* بكثافة (18 إناث: 6 ذكور) / تانك (حجم 2 متر مكعب) بمتوسط وزن 127.5 جرام للإناث و148 جرام للذكور. بينت النتائج أن متغيرات الأداء التناسلي وجود فروق بين مجموعات (الإناث التي تم التبويض بها ، والخصوبة المطلقة والنسبية) وذلك علي مدار أربع دورات تكاثر متتالية لإنتاج الزريعة. ولم تسجل أية حالات نفوق بين إناث أو ذكور الأسماك في المجموعات التجريبية المختلفة. وأوضحت النتائج تأثر ذكور وإناث أسماك البلطي إيجابيا بنظام التربية ومستويات البروبيوتيك ، كما أظهرت النتائج أن قطيع الأسماك التي تغذي علي البروبيوتيك التجاري في تانكات ال biofloc كانت لها أعلى قيم للأداء التناسلي (المجموعة الرابعة :البيوفلوك + البروبيوتيك) ، تليها المجموعة الثالثة (البيوفلوك بدون إضافة بروبيوتيك) ، ثم المجموعة الثانية (مجموعة نظام المياه الرائقة مع إضافة بروبيوتيك) ، وأخيرًا المجموعة الأولي (مجموعة المياه الرائقة بدون إضافة البروبيوتيك).

توصي النتائج بإضافة البروبيوتيك لاكتيول فورت® والذي يحتوي على كل من بكتريا اللاكتوباسيلس ديلبروكي بتركيز 10^{13} / وحدة تشكيل مستعمرة جرام و بكتريا اللاكتوباسيلس فيرمنتوم بتركيز 10^{13} وحدة تشكيل مستعمرة / جرام كمضاف غذائي في علائق قطعان تفريخ أسماك البلطي الأحمر بمعدل 1 جرام / كيلو جرام من العلف أثناء موسم التفريخ حيث يؤدي ذلك إلي تحسين الأداء التناسلي مع معدل إعاشة مرتفع وجودة أفضل للزريعة الناتجة.