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Effect of local probiotic on common carp *Cyprinus carpio* growth performance and survival rate

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Abstract

The aim of this study was to assess the effect of four levels of local probiotics compared to commercial probiotic as a biological control on common carp *Cyprinus carpio* growth parameters, specific growth rate (SGR), food conversion ratio (FCR) and survival rate. Fish (average weight 57.85 ±0.3g) were fed 0.05, 0.1, 0.25 and 0.75 mg kg d.w^{-1} local probiotic compared with 2 mg kg d.w^{-1} fed of commercial probiotic and control group without additive for 42 days. Highest level of probiotics (0.75 mg kg d.w^{-1}) showed significantly increases on the SGR and FCR ($P \leq 0.05$) compared to the other groups. Also, survival rate recorded 94.6% in comparison to control group (72.1%), suggesting that the high level of probiotics induces significant increases on growth performance which have profound may implications for the global aquaculture industry.

Key words: Common carp, *Cyprinus carpio*, Probiotics, Growth parameters, Survival rate.

Introduction

World aquaculture has grown tremendously during the last years, becoming an economically important industry, it is the fastest growing food-producing sector in the world with the greatest potential to meet the growing demand for aquatic food (Subasinghe *et. al.*, 2009) . Disease is a major problem in the fish farming industry with the increasing intensification and commercialization of aquaculture production, (FAO, 2006). Antibiotics used as traditional strategy for fish diseases management but also for the improvement of growth and efficiency of feed conversion, however, the development and spread of antimicrobial resistant pathogens were well documented (Bondad-Reantaso *et. al.*, 2005).

There is a risk associated with the transmission of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of nonpathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens (Sørum, 2006).

On the other hand antibiotics inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem but it also made antibiotic residue accumulated in fish products to be harmful for human consumption (FAO, 2005). Researchers and aquaculture managers began to look at alternative therapeutics and look at aquaculture system in more environmental perspective then that of a production system the aim now is to manage a culture using probiotic, immunostimulants, vaccine and the aquatic environment at large amount without at any stage resorting to inorganic drugs and chemical (WHO, 2006).

Probiotics are defined as microbial dietary adjuvant that beneficially physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (Maroni, 2000) . Most studies with probiotics conducted to date in fish have been undertaken with strains isolated and selected from aquatic environments. There are a wide range of microalgae (Tetraselmis), yeast (Debaryomyces, Phaffia and Saccharomyces) and gram positive

(Bacillus, Lactococcus, Micrococcus, Carnobacterium, Enterococcus, lacobacillus, Streptcoccus, Weisslla) and gram negative bacteria (Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas and Vibrio) that have been evaluated as a probiotics (Villamil *et. al.*, 2000). Several studies have demonstrated certain modes of probiotic action in the aquatic environment, they improved feed conversion ratio and feed utilization, revealed adhesion capacity to the intestinal mucosa that hindered the adherence of pathogenic bacteria, produced extra-cellular antibiotic like products or iron binding agents (siderophores) that prevent the growth of some pathogenic flora. The probiotics achieved improvement in water quality (bioremidation) and facing the problem of red tide planktons, Gatesoupe, (1999) showed improvement in the immune response of fishes treated with probiotices therefore the study aimed to evaluate the effect of local probiotic on fish growth performance and survival rate.

The aim of this study was to assess the effect of four levels of local probiotics compared to commercial probiotic as a biological control on common carp *Cyprinus carpio* growth parameters.

Materials and Methods

Fish and experimental condition: Common carp *Cyprinus carpio* (average body weight of 57.85±0.3g) were obtained from AL-Mesiab farm, Babylon/Iraq. Fish were kept in path trough aquaria measuring (150 × 80 × 50 cm) and maintained in aerated de-chlorinated fresh water at 25 ^{o}C ± 1 for 14 days prior to use in experiments. Water pH was measured using electric digital pH meter and water temperature was recorded daily using a glass thermometer.

Fish diet: A basal diet was prepared at Dept. of Pathology, Veterinary Medicine College, Baghdad University. It contained 26% protein.

Probiotics: Two products containing probiotics, local (table 1) and commercial were used and mixed thoroughly with the prepared basal fish diet during its preparation.

Experimental design: One hundred and eight *Cyprinus carpio* were equally distributed into 12 trough and acclimate-ized for the experimental conditions for 15 days prior to the start. During this period fish were adapted on feeding of control diet (without any additives).

Table (1): Local probiotic microorganism

Microorganism	Count CFU/ml
Saccharomyces cerevisiae	1×10^{10}
Bacillus subtilus	1×10^{9}
Lactic acid bacteria J6	1×10^{11}

Water was changed daily to maintain good water quality. Water temperature and pH were adjusted at 24-26 ^{0}C and 6.5-6.8 respectively during the experimental period. The experimental design is presented in figure (1).

Growth parameters: Growth weight: estimated biweekly throughout the experimental period. Body weight gain: Final fish weight (g)-Initial fish weight (g). Daily gain (D.G) were determined according to (Watson *et. al.*, 1963), Specific Growth Rate %, it was calculated according to (Schmalhausen, 1926), Relative growth ratio (RGR), Feed Conversion Ratio (FCR) and Food Conversion Efficiency (FCE) were determined according to (Pouomonge and Mbonglang, 1993), survival rate, the fish were counted after 42 days from the start of the experiment to determine the survival percentage:

Survival % = (no. of fish counted /no. of stocked fish) × 100.

Results and Discussion

Data on average body weight of *Cyprinus carpio* post treatment with probiotic during 42 days including initial weight at 0, 14, 28 and 42 days were reported in table (2). At the beginning no significant difference was observed in the initial weigh between T1, T2, T3, T4 and T5 and control groups that were 57.85, 57.8, 57.8, 57.9, 57.8 and 58.0 respectively. However the mean final weight were no significant different ($P\leq 0.05$) between T3, T4 and T5 that were 80.3, 80.28, 78.05 respectively also similar trend was noted in T1, T2 that were 74.35, 69.97 but all groups were significantly increased ($P\leq0.05$) from the control group that was 68.56. Body weight gain, Daily gain (DG), specific growth rate (SGR) relative growth ratio (RGR), Food conversion rate (FCR) and food conversion efficiency (FCE) were reported in table (3), There were no significant difference observed in the body weight gain and Daily gain between groups T4 and T3 (22.38, 22.50), (0.53, 0.53) groups however all parameters significantly increased at level ($P\leq0.05$) when compared with

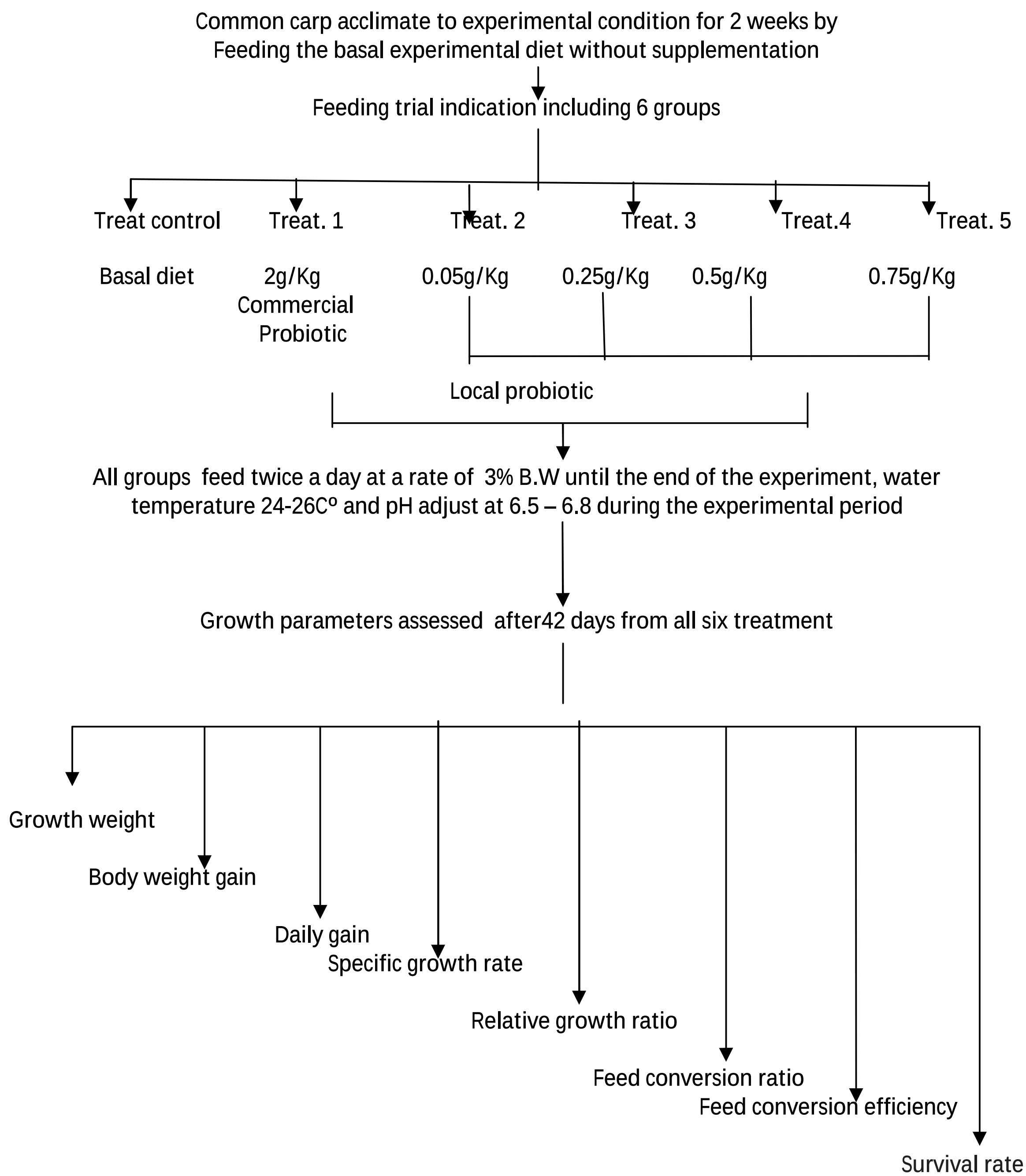

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Fig (1): Experimental design

Table (2): Average body weight of *Cyprinus carpio* post treatment with probiotic during 42 days including initial weight at 0, 14, 28 and 42 days (mean ± SE).

		Weight of fish (gm)		
Treatment	Zero day	14 days	28 days	42 days
Control	58.00 $\pm0.0^{a}$	59.35 $\pm0.35^{c}$	64.25 $\pm1.45^{b}$	68.56 $\pm1.8^{d}$
T1	57.85 $\pm0.3^{a}$	61.80 $\pm0.70^{ab}$	67.3 $\pm2.80^{ab}$	74.35 $\pm1.85^{bc}$
T2	57.8 $\pm0.01^{a}$	61.05 $\pm0.75^{bc}$	65.1 $\pm0.68^{b}$	69.97 $\pm1.35^{cd}$
T3	57.8 $\pm0.015^{a}$	61.00 $\pm1.0^{bc}$	68.5 $\pm0.5^{ab}$	80.3 $\pm0.90^{a}$
T4	57,9 $\pm0.20^{a}$	63.06 $\pm1.06^{ab}$	70.55 $\pm0.05^{a}$	80.28 $\pm0.55^{a}$
T5	57.8 $\pm0.01^{a}$	64.05 $\pm0.45^{a}$	70.8 $\pm0.20^{a}$	78.05 $\pm0.20^{ab}$

means having the different litter in the same column are significantly different at P ≤ 0.05 Control =diet(basal diet),T1=diet (4gm commercial pro/1Kg diet), T2= diet(0.05 g local probiotic /1Kg diet), T3=diet(0.25g local probiotic/1Kg diet), T4= diet(0.5g local probiotic/1Kg diet),T5= diet(0.75g local probiotic /1Kg diet.

control group (10.56, 0.25) while no change was seen between T2 and control.

The obtained results could be attributed to the evaluation of growth on fish accurate by use specific growth rate (SGR) and relative growth rate (RGR) parameter more than the Body weight gain and Daily gain (DG) parameter, due to that the SGR and RGR parameters reduce the effects of variant in the initial weight between fish (13). We can see in table (3) there were significantly difference at level (P≤0.05) between T1, T2, T3, T4 and T5 and control in SGR, RGR, FCR, FCE parameters. The values of SGR and RGR were higher in T3, T4 and T5 (0.78, 0.77,0.71) (38.92,38.65,35.03) than in T1 and T2 (0.59, 0.45), (28.52, 21.05) and control (0.39, 18.20). Also decrease value of FCR (3.49, 3.57, 3.90) values and increase value of FCE (28.6, 27.95, 25.03) values observed with T3, T4 and T5 that means treatment group supplemented diets at dose 0.25g,0.5g,0.75g prepared probiotic /Kg diet respectively improved feed utilization of common carp compared with T2 values by addition prepared probiotic at dose 0.05g/Kg diet, T1 commercial probiotic at dose 4g/Kg diets and control group.

Table (3): Growth parameters of *Cyprinus carpio* treatment with probiotic

Treatment	Body weight gain	Daily gain g/d	Specific growth rate	Relative gain rate %	Food conversion	Food conversion efficiency
control	10.56 $\pm0.0^{c}$	0.251 $\pm0.003^{d}$	0.39 $\pm0.004^{e}$	18.20 $\pm0.2^{e}$	7.22 $\pm0.07^{a}$	13.84 $\pm0.19^{e}$
T1	16.50 $\pm0.50^{c}$	0.39 $\pm0.004^{c}$	0.59 $\pm0.003^{c}$	28.52 $\pm0.1^{c}$	4.75 $\pm0.1^{b}$	21.01 $\pm0.03^{c}$
T2	12.17 $\pm0.17^{c}$	0.28 $\pm0.02^{d}$	0.45 $\pm0.006^{d}$	21.05 $\pm0.5^{d}$	6.34 $\pm0.34^{a}$	15.75 $\pm0.25^{d}$
T3	22.50 $\pm0.5^{a}$	0.53 $\pm0.01^{a}$	0.78 $\pm0.01^{a}$	38.92 $\pm0.04^{a}$	3.49 $\pm0.49^{c}$	28.6 $\pm0.20^{a}$
T4	22.38 $\pm0.38^{a}$	0.53 $\pm0.01^{a}$	0.77 $\pm0.004^{b}$	38.٦٥ $\pm0.03^{a}$	3.57 $\pm0.03^{c}$	27.95 $\pm0.05^{a}$
T5	20.25 ±O.25^{b}	0.48 $\pm0.005^{b}$	0.71 $\pm0.003^{b}$	35.03 $\pm0.03^{b}$	3.9 $\pm2.6^{d}$	25.03 $\pm0.03^{b}$
Significant level	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05

The different litter in the same column are significantly different at P ≤ 0.05 Control =diet(basal diet),T1=diet (4gm commercial pro/1Kg diet), T2= diet(0.05 g prepared probiotic /1Kg diet), T3=diet(0.25g prepared probiotic/1Kg diet), T4= diet(0.5g prepared probiotic/1Kg diet),T5= diet(0.75g prepared probiotic /1Kg diet.

At the end of present experimental period and before the challenge test all groups T1, T2, T3, T4 and T5 received probiotic supplemented diets revealed significant decrease in mortality rate when compared with control group.

Table (4): Survival rate of *cyprinus carpio* post treatment with probiotic during 42 days (mean ± SE).

Fish group	Total number	The number of dead fish	survival %	Mortalities %
control	18	5	72.2[e]	27.1[a]
T1	18	3	83.3[c]	16.6[c]
T2	18	4	77.7[d]	22.2[b]
T3	18	4	88.8[b]	11.1[d]
T4	18	3	94.4[a]	5.5[e]
T5	18	2	88.8[b]	11.1[d]

the different litter in the same column are significantly different at P ≤-0.05 Control =diet(basal diet),T1=diet (4gm commercial pro/1Kg diet), T2= diet(0.05 g local probiotic /1Kg diet), T3=diet(0.25g local probiotic/1Kg diet), T4= diet(0.5g local probiotic/1Kg diet),T5= diet(0.75g local probiotic /1Kg diet.

There were significant differences (P≤0.01) among various groups of fish concerning survival rate of the experimented fish being the best values in T4 which show94.4% Survival rate and then T3,T5 which seem 88.8% Survival rate also followed by T1,T2which have 83.3, 77.7 respectively all groups seemed significantly better than the control group which have 72.2% Survival rat these result are demonstrated in table (4).

Body weight gain and daily gain are important parameters to evaluate types of diet and protein value in it (Uten,1978) while growth was not induce just from protein product but also by increase fat content in the tissue Food conversion ratio parameters means the capability of any species of fish to converted diet to body weight gain with keep healthy (Hepher,1988) that was mean there were relationship between diet intake and weight gain. The probiotic attach in the intestine after transit through the stomach, the bacteria in probiotic are use a large number of carbohydrates for their growth and produce a range of relevant digestive enzymes (amylase, protease and lipase) that increase the digestibility of organic matter and protein, produce a higher growth, prevent the intestinal disorders and produce or/and stimulate a pre-digestion of secondary compounds present principal in plant protein sources (Al-faragi, 2000) .Used lactic acid bacteria and yeast in finfish as probiotic demonstrated beneficial effect on the growth performance, feed efficiency and digestibility of organic matter and protein (EL-Haroun *et. al.*, 2006), So Probiotics may stimulate appetite and improve nutrition by the production of vitamins detoxification of compounds in the diet and by break down of indigestible components (Lara-Flores *et. al.*, 2003).

In our study we agree with the study of (Irianto and Austin 2002) that the growth parameters effect by many factors the dose, strain origin of probiotic and the fish age play important role on growth parameters. Many studies that focus on applying different types of probiotic in order to modify the micro flora of the alimentary tract of fish , explicitly positive effects were obtained with juvenile stages in many farmed fish species, including common carp, (Al-Thalimy, 2010), European eels, *Silurus glanis* L. (Przyby *et. al.*, 2006), Atlantic cod, *Gadus morhua* L. (Bogut *et. al.*, 1998), Mozambique tilapia, *Oreochromis mossambicus* (Gildberg and Mikkelsen, 1997), Atlantic salmon, *Salmo salar* L. and rainbow trout, *Oncorchynhus mykiss* (walbaum) (Naik *et. al.*, 1999). The best growth parameters observed with prepared probiotic supplemented diets on T4, T3 followed by T5 we suggested that, the addition of probiotic improved feed utilization in practical terms, this means that local probiotic can decrease the amount of diet necessary for animal growth. Similar results have been reported by (EL-Haroun *et. al.*, 2006). The results of growth parameters indicated a positive acceptable effect of the used probiotic mainly lactic acid bacteria ,*Bacillus subtilus* and *Saccharomyces cerevisiae* (local probiotic).

The obtained results could be attributed to the effect of lactic acid bacteria on the nutrient value of grain on diet (Hepher,1988) they founded nutrient value of grain increased in present lacto fermentation by LAB and that fermentative acts on increase lysine amino acid in grain in addition to nutrient value to some of amino acid and vitamins so increase the level of riboflavin niacin, tryptophan, methionin also increase the vital level of nitrogen, phosphate and calcium this supported with study (Bagheri *et. al.*, 2008) found the same result including significant increase in Weight gain, Specific growth rate compared with control on *O. mykiss* when added Bacillus bacteria on experimented diet. The ability of *B. subtilus* to adhere to the intestinal mucosa of *Cyprinus carpio* producing a wide range of relevant digestive enzymes (amylase, lipase and protease) which have the ability to denature the indigestible components in the diets, the ability to detoxify the potentially harmful components of feed and the ability to produce a lot of essential vitamin B. complex members particularly Biotin and vitamin B_{12}, the matter of which resulted in high food utilization and an increase in digestibility of different diet components. These results supported those by (Kennedy *et. al.*, 1998) who used *B. subtilus* in the food of common snook, *Centropomus undecimalis* and found that these probiotic bacteria increased the food absorption by enhancing the protease level and consequently gave a better growth.

The incorporation of *S. cerevisiae* as a probiotic in fish diet was investigated by a lot of researchers in which similar results were obtained. (Abd El-Halim *et. al.*, 1989) found that the addition of living yeast in diet improve the performance of *Cyprinus carpio* also (Scholz *et. al.*, 1999) reported that *S. cerevisiae* improved the growth and survival of common carp, they attributed this action to adherence of *S. ceevisiae* cells to the gut and the secretion of amylase enzymes which shared in the increased digestibility of the diet. On the other hand, the increased growth performance of *Cyprinus carpio* treated with local probiotic containing living *S. cerevisiae* with *B. subtilus* and lactic acid bacteria could be also attributed to the inhibition of some intestinal bacterial flora and increasing the non-specific immunity of the treated *Cyprinus carpio* The adherence capacity of *S cerevisiae* , *B. subtilus and* Lactic acid bacteria to the intestinal mucosa inhibits the attachment of the other intestinal bacteria to these binding sites and so preventing the disease occurrence with its negative impact on the fish growth. These results supported the results of (Esteban *et. al.*, 2001) who reported that the cell wall constituents of *S. cerevisiae* play a significant role in stimulation of innate immune response and protect the fish against infection. Pillay and Kutty (2005) reported also that mannose rich proteins from yeast are belonging to the category of compounds which adhere to receptors used by pathogenic microbes and so prevent their colonization in the fish gut, he reported also that the yeast cells could also produce group of substances namely Glutamine, Glutamic acid, Keto glutaric acid which are known to be energy substrates for intestinal cells and which contribute to healthy gut, finally he reported about the peptides contents of yeast cells which regulate the digestive enzymes secretion in fishes.

The result from this parameter has been attributed to the present of (*Lactic acid bacteria* , *Bacillus subtilus* and *Saccharomyces cerevisiae*) that could be inhibited some harmful intestinal bacteria and increasing the non-specific immunity of the treated *Cyprinus carpio,* also the adherence capacity of probiotic micro-organism to the intestinal mucosa inhibits the attachment of the harmful intestinal bacteria to these binding sites and so preventing the disease occurrence with its negative impact on the fish growth. On the other hand the probiotic microorganism are capable of preventing the adhesion of micro-organisms due to factors other than the production of antimicrobial substance such as antibiotics or sidrophore or/and by competition for nutrients and spacebut by alteration of microbial metabolism or by stimulation of host immunity (EL-Haroun *et. al.*, 2006).

Also, Scholz *et. al.*, (1999) reported that the probiotic improved the growth and survival of sea bass fry, They attributed this action to adherence of *S. cerevisiae* cells to the gut and the secretion of amylase enzymes which shared in the increased digestibility of the diet.

Pillay and Kutty (2005) evaluated the nutritional quality of LAB and their possible effect on growth parameters and survival of juvenile *Penaeus indices* four commercially available cultures *L. acidophilus*, *S. cremoris*, *L. bulgaricus*-56 and *L. bulgaricus*-57 were orally administered they showed all the treatment groups recorded higher weight that were 26.76% ,86.8% ,39.0% and 48.37% respectively as compared to the weight gain of 21.32% recorded in control group of shrimp

(P<0.05). Specific growth rates that were 0.008, 0.023, 0.01 and 0.01 respectively compared to control that was 0.006 and survival rates were also better in all the treatment groups that were 95%, 98%, 92% and 95% respectively as compared to control that was 70%.

thus due to seed the gut with harmless bacteria which occupy the attachment sites and prevent infection by pathogenic bacteria by maintaining artificially these bacterial populations at high level by regular intake with feed. The addition of lactobacilli was found to induce an immune response and thereby reestablish the mucosal barrier (Rachel *et. al.*, 2011) again debating over viability it has been demonstrated that live cultures are more efficient at enhancing certain aspects of immune function than the killed culture (Johansson *et. al.,* 1993) the interaction between the bacteria and host defense can be direct and involve attachment of GI tract bacteria to receptor of host cells. Therefore the introduction of probiotic in fish feed should be seen an essential step to improve the sustainability of aquaculture.

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