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Effect of Probiotic and Synbiotic Food Supplementation on Growth Performance and Healthy Status of Grass Carp, *Ctenopharyngodon idella* (Valenciennes, 1844)

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Abstract: This study aimed to investigate the effect of probiotic (yeast *Saccharomyces cerevisiae, bacillus subtilis* and *bacillus cereus*) and synbiotic (Microban aqua®) on growth performance, nutrient utilization, biochemical composition, blood parameters, gut pathogens and stress response of the fingerlings of grass carp *Ctenopharyngodon idella*. Results indicated an enhancement in growth and feed utilization for all fish groups fed by probiotic followed by synbiotic compared with the control group. The biochemical analyses exhibited significant decrease in moisture contents in fish fed probiotic. The obvious increment in lipid contents was reported for in fish fed synbiotic. Serum total protein, albumin and globulin levels indicated positive effects when fish fed probiotic. Also, results exhibited decrease in serum cholesterol levels in the groups that treated with probiotic (*bacillus subtilis, bacillus cereus* and yeast). The tolerance to gut pathogens and stress test has been enhanced in all fish groups fed with probiotic followed by synbiotic compared to the control group. The present results indicated the effectiveness of food Supplementation with Probiotic and Synbiotic in fish diet with the preference of probiotic to improve the growth Performance and Healthy Status of fishes particularly Grass Carp, *Ctenopharyngodon idella*.

Keywords: Probiotic, Symbiotic, Grass Carp, Growth Performance, Blood Parameters, Gut Pathogens and Stress Test

1. Introduction

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance [1]. The use of probiotic products as feed supplements has attracted considerable attention by feed manufactures as means of improving livestock performance. Probiotics have been proven to play positive roles as feed additives in various aspects such as growth performance and disease prevention. Observations in fish show that probiotics can modulate immunologic responses and intestine microbial populations, strengthen the mucosal barrier and improve mucosal defenses of the gastrointestinal tract [2, 3, 4, 5]. Most probiotics used in aquaculture belong to the lactic acid bacteria (e.g. *Lactobacillus* and *Corn bacterium*), the genus Bacillus, the photosynthetic bacteria (e.g. *Rhodobacter sphaeroides*), the yeast, Pseudomonas or Vibrio, and other genera and/or species [6]. Bacillus is the most widely used as probiotic in aquaculture [7], mainly due to its higher resistance to harsh environmental conditions compared to other probiotics, e.g. Lactobacillus [8]. Based on data cited in [9, 10] the use of a feed containing *B. subtilis* for Indian major carp (*Labeo rohita*) showed higher survival and growth rates, as well as enhanced innate immune responses and stronger resistance to *Aeromonas hydrophila* infection. It has also been demonstrated that Bacillus additives positively affect

digestive enzyme activities [11, 12] and help reduce total viable bacterial counts and Vibrio populations [5]. Yeasts are important additives in fish diet, have been evaluated as probiotic properties [13]. From the other hand, the combination of a prebiotic with other probiotic is described as Synbiotic, the addition of an appropriate prebiotic may improve survival and establishment of a probiotic organism by providing a readily available nutritional source that might not be used by competing organisms [14]. Microban aqua® was considered Synbiotic (active enzymes and microorganisms). Grass carp (*Ctenopharyngodon idella*) is one of the main products of freshwater aquaculture in the world; however, it is highly vulnerable to pathogenic infections, which always lead to significant economic losses [2]. Although of great interest and importance of such species, little information have been reported on the utilization of probiotics and synbiotic in grass carp culture. This study was to investigate the effect of the selected probiotics and synbiotic in the culture of grass carp, considering its effects on growth performance, feed and nutrients utilization, carcass composition, blood hematological parameters, intestinal pathogens and stress response.

2. Materials and Methods

2.1. Fish and Experimental Management Design

One Thousand Grass carp (*Ctenopharyngodon idella*) fingerlings were obtained from Nursery earthen pond El-khashaa Farm, Kafr-elshikh governorate. A feeding experiment was conducted in the Fish Nutrition Laboratory, (NIOF), Baltim Research Station Egypt. After acclimation in concrete tank (5×10×1 m) for two weeks, fish specimens were divided into five triplicated groups according to food supplementation of 30 fish per replication, with an average weight of 3.3± 0.4 g/fish. The specimens were stocked randomly in 15circular fiberglass (2 tonnes) with continuous aeration. The fiberglass tank was daily cleaned before the first feeding and excreta were siphoned and were supplied with running fresh water. Water quality parameters were measured weekly included temperature (via a thermometer), PH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter). Ambient water temperature, dissolved oxygen and pH through the experimental period were 20.0± 1.0°C, 7.2 ±1.0 and 7.0 ±0.2 mg/l, respectively. Fish were fed twice daily, at 9:00 and 14:00 hours. Daily feeding rate was about 5% of total body weight and properly regulated according to the actual intake. During the study period, the total amount of feeds consumed by the fish in each fiberglass tank was determined and the feed consumed for each individual fish was calculated accordingly.

2.2. Dietary Treatments

For probiotic and symbiotic, *Bacillus subtilis* and *bacillus cereus* were obtained from Microbiology Laboratory, Marine Environmental Division, National Institute of Oceanography and Fisheries, Alexandria, Egypt. A pure culture of two both bacillus species was inoculated into a conical flask (500 ml) containing nutrient broth and incubated at 30°C for 24 h in a shaker incubator. The culture was concentrated by centrifugation at 3000 g and rinsed three times with sterile water. The suspension was quantified by the spread plate technique (nutrient agar, incubated at 30°C for 24 h). The purified and quantified bacteria were kept at 4°C and used for feed preparation as required. Five experimental diets were formulated; the first one is the control group of diet without additives (26% C). The other experimental diets consisted of three types of probiotic (Y: 2g/kg yeast *Saccharomyces cerevisiae*, BS: 1×10^{9} *Bacillus subtilis* CFU/g and BS: 1×10^{9} CFU/g *bacillus cereus*). The last diet was added to 2g/kg Microban aqua (Mc). The diets formulation and chemical composition are shown in Table (1). All the dietary ingredients and additives were purchased from the markets in Egypt. All ingredients and additives were milled and mixed, then pressed by manufacturing machine.

Table 1. *The compositions of the experimental diets.*

Ingredients	Experimental diets composition/kg.
fish meal	50
Corn gluten	70
Soybean meal	200
Yellow corn	100
Wheat brane	150
Wheat flour	100
Rice brane	280
Soy oil	20
Premix[1]	30
	1000
Dry matter (DM)	93.5
Crud protein (CP)	26.30
Ether extract	7.3
Crude fibre	6.87
Ash	8.5
Nitrogen free extract (NFE)[2]	51.03
Gross energy (MJ/KG DM)[3]	17.87

[1]Premix (mg /kg); p-amino benzoic acid (9.48); D-Biotin (0.38); Inositol (379.20); Niacin (37.92); Ca-pantothenate (56.88); Pyridoxine-HCl (11.38); Riboflavin (7.58); Thiamine-HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); Folic acid (0.76); Cyanocobalamin (0.08); Menadione (3.80), Vitamin A-palmitate (17.85); a-tocopherol (18.96); Calciferol (1.14). K_2PO_4 (2.011); $Ca_3(PO_4)_2$ (2.736); Mg SO_4 $7H_2O$ (3.058); NaH_2PO_4 $2H_2O$ (0.795)
[2]Nitrogen-free extract (NFE) = 100 - [% Ash +% lipid +% protein +% Fiber].
[3]GE (kJ/g) = (protein content × 23.6) + (Lipid content × 39.5) + carbohydrate content × 17.2).

2.3. Experimental Procedures

2.3.1. Proximate Analyses

Five fish specimens were netted from each tank at the end of the feeding trial. Then, they were pooled and homogenized for proximate composition (total of 15 fish per treatment). Moisture, total protein, lipid and ash contents were all determined by Standard Association of Official Analytical Chemist [15] methodology. Triplicates of diet samples were used for proximate analyses (Table 1).

2.3.2. Serum Constituents

Blood samples were collected, transferred to centrifuge

tubes and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 (rpm) for 5 minutes, and stored at -20°C until its use in the followings; Serum total protein (g/dl), albumin (g/dl) and cholesterol (mg/dl) were determined colorimetrically using Kits supplied by El- Nasr Pharmaceutical Chemicals Co. (Egypt). Serum globulin (g/dl) levels were obtained by differences between total protein (g/dl) and albumin (g/dl). Serum amylase (mg/dl) and ALT (alanine aminotransferase activity) were determined colorimeterically using commercial Kits of *Biodiagnostic* Co. (Egypt).

2.3.3. Evaluation of Growth Performance and Feed Utilization Efficiency

Growth performance and feed utilization including weight gain (WG, g), weight gain (%WG), specific growth rate (SGR,%/day), feed conversion ratio (FCR) and protein efficiency ratio (PER) were determined as follows:

$$WG = FW - IW \text{ (g / fish)}$$

$$\%WG = 100 \times [(\text{final fish weight (g)} - \text{initial fish weight (g)}) / \text{initial fish weight}]$$

$$SGR = 100 \times [(\ln \text{final fish weight}) - (\ln \text{initial fish weight})] / \text{experimental days}$$

$$FCR = \text{feed fed (g) (dry weight)/weight gain (g)}$$

$$PER = \text{weight gain (g) / protein fed (g)}$$

2.3.4. Intestinal Pathogens

Preparation of samples: fish gut were removed from freshly specimens and putted in saline solution homogenate vigorously. These methods are well referenced and represent a good minimum standard for food, water and environmental microbiology [16]. Total bacterial counts (TBC) were calculated as follows; 1 ml of each sample were spread using the nutrient agar plate media and incubate at 30°C for 18-24h. The counts were calculated as CFU /100 ml. For differential microbial pathogens: 1 ml of each sample was filtered using 0.45µm membrane filters then separately cultured on four different selective media as follows: m-FC medium: for total coliform, TCBS agar medium: for Vibrio sp., mannitol agar medium: for Staphylococcus sp., Aeromonas isolation medium for *Aeromonas hydrophila*. All plates were incubated at 35°C for 18-24h except the m-FC plates they incubated at 44.5°C for 18-24h. The count was calculated as CFU /100ml. [17, 18].

2.3.5. Stress Test

After 68 days of feeding, 30 fishes were collected from each treatment and observed in 5 aquariums of salt water 30‰ for salinity stress test with continuous aeration. The fish mortality was recorded as death fish/mint in each treatment.

2.3.6. Statistical Analysis

The collected data were subjected to statistical analysis using general linear models procedure as cited in [19] for users guide, with a one-way ANOVA. Means were statistically compared for the significance ($p \leq 0.05$) using multiple range test [20].

3. Results and Discussion

The survival rate of Grass carp *Ctenopharyngodon idella* in all feed treatments was 100% after 68 days culture. The growth and feed utilization indices are illustrated in Table (2), the average initial weights are ranged from 3.354±.08 for BS group to 3.45±.08g for the control group with insignificant differences ($p<0.05$) among the experimental groups. The mean final weight of the control (C) was lower ($P < 0.05$) than those of the rest four trials indicating the positive growth for groups that treated with probiotic and synbiotic additives; SGR in all trials was higher than that in the control (C) fish ($P < 0.05$). Addition of *B. subtilis* (BS) to the diets also decreased FCR ($P < 0.05$) where the group diet BS showed lowest value of FCR (1. 7±.5). At terminal of the experiment, the average percentage weight gain (% wt. gain) was ranged between 89±4.5 and 152±9 for control and *B. subtilis* (BS) respectively. The highest weight gain was recorded in treatment with *B. subtilis* (152±9%), followed by samples treated with yeast (143±24) and Mc (134±15%) in comparing with the control group which reported the lowest weight gain 89±4.5%.

Table 2. *Growth and feed utilization indices of fish at the end of feeding trial for 68 days.*

Indices	Treatments				
	C	Y	Mc	BC	BS
Initial w.t	3.45±0.08	3.40±0.08	3.37±0.08	3.43±0.08	3.35±.08
Final w.t	6.5±0.17 ^{b}	8.3±0.94 ^{ab}	7.9±0.48 ^{ab}	7.7±0.53 ^{ab}	8.5±0.30 ^{a}
weight gain	3.1±0.14 ^{b}	4.9±0.90 ^{ab}	4.5±0.49 ^{ab}	4.2±0.54 ^{ab}	5.1±0.30 ^{a}
%weight gain	89±4.5 ^{b}	143±24^{a}	134± 15 ^{ab}	124±17 ^{ab}	152±9 ^{a}
SGR	1.03±0.03^{b}	1.37±0.16^{ab}	1.33±0.88 ^{ab}	1.23±0.13 ^{ab}	1.43±0.06^{a}
FCR	2.1±0.5 ^{a}	1.8±1.3 ^{b}	1.8±0.8 ^{b}	1.8±.3 ^{b}	1. 7±.5 ^{b}
PER	1.67±0.9 ^{b}	2.37±0. 2 ^{a}	2.23±0.1 ^{ab}	2.1±0.1^{ab}	2.27±0.06 ^{ab}
C. F	1.1±0.27 ^{b}	1.4±0.73 ^{a}	1.2±0.61 ^{b}	1.4±0.38 ^{a}	1.5±0.23 ^{a}

Different letters within the same row indicate significant differences ($P< 0.05$). (C: control with no additives; Y: diet with yeast; BS: diet with *B. subtilis*; BC: diet with *bacillus cereus*; Mc: diet with Microban aqua; SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio.

The results also revealed that, the highest ($P<0.05$) final weights and specific growth rate were recorded in *B. subtilis* followed by *B. cereus* (BC) fish groups. The value of PER of fish fed yeast diet indicated significant improvement in protein utilization comparing with control tested group. The similar increase in growth of different kinds of fish resulted

from the adding probiotics to diet were reported by Wu *et al*. [2], they concluded that incorporation of *B. subtilis* Ch9 as a probiotic supplement in diets gave better growth performances and feed utilization than that in the basal diets. Moreover, the same trend of results were cited in [9] for Indian carp and in [21] for common carp. According to El-Haroun *et al.* [22] the addition of the commercial probiotics indicated the noticeable effect on tilapia growth performance and nutrient utilization. These effects have been also demonstrated on shrimp [12]. The fish fed diets contain 10% of *B. cereus* bacteriocin diet has significantly enhanced feeding and growth rate as compared to fish fed control diet, the FCR in control was higher than the other compared treated groups. Similarly Live yeast *Debaryomyces hansenii* enhanced the growth performance of sea bass *Dicentrarchus labrax* larvae [24]. Chiu and Liu [25] suggested that the best growth rate, food consumption, and food conversion were in Nile tilapia fed a combination of three probiotic bacteria. The biomaterial Lycogen™ increased muscle weight, weight gain, the specific growth rate (SGR) and the feed conversion ratio (FCR) of seawater red tilapia (*O. mossambicus* × *O. niloticus*) [26].

Averages of whole body composition including moisture, crude protein (CP), ether extract (EE) and ash contents due to the dietary treatments effect from start to end of the experimental period are presented in Table (3). Results reveled that CP and EE contents in fish whole bodies, at the end of the experimental period, were significantly (P<0.05) higher in the treated groups compared with the corresponding values at the beginning experimental meanwhile, moisture and ash contents were significantly decrease in tested groups comparing with starter group. The highest values of total protein content were recorded in BS and C groups (63.9 and 63.2, respectively). Cp contents were significantly decreased in treated group (Mc) comparing with all other groups. The increment in lipid contents was pronounced as the level of MC diets treatments. These results are in agreement with the findings of [22] who evaluated the effect of different dietary probiotic levels on chemical proximate analysis of whole carcass, no statistical differences were observed in carcass moisture, ash and protein content among the different treatments. Differences were observed in carcass lipid and gross energy content, with the highest value recorded in fish fed a control diet, while the lowest overall lipid and gross energy content also. According to Abdel-Tawwab *et al*. [27], the yeast supplementation has improved the protein of the whole fish body composition giving higher values than that in control; the same authors illustrated that there was no significant difference in lipid content in fish body observed when fed 0.0–1.0 g yeast/kg, whereas fish fed 2.0–5.0 g yeast/kg diet had the lowest lipid contents. Ash content increased significantly with the increase of dietary yeast giving the highest ash content in fish fed 5.0 g yeast/kg, while the lowest value was obtained in fish fed the control diet; the ether extract (EE) in whole fish decreased significantly ($P \leq 0.05$) as inclusion levels of fish fed 2.0–5.0 g yeast/kg diet increased. These findings also were in agreement with the present trend of results.

Table 3. *Biochemical composition of fish at end of feeding trial (dry matter weight basis).*

Composition	Treatments					
	Initial	C	Y	Mc	BC	BS
Moisture	78.6±0.3	754±.23 ^{ab}	74.3±.26 ^{b}	76. 3±.52^{a}	74.7±.67 ^{b}	74.1±0.35 ^{b}
CP	50.2±0.8	63.2±0.23 ^{a}	61.4±0.3 ^{ab}	57.8±1.6 ^{c}	60.3±0.55^{b}	63.9±0.26 ^{a}
E. E.	10.3±0.45	24.1±0.03^{c}	27.0±0.78^{b}	31.7±1.7^{a}	28.9±0.57^{ab}	23.4±0.24 ^{c}
Ash	17.3±0.05	11.5±0.18 ^{a}	11.1±0.4 ^{ab}	11.6±0.4 ^{a}	10.3±0.36 ^{b}	11.7±0.67 ^{a}

Different letters within the same row indicate significant differences (P< 0.05). (Cp: crude protein, EE: ether extract)

Results represented in Table (4) showed that serum total protein values in fish fed probiotic diets were enhancement comparing with other fish groups. Albumin values showed no significant variations among treatments except for BS group where significant depletion was recorded (2.3 g/dl). Additionally, an obvious increase in globulin concentration was observed for treatments Y, BS and BC giving 0.7± 0.03, 0.7±0.05 and 0.7±0.03 respectively with a significant increase for probiotic than other treatments. These results suggest an improvement of fish health in case of administrating probiotic supplement feed diets. The present findings confirm those reported by Khattab *et al*. [28], they revealed that blood hematological parameters (hemoglobin, erythrocytes count) in fish fed diets containing (commercial probiotic Biogen® consists on *Bacillus licheliformes* and *Bacillus subtilis*) were significantly higher compared to the control group. Marzouk *et al*. [29] found a positive effect of probiotics represented by a significant increase in RBCs count and Hb concentration in both fish groups fed with probiotics supplemented diets yeast and both live *B. subtilis* and *Saccharomyces cerevisiae*), compared to the control group, fed with probiotic free diet.

The results presented in table (4) also show decrease in cholesterol level from control treatment which recorded highest value (176±4) towards fish fed probiotic diets treatments where the lowest value was recorded for BS (107±3). For blood urea, a significant increase was observed in treatments of bacteria (BS and BC). ALT analysis showed that, the effects of probiotic or synbiotic as supplementation in fish diet gave values lower than the control group which reported the highest value (152±2.3), this indicates no effect of probiotic addition to fish diet on ALT. Similarly, as cited in [2] the amylase activity of the foregut increased significantly from days 14 to 56, when fish were fed diets containing *B. subtilis* Ch9. In all experimental groups amylase activity of the mid gut and hindgut increased significantly during the period from days14 to 56 and the

highest activity was observed in high doses. Amylase activity in the hepatopancreas was significantly higher from days 14 to 42. Wang [30] reported that, the amylase activity in intestine of grass carps fed with probiotics was higher than those fed with basal diet. The probiotics except *L. acidophilus* also improved significantly the amylase activity in the hind intestine compared with the control. As for probiotics treated groups, there was no significant difference in amylase activity of fore intestine compared to that of hind intestine, even with the presence of a tendency for increased activity. However, a significant difference ($P < 0.05$) between amylase activity of fore intestine (8.54 ± 0.52 Ug)1) and hind intestine (10.76 ± 0.75 Ug)1) was detected in individuals fed with the basal diet.

Table 4. *Serum constituent of fish blood at end of feeding trial.*

Parameters	Treatments				
	C	Y	Mc	BC	BS
Total protein	2.2±0.1[b]	2.4±0.0[b]	2.2±0.03[b]	2.4±0.0[b]	3±0.11[a]
Albumin	1.6±0.1[b]	1.7±0.0[b]	1.6±.05[b]	1.7±0.0[b]	2.3±0.2[a]
Globulin	0.6±0.03[b]	0.7± 0.03[a]	0.6±0.03[b]	0.7±0.05[a]	0.7±0.03[a]
A/g	2.1±.2[bc]	2.9±0.1[ab]	1.9±.14 [c]	3.4±0.4[a]	3.1±0.3[a]
Urea	18.7±0.[c]	19.0±0.6[c]	18.3±.9 [c]	21.3±0.[b]	24.0±.6[a]
Cholesterol	176±4[a]	148±2.6 [c]	161±4[b]	122±2.[d]	107± 3[f]
ALT	152± 2.3[a]	68±2.6[d]	113± 2.6[b]	55±2.0[f]	93±2.3[c]
Amylase	154±5.6[d]	523±5.2[a]	463±16[b]	533±6 [a]	252±3[c]

Different letters within the same row indicate significant differences ($P< 0.05$).

A healthy digestive system is fundamental for ideal animal growth. Determining alterations that may occur in the intestine is crucial to guarantee the nutritional efficiency of the diet as well as animal health. For this reason, the detection of pathogenic bacteria count related to gut of fish fed experimental diets was studied and the results are shown in table 5. From this table, the total bacterial count in the gut of fish groups fed probiotic and synbiotic in its diet had gave a decreasing trend compared to the control group. For specific pathogens, the detected *Faecal coliform* showed sharp decrease in groups of Y (3) and BS (9) compared to control group (48), moreover the *Staphylococcus spp* showed obvious declining in all groups containing probiotic and synbiotic treatments, the same trend was observed for detected *Vibrio spp* in all treated groups except the group BC which was higher (28) than control group (19). Meanwhile the *Aeromonas hydrophila*, were absent in all treatments. These results indicated that using of probiotics is useful as fishmeal additives and may be used to check their effectiveness and antimicrobial potency against the fish pathogens in accordance with Pannu1 [31] who stated that the probiotics (with single and multiple strains of non-pathogenic bacteria), plant extracts, different oils, and more potent the bacteriophage therapy can be used to control fish pathogens. However, further *in vitro* as well as *in vivo* studies need to be conducted to know more specifically about the effect and doses of these compounds that prove to be used in fish farming and management. Apart from probiotics the marine actinomycetes has been evaluated for antagnostic activity against fish bacterial pathogens *viz*. *Aeromonas hydrophila, A. sorbia* and *Edwardsiella tarda* [32]. The absence of expected *Aeromonas hydrophila* in all present experimental treatments, even the control group may suggest that, it has no ability to grow in the intestine of grass carp *Ctenopharyngodon idella* and may be found in another fish farm species. Recent research on probiotics has focused on responses to pathogenic agents such as *Aeromonas sp.* [34] or *Edwardsiella tarda* [35].

Table 5. *Pathogen count in intestinal microflora of feeding trial.*

Treatment	Total count/ml	Pathogen counting (CFU/ml)			
		Faecal coliform	Aeromonas hydrophila	Vibrio spp.	Staphylococcus spp.
C	640	48	0	19	23
Y	316	3	0	0	1
Mc	264	32	0	0	0
BS	256	9	0	7	0
BC	396	46	0	28	5

The stress resistance is the key to improve the health and the quality of the end products, since it would diminish the need for therapeutic agents. Probiotic bacterial dietary supplements have been widely studied for their ability to enhance the quality of life of aquaculture animals.

Fish in aquaculture systems are very often under chronic stress; consequently, the determination of potential protective benefits of probiotics for animals living in stressful conditions would be a major breakthrough. Rollo *et al*. [36] reported increased fry resistance to pH stress in Sea bream *Sparus aurata* fed a diet supplemented with *Lactobacillus fructivorans* and *L. plantarum*. Taoka, *et al*. [37] measured a higher tolerance to heat shock stress in Japanese flounder *Paralichthys olivaceus* fed a commercial probiotic. Recently, Hernandez [35] demonstrated that a commercial probiotic containing *Lactobacillus casei*

improved air dive stress resistance in juvenile porthole livebearer *Poecilopsis gracilis*.

For salinity stress resistance at 30‰, the mortality rates were reported in table (6). From this table, the percentages of dead fish populations were categorized in five groups, the lowest dead group (0-20%) of the total collected fishes in each groups showed the lowest death time for group C (after 2-23 min.) while the higher death time was reported for group BS (after 58-64 min). For the highest dead group (80-100%), the same trend was observed where the lowest death time was recorded for group C (2 after 8-30 min.) while the higher death time was reported for group BS (after 95-112 min.). Generally, the present results gave the elevation trend in death time for fish population from the control group towards groups containing probiotics for all percentages of dead fishes from 0-20% to 80-100%. This indicates, the probiotic supplementation in fish diet particularly grass carp is more effective in stress resistance especially to salt water, and this may useful as biological control for aquaculture animals. This agreed with that cited in [33, 8], they stated the most common probiotics proposed as biological control agents in aquaculture are lactic acid bacteria (*Lactobacillus and Carnobacterium*) or members of the genera *Bacillus* and *Pseudomonas*, among others, where the present study has used *Bacillus spp*. as probiotics.

Table 6. *Stress test for the effect of probiotics in treatments of Grass carp tolerance to salt water at 30 ppt. (Dead fish /mint for each treatment).*

Treatment	Dead population% in min.				
	0-20%	20-40%	40-60%	60-80%	80-100%
C	2-23	23-24	24-26	26-28	28-30
Y	8-23	23-30	30-34	34-37	37-41
Mc	28-38	38-57	57-75	75-80	80-85
BC	8-48	48-65	65-70	70-76	76-80
BS	58-64	64-73	73-80	80-95	95-112

4. Conclusion

In conclusion, the addition of probiotic in grass carp diets is more effective in growth performance than synbiotic and to improve feed utilization, fish integrity, health status, pathogens & stress resistance with the increase in economic efficiency. More studies are recommended to investigate the long run effects on fish performance and maximization the use of probiotics.

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