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# Effect of probiotics on the growth performance and survival rate of the grooved carpet shell clam seeds, *Ruditapes decussatus*, (Linnaeus, 1758) from the Suez Canal

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**ABSTRACT**

The carpet shell clam, *Ruditapes decussatus* is one of the most popular and commercially important bivalves in Egypt. This study aimed to evaluate the addition of probiotic to fresh algae, yeast and bacteria on growth performance and survival rates of the studied species. The experiment was performed in the Mariculture laboratory, at the Department of Marine Science, Suez Canal University, Ismailia, for 126 days from February 17$^{th}$ to June 21$^{th}$ 2018. The experiments were implanted in seven square fiber glass tanks with upwelling closed recirculating systems. Six diets were used in this experiment in addition to a control. The growth performances were determined by measurement of all biometric parameters including shell length, height, width, total weight, soft body weight and dry weight. The highest specific growth rate and growth gain of shell length, height, width and total weight were recorded in diet F (mixed algae + probiotic) while the highest SGR and GG of soft weight and dry weight were recorded in diet D (bacteria + probiotic).

## INTRODUCTION

The grooved carpet shell clam, *Ruditapes decussatus*, together with other clams present one of the main components of bivalve production and make up about 25 % of total mollusk production in the world **(FAO, 2010).** It is the most appreciated native clam species in Spain, France, Italy, Portugal, Tunisia and Egypt. According to **FAO (2016)**, the world production of *Ruditapes decussatus* were 6324.34 tons. The Egyptian production of *Ruditapes decussatus* ranged from 690 ton. to 4036 ton**. (Pinello *et al.*, 2020).**

Nutrition is one of the dominant factors influencing bivalve growth and survival, and has been extensively reviewed by **Marshall *et al.* (2010)**. Live microalgae are traditionally used as food for bivalves in mollusk hatcheries or bivalve culture **(Pernet *et al.*, 2003).** The mass production of microalgae is identified as a major cost in mollusca rearing in hatcheries and nurseries and grow-out. In fact, this practice requires special

expertise, manpower and devoted facilities; covering 20-50% of the overall seed production costs **(Coutteau and Sorgeloos, 1992; Borowitzka, 1999)**.

Knowledge of nutritional requirements is important to optimize the culture system. Nevertheless, feeding still forms the basic problems in shellfish culture **(Matias, 2013)**, while others believe that knowledge of the digestion, absorption and feeding mechanisms is the best way to understand these requirements **(Flye-Sainte-Marie *et al.*, 2007)**. In order to reduce the expenses for producing algae in hatcheries and seed rearing, many authors have tried to find alternative feeds. There would be many benefits in using substitute products in mollusca feeding: a guaranteed food supply, even during peak demand or in case of a loss of fresh algae cultures, standardized feed quality, better control of food bacteria load, a reduction in overhead costs, a reduction in space and labour demands **(Bonaldo *et al.*, 2005)**.

The development of bivalve aquaculture would be greatly enhanced by the total, or even partial, replacement of live algal food by a cheap, easily handled food that has the same nutritive qualities as live food **(Albentosa *et al.*, 1999)**. A suitable alternative diet for bivalves must meet the following requirements: stability within the culture system, particle size suitable for the filtration mechanism of these animals, digestibility, null toxicity and a biochemical composition that covers the nutritional needs of each species**.** In accordance with these criteria, experiments have been conducted with dry microalgae **(Curatolo *et al.*, 1993),** yeasts (**Nell, 1985)**, modified yeasts **(Coutteau and Sorgeloos, 1992)**, microcapsules of different kinds **(Laing, 1987)** or bacterial proteins **(Doulliet and Langdon, 1993; 1994)**. In spite of the large number of studies that have been carried out, no substitute has as yet been found for the totality of the live diet, although substitutions of 40–50% of the live diet have been described.

Most of the studies about probiotics in aquaculture are focused on fish and crustaceans cultures, with scarce literature considering their application in bivalve molluscs**. Lodeiros *et al.* (1989)** published a first work on the antibiotic effect of marine bacteria on the larval survival of scallop *Pecten ziczac***. Lilly and Stillwell (1965)** referred to probiotics as Microorganisms promoting the growth of other microorganisms". Probiotics as feed supplements benefit the host by improving the feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth promoting factors and increasing immune response **(Harikrishnan *et al.*, 2010)**. The current study aims to evaluate the addition of probiotic to fresh algae, yeast and bacteria on growth performance and survival rates of the studied clam.

## MATERIALS AND METHODS

### Preparation of experimental tanks

Seven square fiber glass tanks (large tanks) with dimension of 60 X 60 X 30 cm (length X width X height) and seven plastic tanks (small tanks) with dimension of 25 X 20 X 15 cm (length X width X height) were used in this experiment. In order to

implement the upwelling closed recirculating systems, a PVC tubes (0.5 inch) fixed in the center of the large tanks. The lower part of this tubes branched to six small plastic tubes. The upper part of the large tanks opened and fixed with PVC tubes (2 inch) as drainage tube. The tanks were disinfected with formaldehyde (38%), washed and then left to dry. The large and small tanks filled with 80 and 15 liter, respectively. We used peristaltic pumps to flow the water from small tanks to PVC tubes (0.5 inch) with flow rate 7 liters/ min. via plastic hoses. The excreted feces from seed removed from small tanks using siphonation each three day to minimize nitrogen wastes. Water salinity was maintained constant at 25 ‰. The tape water added to the small tanks to replace or compensate the raise of salinity due to evaporation. The seeds of *R. decussatus* were grown on wooden trays of 53 X 47 cm (length X width) with 2 mm plastic netting at the bottom. This tray divided into four equal parts (four replicates). The trays were placed in the large tanks.

**Experimental setup**

The experiment was performed in the Mariculture laboratory, at the Department of Marine Science, Suez Canal University, Ismailia, for 126 days from February 17$^{th}$ – June 21$^{th}$ 2019. The experiments were carried out (four replicates) for each tested diet. Specimens were purchased from clam collectors working at Lake Timsah in February 2019. Their shell lengths were measured by a digital vernier caliper with an accuracy of 0.01 mm and their weights were measured using Sartorius balance with an accuracy of 0.01 g. After one week of acclimatization, 40 specimens were stocked per large tank (10 individual / replicate).

**Diets**

The daily feeding rate was 2 % of seed dry weight. Six diets were tested; diet A (Baker's yeast), diet B (Baker's yeast + probiotic), diet C (bacteria), diet D (bacteria + probiotic), diet E (mixed algae) and diet F (mixed algae + probiotic). There is diet G (control) was cultured in natural seawater (free of any treatment or diet); the water in this tank changed each two days. We used commercial probiotic in this experiment (Sanolife PRO-W® - INVE Aquaculture powder). To use this product in diets B, D and F, 3 g of probiotic and 10 g of sugar were taken and added to 3 liters of sterilized sea water at temperature 28 °c for 24 h. Then, 1 liter from this mixture was added to each tank (diets B, D and F) per three days.

**Bioreactor**

The function of bioreactor was production of the bacteria that used in feeding of seeds of *Ruditapes decussatus* in diet C and D. The square large tanks with dimension of 60 X 60 X 30 cm (length X width X height) filled with 50 liters of stagnant or old sea water (30 ‰). The dechlorinated tape water added to the large tank to replace or compensate the raise of salinity due to evaporation. The tanks aerated using a blower (Model BOYU ACQ-009 Air compressors electromagnetic, China) to increase the dissolved oxygen. Sugar (as source of carbon) and ammonium chloride (as source of nitrogen) were added with ratio of 15:1, C: N ratio) to activate and stimulate the growth of bacteria. Water

temperature in large tank was kept constant at 29°C by means of an aquarium heater (Model MINJIANG HK-300, China).

**Algal culture**

*Tetraselmis suecica* and *Nannochloropsis* sp were cultured in 5 L and 20 L glass and plastic vessels in mariculture laboratory (at temperature 21°C) with continuous illumination by vertical "daylight" fluorescent lamps. Salinity was fixed at 30‰. The culture medium described by Walne (1966) was used. Culture medium was added constantly (1 mL per L of algal culture). Continuous aeration was provided to prevent the algae from settling. Microalgae were harvested during the initial stationary phase of growth. Before being used as food, algal cells were counted with counter cell to determine the amount of algae which used in daily feeding.

**Water quality parameters**

Water temperature in the experimental tanks was monitored daily between 1.00 pm to 3.00 pm. pH was monitored once a week. Temperature and pH were measured by means of multimeter (Model AD1030, pH/mv).

## Seed sampling

Samples were taken at six different times (day 0 — $T_0$; day 21— $T_1$; day 42— $T_2$; day 63— $T_3$; day 84 — $T_4$; day 105 — $T_5$ and day 126 — $T_6$) with an interval of three weeks. In each interval, shell length and total live weight were measured for each replicate while the height, width, dry weight and soft body weight were measured in the beginning and the end of the experiments. The total weight, dry weight and soft body weight measured by means of electronic balance (with an accuracy of 0.01g). Before weighing the seed, they were dried on absorbent paper for 10 min, to remove surface water. Dry weight was determined by drying the meat or soft body at 80 °C for 48 h.

**Growth performance analysis**

The growth of all individuals was estimated based on six biometric parameters: length (L), height (H), width (W), total live weight (TW), dry weight (DW) and soft body weight (SW). To evaluate the individual's total growth rate, the instantaneous (specific) growth rate (K) was calculated using the following equation (Malouf and Bricelj, 1989):

$K= (\ln W_2 - \ln W_1)*100 / (t_2-t_1)$ [1]

Where $W_2$ and $W_1$ are the values of the different variables H, W, SW, DW, TW and L. at the end and beginning of each experiment, respectively and *t* is the number of days.

Survival (%) was estimated as $(N_t / N_0) \times 100$, where $N_t$ is the number of live clams removed from the culture tank after t and $N_0$ is the number of clams at the beginning of the experiment.

Length gain (LG), height gain (HG), width gain (WG), the total live weight gain (TWG), soft weight gain (SWG) and dry weight gain (DWG) were calculated according to the following equations, respectively

LG = FL – IL [2] HG = FH – IH [3]

WG = FW – IW [4] TWG = FTW. – ITW [5]

SWG = FSW – ISW [6] DWG = FDW – IDW [7]

Where F and I represent the value of variables at the end and beginning of experiment, respectively.

**Statistical analysis**

Statistical analysis was performed using SPSS (Version 22). Differences in specific growth rate and growth gain of shell length, height, width, total live weight, dry weight, soft weight and survival rate among different diets were determined using a one-way ANOVA. If significant differences were present, Tukey's HSD test was employed to check for differences between means. Significant levels for all analysis were set at $p<0.05$.

## RESULTS

### 1. Temperature and pH

Water temperature ranged between 19.98 ± 1.42 ºC in March 2019 and 26.67±1.14 ºC in June 2019. While pH ranged between 7.65 and 8.23 through the experimental period with no significant differences between tanks.

### 2. Growth performance analysis

#### 2.1. Specific growth rate (SGR) and total growth gain

Differences in specific growth rate (SGR) and growth gain (GG) of shell length (SL), height (H), width (W), total weight (TW) soft body weight (SBW) and dry weight (DW) with different tested diets are shown in Figs. 1-12. The highest SGR and GG of SL, H, W and TW were recorded in Diet F while the highest SGR and GG of SW and DW were recorded in Diet D. The lowest values of SGR and GG of all biometric measurements were recorded in Diet A. The differences in SGR and total increment of all biometric measurements were highly significant ($p<0.001$).

The specific growth rate of TW for Diet F is significantly higher than the Diets A, B and G but there are no significant differences between Diet F and Diets C, D and E while The growth gain of TW for Diet F is significantly higher than tested diets except Diets D and E. However the specific growth rates and growth gain of SL, W and H for Diet F are significantly higher than the other diets except Diets D. The specific growth rates and growth gain of SW and DW for Diet A are significantly lower than the other diets. While the specific growth rates and growth gain of SW and DW for Diet D are not significantly higher than the other different tested diets except Diets A and G.

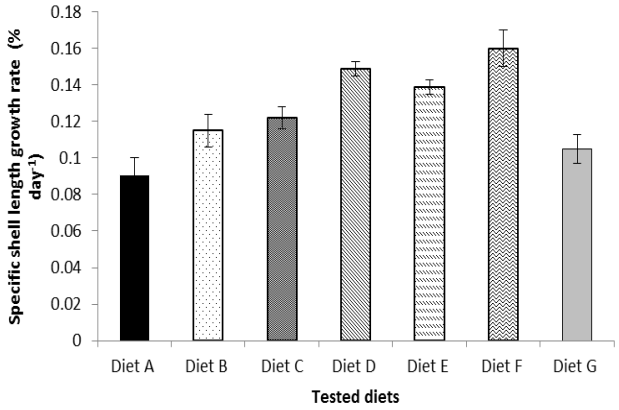


**Fig. 1:** Specific shell length growth rate of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast + probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

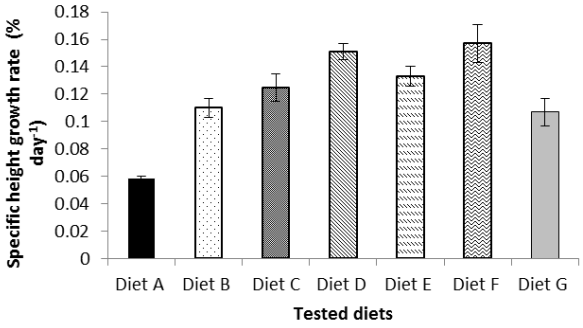


**Fig. 2:** Specific height growth rate of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast + probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

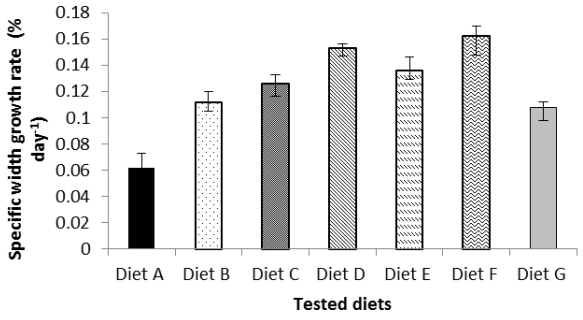


**Fig. 3:** Specific width growth rate of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast+ probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

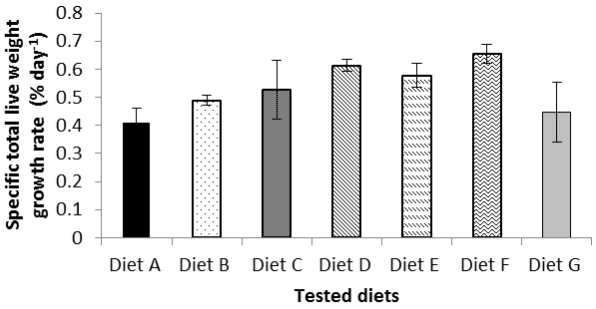


**Fig. 4:** Specific total live weight growth rate of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast + probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

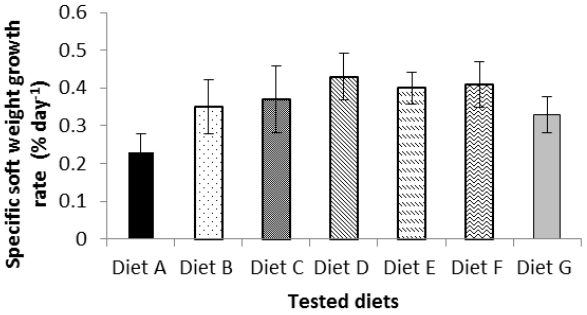


**Fig. 5:** Specific soft weight growth rate of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast+ probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

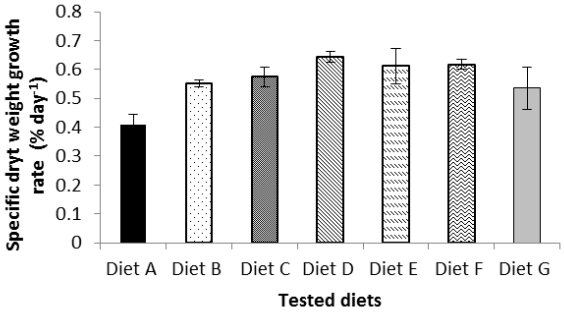


**Fig. ٦:** Specific dry weight growth rate of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast+ probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

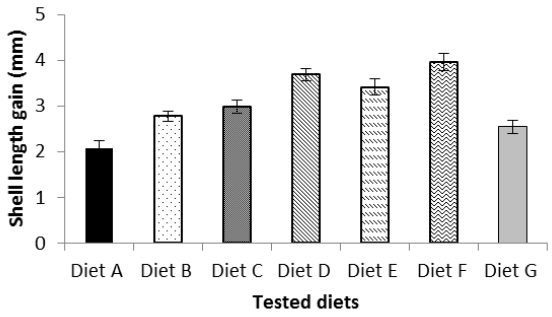


**Fig. 7:** Shell length gain (LG) of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast+ probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

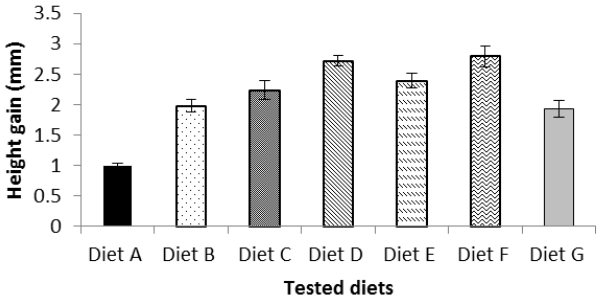


**Fig. 8:** Height gain (HG) of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast + probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

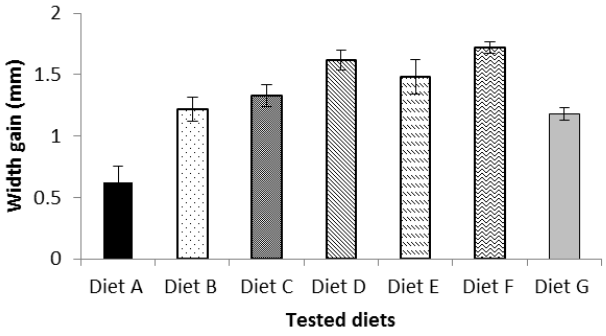


**Fig. 9:** Width gain (WG) of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast+ probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

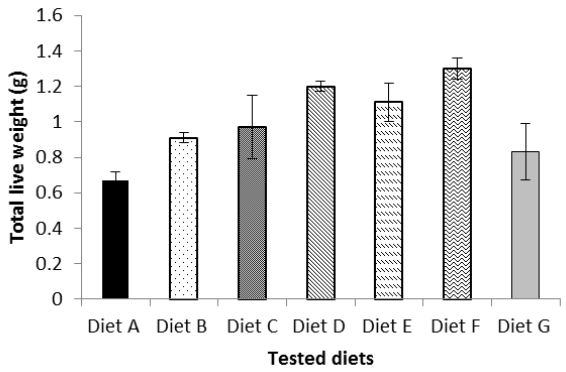


**Fig. 10:** Total live weight gain (TWG) of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast + probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

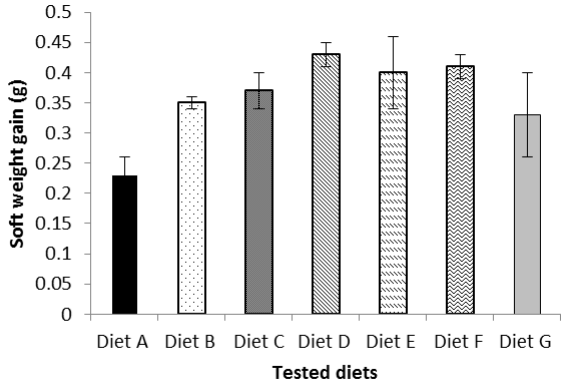


**Fig. 11:** Soft weight gain (SWG) of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast+ probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

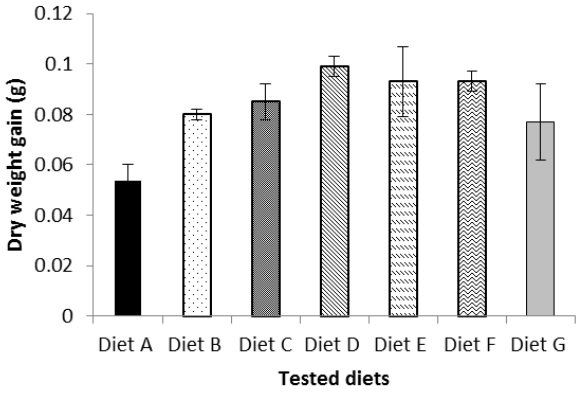


**Fig. 12:** Dry weight gain (DWG) of seeds reared in the different tested diets (Diet A=baker's yeast; Diet B= baker's yeast + probiotic; Diet C= Bacteria; Diet D= bacteria+ probiotic; diet E= mixed algae; Diet F= mixed algae+ probiotic and Diet G =control).

## 2.2. Cumulative growth and growth increment

The cumulative growth and growth increment with interval experimental periods ($T_0$ – $T_6$) for shell length and total live weight are illustrated in Figs. (13 -26) for seeds reared in the different tested diets. The figures clarify the following:

- the initial length of seeds reared in tested diets (Diet A, Diet B, Diet C, Diet D, Diet E, Diet F and Diet G) were 17.25, 17.68, 17.88, 17.80, 17.86, 17.74mm and 18.06, respectively, and reached to 19.34, 20.64, 20.86, 21.49, 21.27, 21.70 and 20.60 mm, respectively, at the end of experiment (126 days).

- The initial total live weight of seeds reared in tested diets (Diet A, Diet B, Diet C, Diet D, Diet E, Diet F and Diet G) were 0.99, 1.08, 1.03, 1.03, 1.04, 1.02 and 1.12 g, respectively, and reached to 1.67, 199, 2, 2.23, 2.15, 2.31 and 1.95 g, respectively, at the end of experiment (126 days).

The cumulative height, width, soft weight and dry weight illustrated in tables (1-4). Most of these results are more or less similar to those recorded with shell length and live weight.

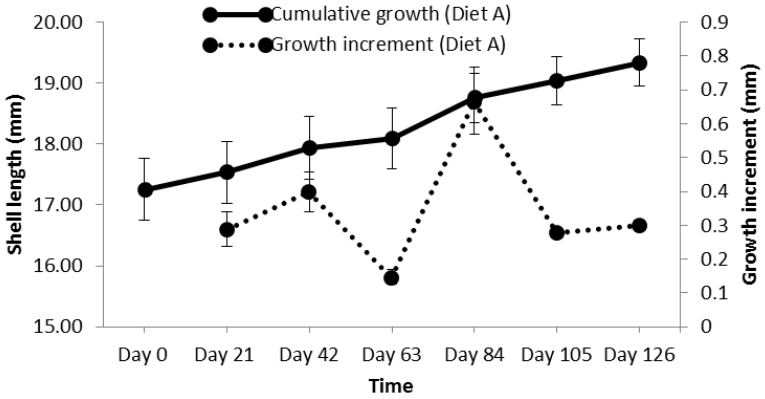


**Fig. 13:** The cumulative growth and growth increment of shell length for seeds reared in Diet A during experimental time intervals.

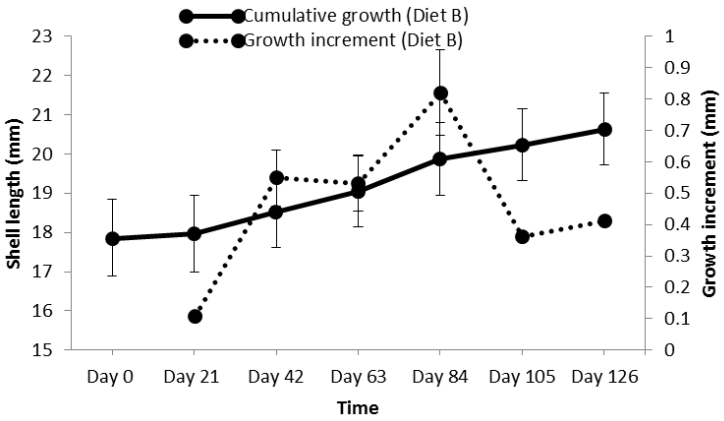


**Fig. 14:** The cumulative growth and growth increment of shell length for seeds reared in Diet B during experimental time intervals.

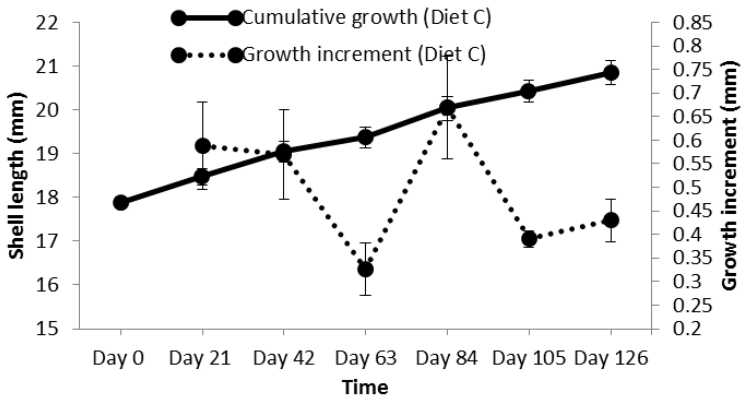


**Fig. 15:** The cumulative growth and growth increment of shell length for seeds reared in Diet C during experimental time intervals.

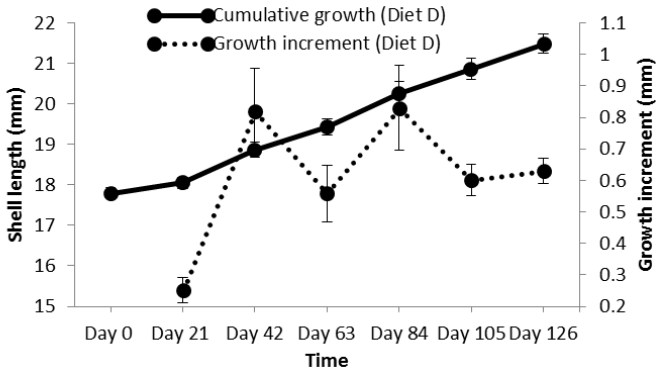


**Fig. 16:** The cumulative growth and growth increment of shell length for seeds reared in Diet D during experimental time intervals.

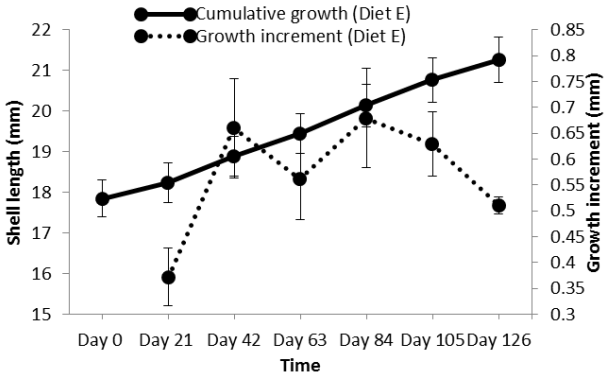


**Fig. 17:** The cumulative growth and growth increment of shell length for seeds reared in Diet E during experimental time intervals.

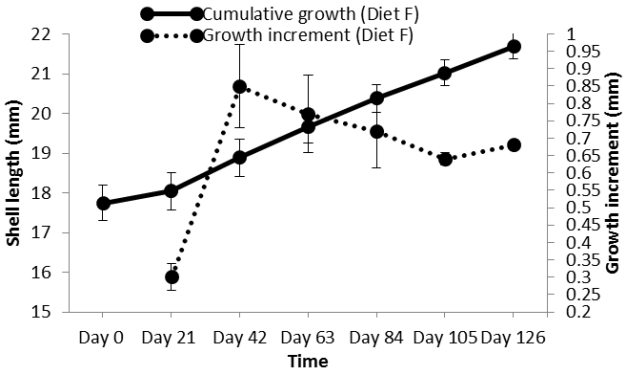


**Fig. 18:** The cumulative growth and growth increment of shell length for seeds reared in Diet F during experimental time intervals.

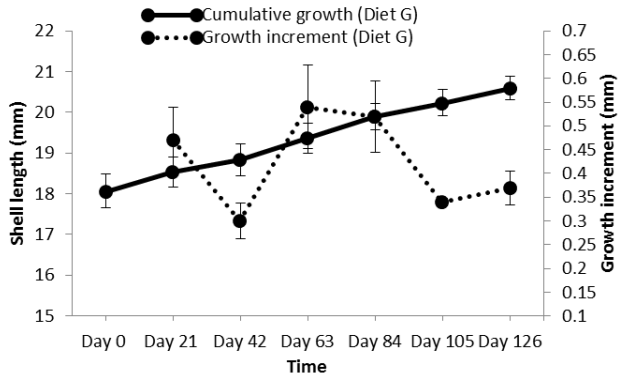


**Fig. 19:** The cumulative growth and growth increment of shell length for seeds reared in Diet G during experimental time intervals.

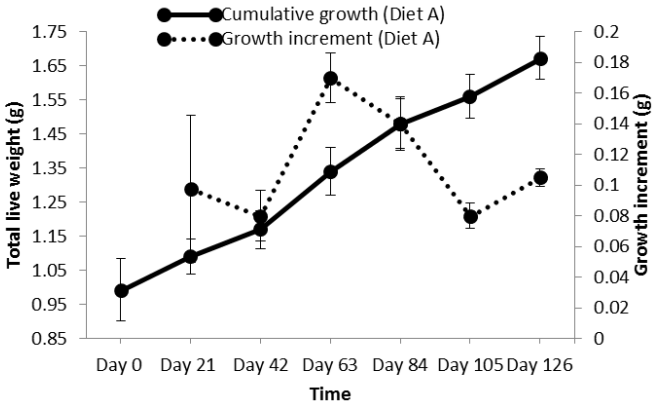


**Fig. 20:** The cumulative growth and growth increment of total live weight for seeds reared in Diet A during experimental time intervals.

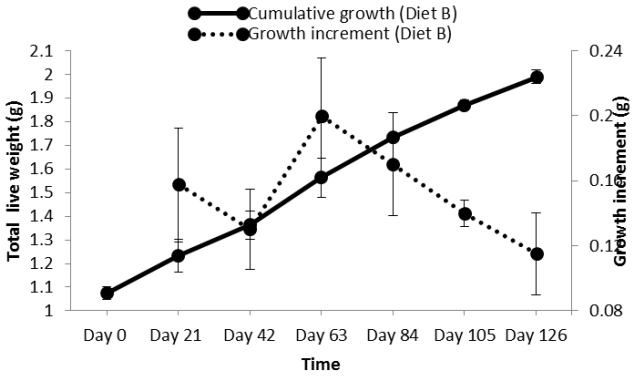


**Fig. 21:** The cumulative growth and growth increment of total live weight for seeds reared in Diet B during experimental time intervals.

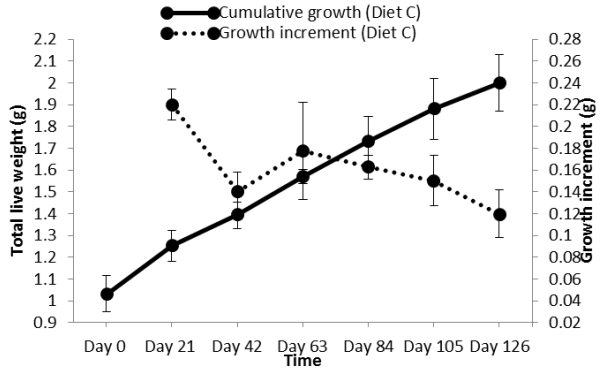


**Fig. 22:** The cumulative growth and growth increment of total live weight for seeds reared in Diet C during experimental time intervals.

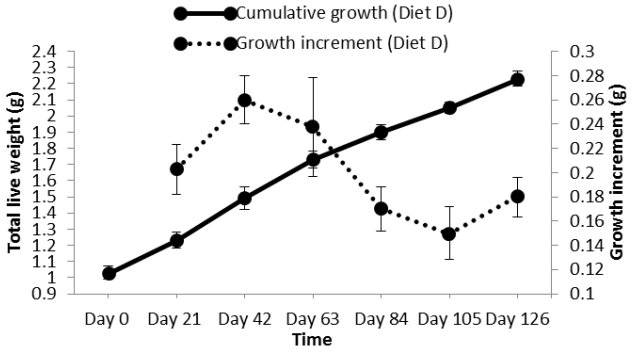


**Fig. 23:** The cumulative growth and growth increment of total live weight for seeds reared in Diet D during experimental time intervals.

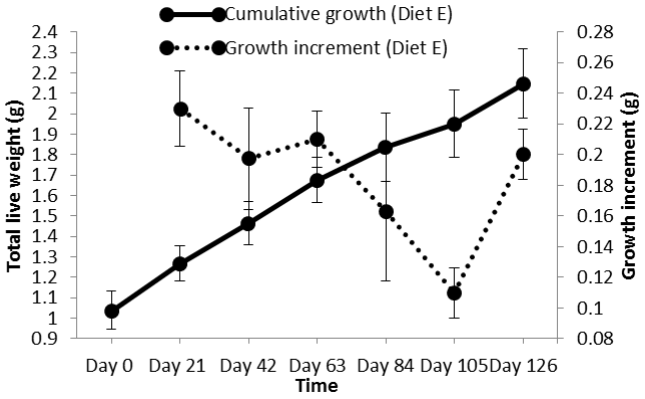


**Fig. 24:** The cumulative growth and growth increment of total live weight for seeds reared in Diet E during experimental time intervals.

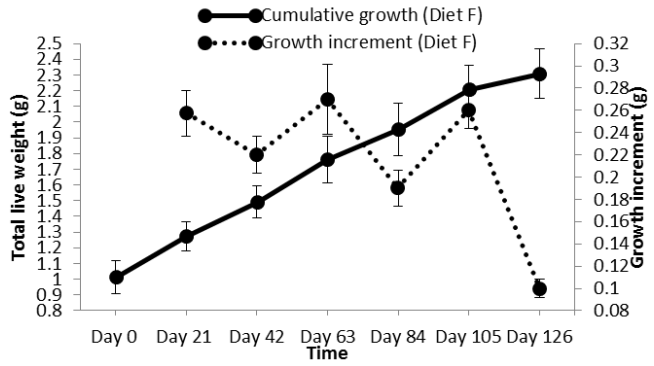


**Fig. 25:** The cumulative growth and growth increment of total live weight for seeds reared in Diet F during experimental time intervals.

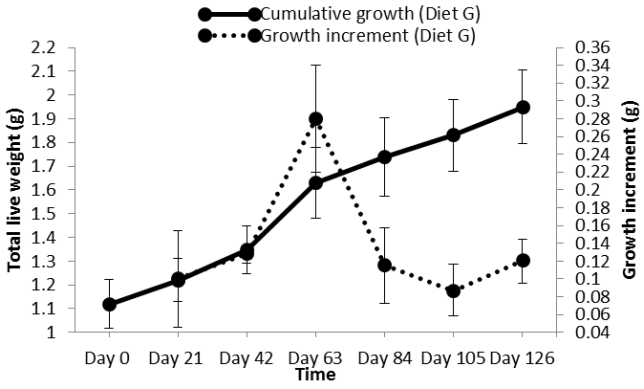


**Fig. 26:** The cumulative growth and growth increment of total live weight for seeds reared in Diet G during experimental time intervals.

**Table 1:** Means of initial and final height (mm) for seeds fed with different tested diets.

| | | |
|---|---|---|
| Diet A | Initial height (mm) | 13.05 ± 0.26 |
| | Final height (mm) | 14.06 ± 0.27 |
| Diet B | Initial height (mm) | 13.30 ± 0.28 |
| | Final height (mm) | 15.28± 0.22 |
| Diet C | Initial height (mm) | 13.16 ±0.20 |
| | Final height (mm) | 15.40± 0.07 |
| Diet D | Initial height (mm) | 12.99 ± 0.23 |
| | Final height (mm) | 15.71± 0.19 |
| Diet E | Initial height (mm) | 13.09 ± 0.36 |
| | Final height (mm) | 15.48± 0.30 |
| Diet F | Initial height (mm) | 12.84 ± 0.44 |
| | Final height (mm) | 15.63 ± 0.27 |
| Diet G | Initial height (mm) | 13.43 ±0.35 |
| | Final height (mm) | 15.36 ± 0.22 |

**Table 2:** Means of initial and final width for seeds fed with different tested diets.

| | | |
|---|---|---|
| Diet A | Initial width (mm) | 7.84± 0.18 |
| | Final width (mm) | 8.48± 0.17 |
| Diet B | Initial width (mm) | 8.01± 0.06 |
| | Final width (mm) | 9.23± 0.15 |
| Diet C | Initial width (mm) | 7.76± 0.09 |
| | Final width (mm) | 9.09± 0.18 |
| Diet D | Initial width (mm) | 7.62± 0.22 |
| | Final width (mm) | 9.24± 0.30 |
| Diet E | Initial width (mm) | 7.91± 0.29 |
| | Final width (mm) | 9.39± 0.38 |
| Diet F | Initial width (mm) | 7.59± 0.29 |
| | Final width (mm) | 9.31± 0.27 |
| Diet G | Initial width (mm) | 8.11± 0.27 |
| | Final width (mm) | 9.29± 0.29 |

**Table 3:** Means of initial and final soft weight for seeds fed with different tested diets.

| | | |
|---|---|---|
| Diet A | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.61± 0.03 |
| Diet B | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.72± 0.01 |
| Diet C | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.75± 0.03 |
| Diet D | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.81± 0.02 |
| Diet E | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.78± 0.06 |
| Diet F | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.79± 0.02 |
| Diet G | Initial soft weight (g) | 0.38± 0.03 |
| | Final soft weight (g) | 0.71± 0.07 |

**Table 4:** Means of initial and final dry weight for seeds fed with different tested diets.

| | | |
|---|---|---|
| Diet A | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.13± 0.01 |
| Diet B | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.16± 0.00 |
| Diet C | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.16± 0.01 |
| Diet D | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.18± 0.00 |
| Diet E | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.17± 0.01 |
| Diet F | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.17± 0.00 |
| Diet G | Initial dry weight (g) | 0.08± 0.01 |
| | Final dry weight (g) | 0.16± 0.01 |

**3. Survival rates:**

Survival rates of seeds reared in tested diets are shown in Fig. 27. The survival rates of seeds reared in tested diets ranged from 55to 92.5% with significant differences among tested diets ($P<0.05$). The differences between diets D, C, E and F were not significant, however, they showed significant higher survival rates than Diets A, B and G.

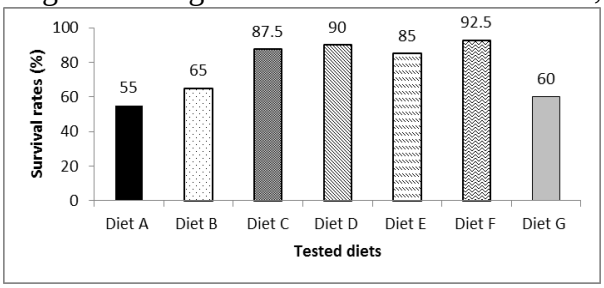


**Fig. 27:** Survival rate (%) for seeds reared in tested diets during experimental period.

## DISCUSSION

The specific growth rate (SGR) ranged from 0.091 - 0.16 % day$^{-1}$ for shell length and 0.412 - 0. 655 day$^{-1}$ for total weight at different tested diets. According to **Gabr and Gab-Alla (2008); Chessa *et al.* (2013); Matias (2013); Sami *et al.* (2016)**, their results were higher than the present study. The low results in the present study can be attributed to availability of foods. The shell length and weight gain ranged from 2.08 – 3.96 mm and 0.67 – 1.3 g, respectively for at different tested diets. The overall growth performance in the present study was higher than that of **Breber (1985)** (5 mm in 8 months), **Shpigel and Fridman (1990); Lake (1992)** (6-10 mm year $^{-1}$), **Serder *et al.* (2007); Dhraief *et al.* (2016)** (7-13 mm in 31 months). The high results in the present study can be attributed to seeding size and different diets and stocking density and temperature.

The difference found in seeds fed algae than other diets is not surprising as previous studies had shown that microalgal pastes and dried microalgae yield lower growth rates than fresh microalgal diets in bivalves (**Robert and Trintignac, 1997**). Prior studies had also shown that algae species differ among themselves in terms of nutritional value to bivalve filter feeders (**Chretiennot-Dinet *et al.*, 1986**). In the present study, the higher growth showed in seeds fed with Diet F (mixed algae + probiotic) but this increasing was not significant with seeds fed with Diet C (bacteria). These results were agreed with Enes and Borges (2003) and Pe´rez Camacho (2004). According to Espinosa and Allam (2006), the live cultured *Isochrysis galbana* generates higher growth rates in juvenile hard clams, *Mercenaria mercenaria*, than any tested commercial diet. This result agreed with the present study.

**Albentosa *et al.* (1999, 2002); Enes and Borges (2003); Perez Camacho *et al.* (2004); Willer and Aldridge (2019)** used cornstarch, cornmeal, wheat germ, wheat flour, corn flour cheese whey, single cell detritus and microcapsules as partially substitute to

live food. Their results demonstrated that the dried or non-live foods can only be used as partial substitutes for live phytoplankton in feeding of clams. However, the present study shows that bacteria can be used as total substitute for phytoplankton ~~in~~ for *R. decussatus*.

The uses of yeast, wheat flour, and corn flour have been reported as potential substitutes for microalgae in other bivalve species. **Albentosa *et al.* (2002)** found no significant difference between spat fed 100 % *I. galbana* and a mixed diet consisting of 50 % *I. galbana* and 50 % wheat germ flour, in the Manila clam (*Ruditapes philippinarum*). With respect to substituting yeast for microalgal diets, many studies have shown positive results for growth in *R. philippinarum* **(Coutteau and Sorgeloos, 1992).** However, growth of seed fed only on diet A decreased compared with the other different tested diets and this agree with **Epifanio (1979)** who recorded absence the growth in the soft tissue of *C. gigas* oysters with the increasing of yeast concentration.

**Pe´rez Camacho *et al.* (2004)** mentioned that the single cell detritus (SCD) prepared through the combined action of enzymes and bacteria (SCD), when used as the sole component of the diet, proved to be of moderate value as a food for juvenile specimens of *R. decussatus*. Nevertheless, the diet is worth considering because it is a non-phytoplankton food. Increases of 50% in LW and 68% in length were obtained for this SCD, expressed as a proportion of the increase given by a 2% diet of live phytoplankton, which is considered to be the diet that produces maximum increase rates **(Albentosa *et al.*, 1999)**. The results obtained in the present study were higher than their results. The differences in the results can be attributed to different density and concentration of bacteria and studied species.

During the past few years, the use of certain beneficial bacteria in-human and animal nutrition has received widespread attention, and there has been increasing interest in the application of probiotics in aquaculture. In recent years, numerous studies have been carried out about the use of probiotics in farmed aquatic animals to increase the growth rates and control potential pathogens **(Kim *et al.*, 2015; Zheng; Wang, 2016)**.

The current study was undertaken as part of the research for potentially probiotic which could enhance the growth performance of the bivalve. The growth parameters, such as survival, weight gain, shell length gain and specific growth rate were higher in diets supplemented probiotics (Diets B, D and F) compared with control and other different tested diets. These results agree with, **Munirasu *et al*. (2017); Jamal *et al*. (2019); Bahnasawy *et al*. (2020)**. They found that probiotic enhance and increase the growth in bivalves (Pacific oyster), shrimps and fishes.

**Silverman *et al*. (1996)** indicated that bacteria alone are not sufficient for growth of the mussels. For instance, the bacteria appear to be lacking some fatty acids essential to the mussel. However, they are not postulating utilization of bacteria as a sole nutrient source. The results obtained in the present study disagree with their results where the Diet C (bacteria) is comparable to Diet E. On the other hand, Diet D gives high growth rate compared to Diet E.

**Subhash and Lipton (2007)** investigated the effect of a probiotic bacterium, *Lactobacillus acidophilus*, on the growth and survival of pearl oyster, *Pinctada margaritifera*, spat. The probiotic bacteria were fed together with a microalgal feed at 1:1 or 2:1 while control groups received no probiotic supplementation. Weight and length also increased significantly. The weight gains in the probiotic groups were 349.8±0.44 mg (1:1 level) and 396.8±0.49 mg (2:1 level) mg, compared to 300.9±0.51 mg in the control. This result agreed with the present study where the weight gain and shell gain in diets D (1.2 g and 3.69 mm, respectively) and F (1.3g and 3.96, respectively) were higher than control and other different diets.

According to **Krishnamurthy *et al*. (1966)**, bacteria, yeast and diatoms could be utilized as food by bivalves. Bivalves fed that yeast has greater food value than diatoms or bacteria. Next to yeast, bacteria are important in food value. These results differed with those obtained in the present study where the lowest growth rate recorded in Diet A (yeast) compared to other diets. These can be attributed to the increasing levels of yeast which seems to be less digestibility in bivalves. The probiotic enhance the growth when added to yeast or bacteria, revealing its role in enhancement the digestibility of bacteria and yeast. **Rodina (1948)** recorded an increase in weight up to sixty percent in the fresh water bivalves, *Sphaerium, Mtiseulum* and *Pisdium* fed on pure culture of bacteria and yeast, isolated from a lake.

In general, the survival rates in the present study were high compared to the other previous studies where the highest survival rate recorded in Diets (F and D) compared to other diets. This can be attributed to the use of probiotic in the experiment where can facilitate depuration of seeds from pathogenic bacteria and viruses **(Prieur *et al*. 1990; Bellou *et al*., 2013)**. These results agreed with **Subhash and Lipton (2007)**. They found that the probiotic groups had significantly higher survival than the control groups.

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