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Original Article

Dry feed for *Artemia*: its effect on performance, physiology, immune responses and bacterial resistance

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Abstract: The larviculture of fish and shellfish is inevitably dependent on live food such as *Artemia* and one of the most important issues in rearing *Artemia* is the food supply. *Artemia* culture is mainly dependent on freshly prepared unicellular algae, which is not accessible easily. This research focused to prepare a dry feed that can support the survival and growth of *Artemia* and improve its resistance against pathogenic bacteria. The nauplii of *Artemia* were fed seven feeding treatments, including the control diet (algae + yeast) and six experimental dry feed containing different levels of probiotic bacteria. The results showed that *Artemia* fed a diet containing 10% algae+1.25% probiotic bacteria performed slightly better in terms of growth, but significantly higher survival and increased alkaline protease activity was detected compared to control. The lipase activity was significantly higher only in *Artemia* fed 5% algae + 0.625% probiotic bacteria, and the highest Amylase activity was detected in the control group. The activity of the antioxidant enzymes superoxide dismutase (SOD), Glutathione reductase (GRed), Glutathione peroxidase (GPx) and Malondialdehyde (MDA) presented a significant increase as a function of culture time and probiotic administration. The challenge with the pathogen resulted in significantly higher survival in all tested life stages of *Artemia* (nauplii, juvenile, and adults) in negative and positive controls compared to the control diet group. It is concluded that pathogen induces an oxidative stress response in almost all stages of *Artemia* growth and probiotic bacteria *Bacillus coagulans* and *B. subtilis* protects *Artemia* when challenged with *Vibrio anguillarum* by enhancing immune responses.

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Introduction

The brine shrimp *Artemia* has been widely used in aquaculture as a primary food source in the larval rearing of marine fish and crustaceans. *Artemia* is a favorite food because it can be cultured easily, has a suitable size, has high nutritional values, and can patronage a wide variety of various larval stages of aquaculture species. *Artemia* is a continuous, non-selective, obligate phagotrophic filter feeder (Provasoli and Kagehide, 1959; Reeve, 1963b; D'Agostino, 1980) which starts to feed at Instar II (Barlow and Sleigh, 1980). However, it has been shown that the feeding rate of *Artemia* changes as a function of food concentration (Evjemo et al., 1999). Various factors affect the feed behavior of *Artemia*, such as food filtration rate, feed quality and quantity, rate of digestion and absorption, stage of life, and culture conditions (Coutteau and Sorgeloos, 1989). Although *Artemia* rearing has been successfully conducted using a wide range of microalga types (Vanhaecke and Sorgeloos, 1989), cultivation and preparation of microalgae require high costs and economically are not affordable (Sorgeloos, 1982). In the meantime, the agricultural by-products can be used as a convenient alternative feed, easily available worldwide (Dobbeleir et al. 1980; Zmora and Shpigel, 2006).

Agricultural by-products and their impact on the growth and survival of *A. urmiana* and parthenogenetic *Artemia* have been studied (Ownagh et al., 2015). Wheat and rice brans possess high fiber content and can substitute adequate part of

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the brine shrimp (*Artemia*) and other live food organisms and monogastric animals' diets (Piccioni, 1965; Sorgeloos et al. 1980; Fuller, 2004). In addition, soymeal is a rich source of protein that has a better balance of amino acids but contains less than 3 percent crude fiber (McDonald et al., 2002). However, low growth and survival rates were reported when agricultural by-products (e.g., wheat bran, rice bran, and soybean meal) were used as the sole diets for *Artemia* (Sorgeloos, 1980; Anh et al., 2009; Ownagh et al., 2015). Moreover, algae and bacteria are characterized by specific fatty acid profiles, and the dietary fatty acids are transferred conservatively into *Artemia* lipids (Intriago and Jones, 1993; Zhukova et al., 1998). Recent aquaculture studies have shown that diets with incorporated probiotics improve the gut microflora, enhance the secretion of digestive enzymes, improve digestion and absorption of nutrients, and increase the immune response in the host animal (Das and Tripathi, 1991; Verschuere et al., 2000). Several studies have reported that microalgae-enriched diets have been used to boost the nutritional quality of *Artemia* (Seixas et al., 2010). In *Artemia*, the enzymatic content depends on the nutritional state and the developmental stage (Munilla-Moran et al., 1990).

Extensive cultivation and populations in the natural habitats depend on natural microalgae, while in small-scale, semi-intensive, and intensive cultivation, by-products of agricultural and food industry, including organic fertilizers, rice bran, corn bran, whey, etc. are used as sole feeds or supplemental feeds (Wear and Haslett, 1987; Wurtsbaugh and Gliwicz, 2001; Zmora et al., 2002). Application of live, non-pathogenic useful bacteria to the *Artemia* culture can improve the nutritional value of *Artemia* and positively affect the performance of cultured fish species by improving the intestinal microbiota and eliminating the harmful bacteria (Havennar et al., 1992; Ringo et al., 1992).

The use of specific biological compounds that enhance immune responses of target organisms, rendering animals more resistant to diseases, may be an excellent preventive tool against pathogens (Anderson, 1992). Several biological compounds and bacteria (Takahashi et al., 2000) are being applied in vertebrate and invertebrate cultures to induce and build up protection against a wide range of diseases. *Saccharomycess cerevisiae*, a good immune enhancer in some aquatic organisms (Li and Galtin, 2003, 2004), is an excellent source of β-glucans and chitin, which are mainly present in the yeast cell wall as major compounds together with mannoproteins (Magnelli et al., 2001).

Artemia has developed a set of antioxidant defense systems to protect from reactive oxygen species accumulation, including antioxidant enzymes such as superoxide dismutase (SOD), GSH redox cycle enzymes, and glutathione S-transferase (GST) (Yu, 1994). A combination of bacteria (synbiotic) with other nutritional feed ingredients may influence the antioxidant enzyme activity in *Artemia* challenged with pathogenic bacteria. Hence, this study aimed to prepare a high-quality and cost-effective dry feed for *Artemia* to enhance performance, the activity of digestive and anti-oxidative enzymes, nutritional value, and resistance to bacterial challenges in cultured *Artemia*.

Material and methods

Artemia culture

Preparation of experimental feed*:* Six experimental dry feed were prepared using rice and wheat bran, dried algae, yeast, probiotic bacteria, starch, lecithin, and astaxanthin. For this purpose, rice and wheat brans were ground, hydrated, and aerated strongly in saltwater (40 g/l) for 30 minutes. Next, the mixture was filtered with a 50 µ mesh. Then dried in an oven at 40°C for 24 hours and ground well. Unicellular algae (*Chlorella*, *Dunaliella*, and *Nannochlorpsis*) were cultured under the standard condition at (12:12hours) photoperiod and then harvested, washed, and dried in the oven fan at 35°C for 8 hours. The bacteria, yeast, starch, astaxanthin, and lecithin were purchased from the local market. The ingredients were mixed and analyzed to confirm that all

treatments contain similar protein, lipid, carbohydrate, and ash content.

Hatching and rearing of *Artemia:* *Artemia franciscana* cysts were hatched under optimal conditions (29°C, 33 ppt, pH: 8.5 and severe aeration) (Sorgeloos et al., 1986). Five hundred nauplii instar I with the initial density of two nauplii L^{-1} were transferred into one-liter conical flasks (21 flasks, seven treatments, and three replicates) filled with 80 ppt saline water. Animal density was 1 nauplii per 2 ml during the first eight days of culture dropped to 1 *Artemia* per 4 ml of culture water (Coutteau et al. 1990). Gentle aeration was conducted during the period of rearing.

All treatments were under 12 hours light and 12 hours darkness. *Artemia* was fed following Coutteau et al. (1990) feeding protocol. The physicochemical parameters of the rearing medium were as follows: temperature 26±0.5°C, dissolved oxygen 5.9-6.1 mg L^{-1}, pH 7.8-8.0, salinity 80 g L^{-1}. Experimental diets were prepared using micronized wheat and rice bran, yeast, algal mixture, commercial probiotic bacteria, starch, lecithin, and astaxanthin. A combination of freshly cultured algae (25%) and yeast (75%) is used as the control diet (Table 1).

Growth and survival: The total length (mm) of 10 *Artemia* from different feeding treatments was measured on days 1, 8, 11, 14, 17, 20, and 23 of the rearing periods using a stereomicroscope equipped with a drawing tube (Model; Nikon SMZ, 1500, Tokyo, Japan). The survival rate was calculated in each replicate on the same days according to the following equation:

Survival rate (%) = (final number of *Artemia* / initial number of stocked Nauplii) × 100

The sex ratios of *Artemia* in each replicate were determined based on the number of males and females at different treatments (Abatzopoulos et al., 2003).

Body biochemical composition and total carotenoids: *Artemia franciscana* was cultured in 300-liter containers with three replicates using the feeding mentioned above treatments under standard rearing conditions. From each container, about 250 g (±10%) of *Artemia* were harvested after 14 days. The cultured *Artemia* were washed, partly dried, and partly preserved wet at -80°C until analyses are done.

Crude protein content: To determine the protein content in the whole body of adult *Artemia*, two grams of *A. franciscana* dry matter from each replicate were minced for analysis according to AOAC (2005). The Kjeldahl method determined the crude protein (N×6.25) after acid digestion using an auto-Kjeldahl System (Gerhardt, Germany).

Lipid and ash contents analysis: The lipid content of *Artemia* was measured by ether extraction (AOAC, 2002). Two grams of dry samples from each replicate were weighted and homogenized and transferred to 98% diethyl ether for 12 hours. To calculate the ash content, two grams of dry samples from each replicate were weighed and placed in an electric furnace for 6 hours at 550°C (AOAC, 2002).

Total Carbohydrate content: The Carbohydrate content of the samples was measured according to Hedge and Hofreiter (1962). Briefly, 100 mg of *Artemia* is hydrolyzed to simple sugar by adding 5 ml of 2.5 N HCl and incubated in a boiling water bath for 3 hours. Solid Na_2CO_3 neutralized the solution until the effervescence ceased. The volume was made up to 100 ml using distilled water and centrifuged at 5000 rpm for 5 min. The supernatant was collected, and 0.5 ml of it was diluted with an equal volume of distilled water. Then 4 ml of 0.2% anthrone reagent was added to the reaction mixture and heated in a boiling water bath for 8 min. The sample was cooled rapidly, and the optical density was read at 630 nm. The standard solution was prepared at different concentrations of 100 $\mu g.ml^{-1}$ glucose (Hedge and Hofreiter, 1962).

Total carotenoids: The adult *Artemia* from each treatment were transferred into the tubes containing 1.5 mL of pure ethanol and kept in the dark at 5°C for 24 hours. Total carotenoids ($\mu g mg^{-1}$) for each treatment were measured using a spectrophotometer at 450 nm based on the following formula (Britton, 1995):

$$\text{Total Carotenoids } (\mu g/mg) = 1\times10^4 \text{ (OD450 / } 2.62\%) \times (V/W)$$

Table 1. Ingredients and proximate composition of experimental dry feed.

Experimental diets	Wheat barn & rice barn (50:50)	yeast	Astaxanthin	Algae*	Probiotic Bacteria**	Starch	Lecithin
1	Algae 25% + yeast 75% (Control Group)						
2	77.050%	15%	0.001%	5%	0.625%	2%	0.1%
3	76.400%	15%	0.001%	5%	1.25%	2%	0.1%
4	77.650%	15%	0.001%	5%	-	2%	0.1%
5	72.050%	15%	0.001%	10%	0.625%	2%	0.1%
6	71.400%	15%	0.001%	10%	1.25%	2%	0.1%
7	72.650%	15%	0.001%	10%	-	2%	0.1%

Experimental diets	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)
1	30.73	11.90	33.92	1.31
2	14.99	38.81	36.88	8.60
3	14.96	38.56	37.18	8.55
4	15.01	39.04	36.55	8.65
5	16.63	37.84	36.28	8.52
6	16.60	37.59	36.58	8.46
7	16.65	38.07	35.95	8.57

*The algae powder was used includes *Dunaliella tertiolecta*, *Nannochloropsis oculata* and *Chlorella* sp. at 1:1:1 ratio. **The freeze-dried commercial probiotic bacteria used in diets include *Bacillus coagulans* and *B. subtilis* at the ratio of 50:1 (Parsilact, Shiraz, Iran).

Where OD is the optical density (at 450 nm of 1.0 cm cuvette path), V is the volume of fluid in the cuvette (1 mL), W is the weight (mg), and 2.62% is the absorption coefficient of 1.0% beta-carotene at 450 nm.

Bacterial load of the culture media: The bacterial load of the culture water at different feeding treatments was determined on days 1, 8, 14, and 21 by sampling 1.0 mL from each replicate, diluted in five sequential intervals. Then, the resulting cultures of the last dilution were pour-plated in plate count agar and incubated at 37°C for 48 h. Finally, the numbers of aerobic bacterial colonies were counted and calculated using the following equation expressed as log10 CFU mL^{-1}.

CFU = colony count × inverted dilution factor

Analysis of digestive and oxidative stress enzymes: The whole body of *Artemia* was rinsed well with distilled water and homogenized by Polytron PT 1300 D homogenizer (15,000 rpm, 3 x 30 sec) (Switzerland) in ice-cold 50 mM Tris-HCl buffer, pH 7.5 (1:3 weight to volume). The homogenate was centrifuged at 10,000 g for 20 min at 4ºC, and the supernatant was collected (Chong et al., 2002) and aliquoted and stored at -80ºC for enzyme analysis (Chong et al., 2002).

Lipase activity was determined by hydrolysis of p-nitrophenyl myristate to p-nitrophenole and myristate (Iijima et al., 1998). The total proteolytic activity of the samples was assayed in quadruplets using 2% azocasein prepared in 50mMTris-HCl, pH 7.5 as a substrate García-Carreño and Haard (1993). The alpha-amylase activity was determined by incubating the enzyme extract with 1% starch solution prepared in 20 mM sodium phosphate buffer and 6 mM sodium chloride (pH 6.9) at 25°C (Worthington, 1991).

Bacterial Challenge test: *Artemia franciscana* were hatched and grown using control diet and diet No. 6 in 1 L conical containers. A freshly prepared sterile culture medium was changed every day until exposure to pathogens. The animals were challenged with *Vibrio anguillarum* at three life stages (metanauplii, juvenile, and adult), according to a modified method of Touraki et al. (2012). Pathogens were administered to the culture medium at two doses, each corresponding to $2x10^5$ CFU mL^{-1}, at 96

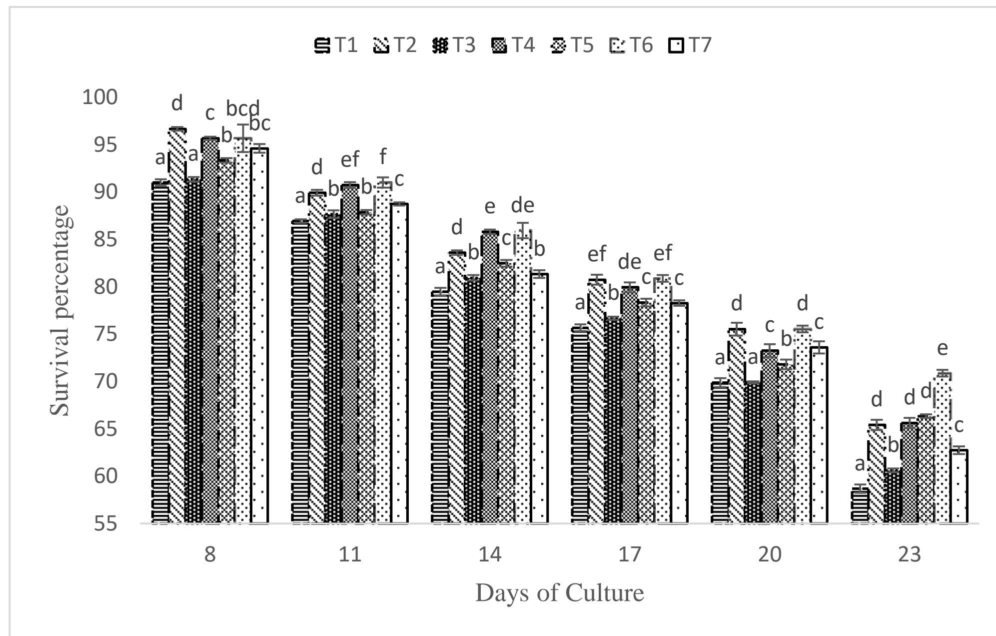

Figure 1. Survival (%) of *Artemia franciscana* cultured at different treatments until the pre-reproduction period for 23 days post-hatch.

and 120 h post-hatch (meta-nauplii), at 216 and 240 h post-hatch (juvenile) and 336 and 360 h post-hatch (adults). Treatments in this stage were; control diet (fed algae 25% + yeast 75% and exposed to pathogen), negative control (fed diet number 6, not exposed to pathogen), and positive control (fed diet number 6, exposed to pathogen). Diet number six was the best diet for growth, survival, and reproductive characteristics.

There were eight replicates, and survival rates were monitored throughout the experiment (24 to144 h post-hatch daily for meta-nauplii; 72, 144, 216, 240, and 264 h for juveniles and 72, 144, 216, 288, 336, 360, and 384 h for adults). *Artemia franciscana* were collected at 144, 264, and 384 h and used for biochemical evaluation. The presence of the pathogen in *A. franciscana* was confirmed at the end of each experiment as described above, and inoculation was performed on plates with BHI agar containing 2% NaCl followed by incubation at 25°C for 24-48 h.

The activity of oxidative stress enzymes: In experiments, enzymatic activities were determined in freshly homogenized samples collected after the challenge tests. For determination of superoxide dismutase (SOD), Lipid Peroxidation (MDA), Glutathione reductase (GRed) and Glutathione Peroxidase (GPx), Nasdox™–Superoxide Dismutase Assay Kit (NS-15032), Nalondi™-Lipid Peroxidation Assay Kit (NS-15022), Naglur™ Glutathione Reductase Assay Kit (NS-15087) and Nagpix™ Glutathione Peroxidase Assay Kit (NS-15082) were used respectively from Navand Salamat Co. Iran.

Statistical analyses: Before analysis, Kolmogorov-Smirnov and Bartlett's tests were applied to verify the normality and homogeneity of variances. Then, the results of growth, body composition, and reproductive performance were analyzed using standard one-way analysis of variance (ANOVA) by SPSS software (Version 22). Tukey test was applied to determine the significant differences between the means at a significance level of $P<0.05$.

Result

Survival and growth: At the end of day 23, *Artemia* fed the experimental diets exhibited more than 60% survival rate, while it was lower than 60% in control. The highest survival was observed with feeding treatment 6 (70.87%) and lowest in the control diet (58.67%) ($P<0.05$) (Fig. 1). *Artemia* fed diet 6 also grew bigger on day 23 (11.66 mm) compared to all

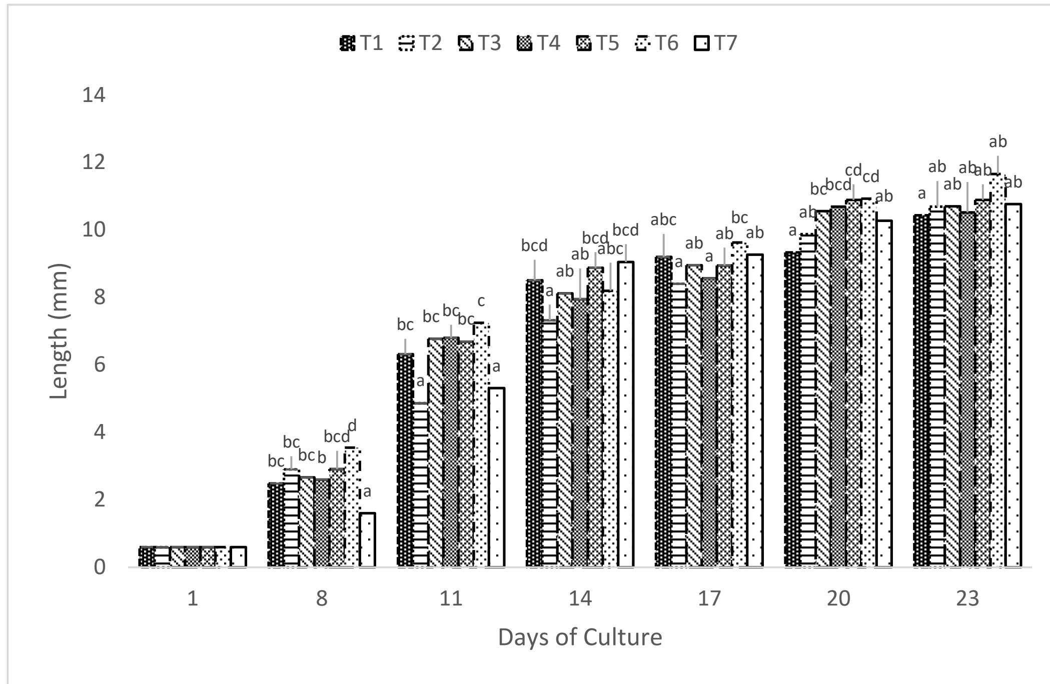

Figure 2. Total length (mm) of *Artemia franciscana* cultured at different treatments until pre-reproduction period for 23 days.

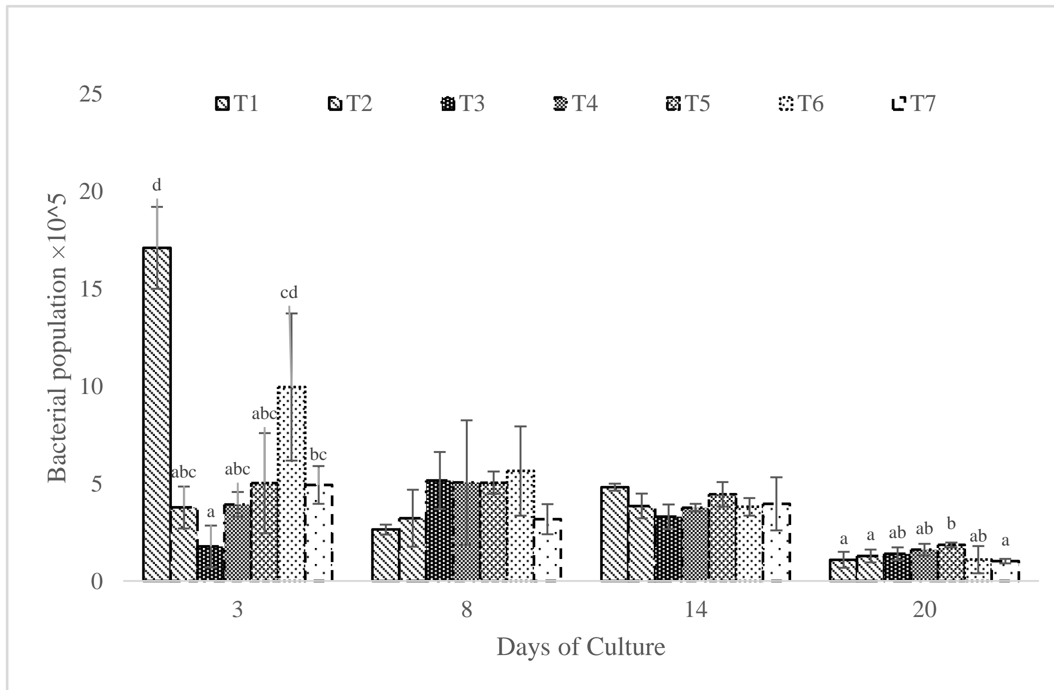

Figure.3. Total bacterial load (n $\times 10^5$ CFU mL^{-1}; mean ± SD) in the culture medium of *Artemia franciscana* at different treatments on days 3, 8, 14 and 20.

other treatments, including the control, although there were no significant differences between them (P>0.05) (Fig. 2).

Body biochemical composition, fatty acid profiles, and total carotenoid content: The protein content in the whole body of *Artemia* (40.68, 40.91, and 40.46%, respectively) fed on feed 5, 6, and 7 were significantly higher than those treatments of 2, 3 and 4 (P<0.05), but no significant differences with the control group. The highest lipid content (17.15%) was detected in *Artemia* fed diet 6, significantly different from other treatments (P<0.05). No differences (P>0.05) were found in the ash content between treatments. Carbohydrate level was

Table 2. Proximate composition of whole-body (expressed in percent dry weight) and total carotenoids (μg.mg^{-1}) of *Artemia franciscana* fed diets containing different experimental diets.

	1	2	3	4	5	6	7
Carbohydrate (%)	42.80±1.19^{c}	36.01±0.59^{b}	36.85±0.83^{b}	37.26±1.14^{b}	34.36±0.19^{b}	32.18±0.23^{a}	35.15±1.54^{b}
Ash (%)	9.26±0.52^{a}	10.23±0.49^{a}	9.63±0.06^{a}	9.47±0.11^{a}	9.11±0.90^{a}	9.13±0.26^{a}	8.14±0.64^{a}
Lipid (%)	7.66±0.60^{a}	14.81±0.07^{b}	14.73±0.53^{b}	15.03±0.75^{b}	15.45±0.33^{b}	17.15±0.74^{c}	15.46±0.22^{b}
Protein (%)	39.92±0.19^{ab}	38.45±0.26^{a}	38.14±0.58^{a}	37.59±0.49^{a}	40.68±0.71^{ab}	40.91±0.66^{ab}	40.46±1.09^{ab}
Carotenoids (μg mg^{-1})	43.80±2.19^{a}	65.52±0.09^{d}	71.35±0.67^{f}	60.76±0.15^{b}	68.36±0.19^{e}	66.18±0.76^{d}	62.65±2.95^{c}

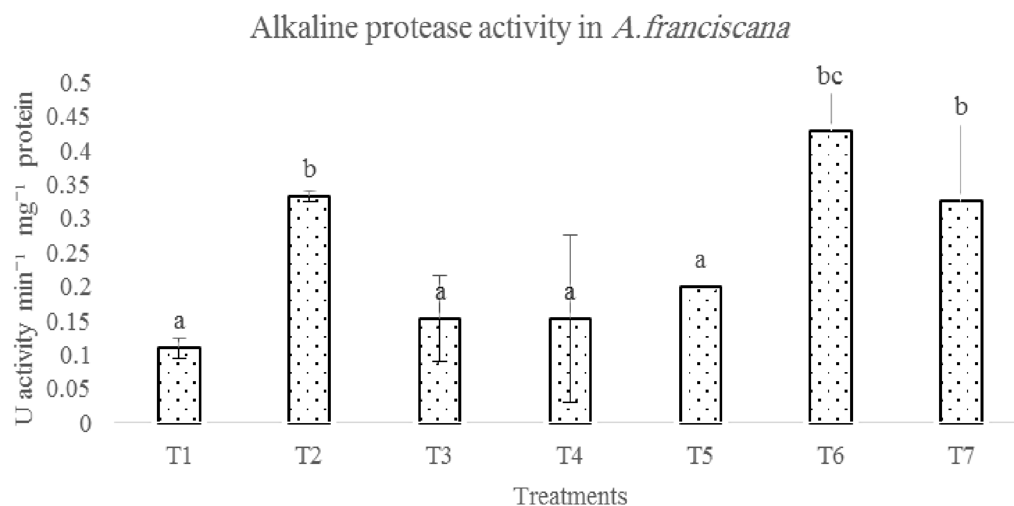

Figure.4. Alkaline protease activities (U activity min^{-1}mg^{-1} protein) in adult *Artemia franciscana*. Means±S.D. (n=9).

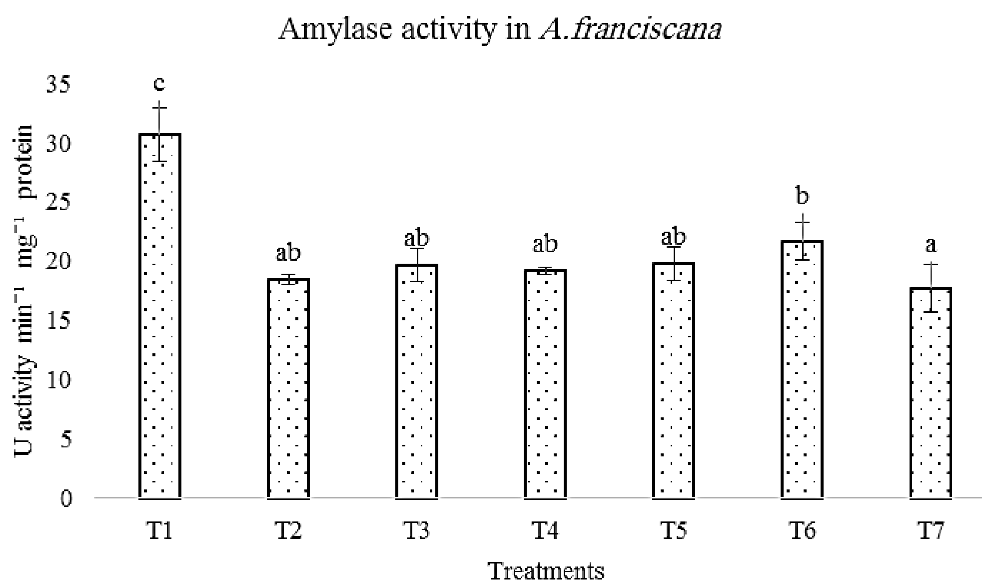

Figure.5. Amylase activities (U activity min^{-1}mg^{-1} protein) in adult *Artemia franciscana*. Means ± S.D. (n=9).

significantly higher in control (42.80%) compared to other groups ($P<0.05$). Total body carotenoids were significantly higher in all groups fed experimental diets, highest in treatment 3 fed diet containing 5% algae and 1.25% bacteria ($P<0.05$) (Table 2).

Bacterial load of culture medium: Bacterial load of the culture medium was significantly different between different groups only on day 3, but the differences gradually decreased between treatments, and no significant differences were found on day 20

Table 3. Effect of experimental dry diet on the survival of *Artemia* with or without experimental challenge with *Vibrio anguillarum*. Challenge was performed for 96 and 120 h for meta-nauplii, 216 h and 240 h for juvenile and 336 h and 360 h for adult *Artemia*.

Experimental	48h	72h	96h	120h	144h	216h	240h	264h	288h	336h	360h	384h
Meta-nauplii control diet	99.67±0.21	98.82±0.27	97.77±0.14	39.45±0.93[a]	17.68±1.32[a]	*	*	*	*	*	*	*
Meta-nauplii- negative control (diet No. 6)	99.56±0.10	98.15±0.18	97.30±0.36	95.71±0.11[c]	93.87±0.36[c]	*	*	*	*	*	*	*
Meta-nauplii-positive control (diet No. 6)	99.72±0.10	98.56±0.44	97.73±0.33	89.70±1.10[b]	82.89±0.44[b]	*	*	*	*	*	*	*
Juvenile control diet	*	98.82±0.87	*	*	92.88±0.13	90.56±0.41	47.06±0.54[a]	41.36±0.60[a]	*	*	*	*
Juvenile- negative control (diet No. 6)	*	99.13±0.19	*	*	92.44±0.35	90.59±0.24	88.66±0.05[c]	87.30±0.09[c]	*	*	*	*
Juvenile-positive control (diet No. 6)	*	99.55±0.45	*	*	92.85±0.31	90.53±0.23	80.74±0.64[b]	77.72±0.23[b]	*	*	*	*
Adult control diet	*	98.82±0.27	*	*	93.81±0.13	91.00±0.13	*	*	84.46±0.13	79.22±0.23	46.22±0.69[a]	43.74±0.20[a]
Adult- negative control (diet No. 6)	*	98.63±1.53	*	*	93.36±0.35	91.50±0.34	*	*	84.96±0.34	79.72±0.43	77.09±0.64[c]	73.90±0.83[c]
Adult-positive control (diet No. 6)	*	98.06±1.02	*	*	93.78±0.32	90.96±0.31	*	*	84.42±0.31	79.18±0.13	71.09±0.50[b]	64.31±0.96[b]

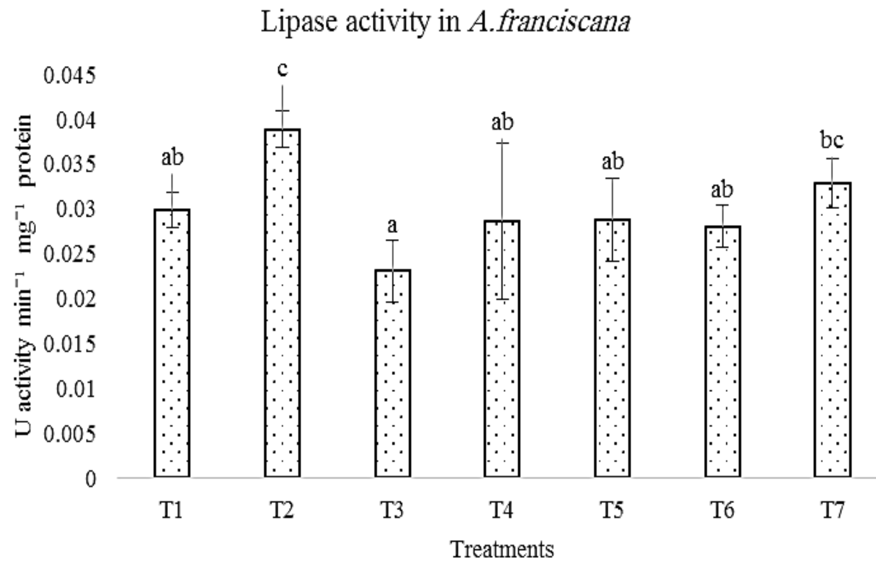

Figure.6. Lipase activities (U activity $min^{-1}mg^{-1}$ protein) in adult *Artemia franciscana*. Means ± S.D. (n=9).

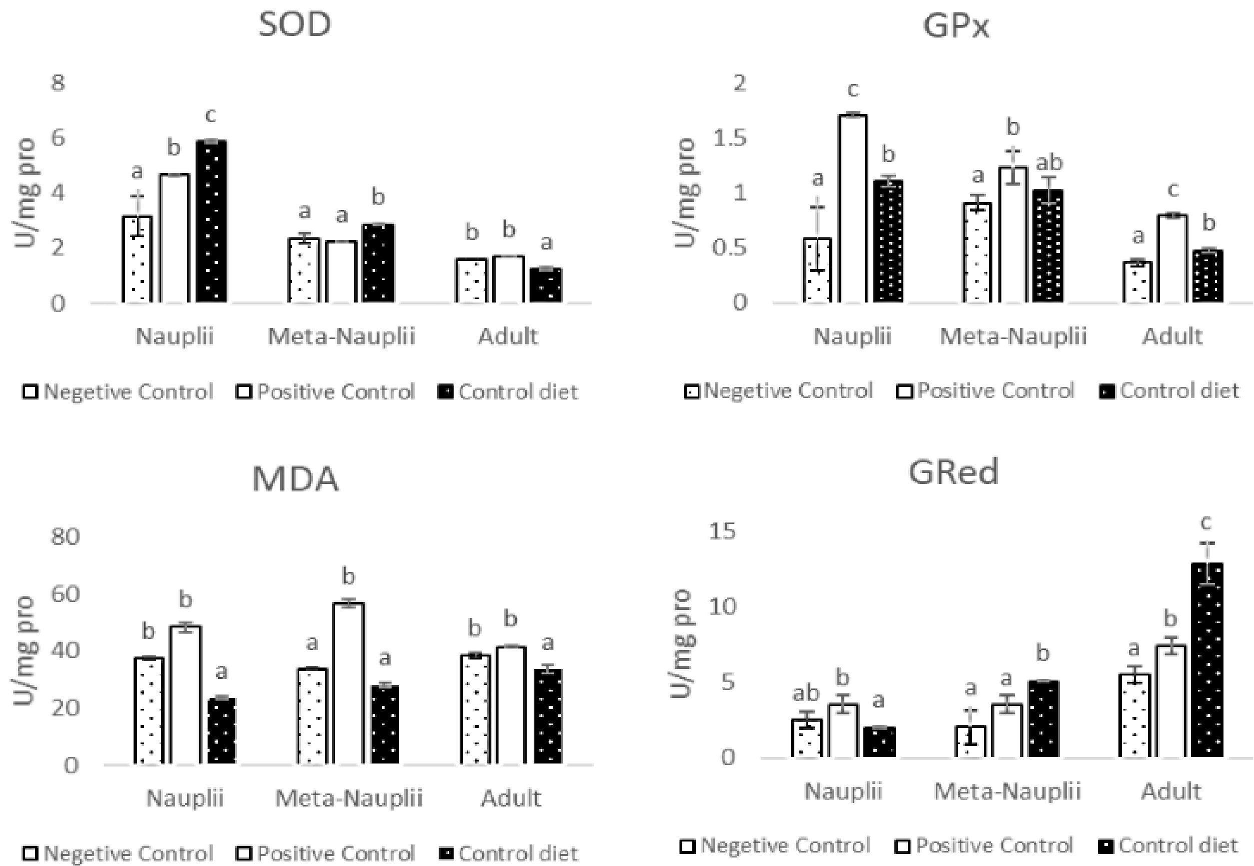

Figure.7. Enzymatic activities in *Artemia franciscana* following a challenge with *Vibrio anguillarum*. Positive control series received *Artemia* diet with a pathogen; Negative control series received *Artemia* diet with no pathogen and Control group series received algae + yeast with the pathogen. Each point is the mean ± SD of eight separate Samples resulting from three replicate experiments.

of culture (Fig. 3).

The activity of digestive enzymes: The results showed that the alkaline protease activity in *A. franciscana* increased significantly in treatments 2, 6, and 7 compared to control (P>0.05) (Fig. 4).

The amylase activity in *A. franciscana* was significantly higher in the control group (30.69 U) compared to experimental treatments (P>0.05) (Fig.

5). The activity of the lipase enzyme in *A. franciscana* was higher in T2 (0.038U) compared to all treatments except T7 (P<0.05) (Fig. 6).

Activity of oxidative stress enzymes: Significantly higher SOD activity was detected in treatments challenged with the pathogen (positive control and group control diet containing pathogen, Fig. 7) in nauplii and meta-nauplii (P<0.05), but significantly lower in adults (P<0.05) compared to the corresponding negative control. The largest decrease in activity was found for GRed as 40% in negative control for meta-nauplii and adult (P<0.05) and the lowest for SOD as 17.19% in positive control for meta-nauplii (P<0.05). Lipid peroxidation presented an increase for negative and positive controls, respectively (P<0.05) compared to the control diet group containing pathogen in all three age groups of *A. franciscana* tested (Fig. 7). On the other hand, GPx revealed an increase in positive control and dry feed group (P<0.05) compared to negative control in all age groups of *A. franciscana* (P<0.05) (Fig. 7).

Challenge test: The survival percentages of *A. franciscana* challenged with pathogenic bacteria in different experimental treatments are reported in Table 3. Significantly higher survival rates were observed in the control diet group than in the negative control and positive control (Table 3).

Discussion

Growth, survival, biochemical composition, fatty acid profiles and total carotenoids of *Artemia*: This study showed that the type of diet affects the total body length of *Artemia* in the pre-reproductive period. Significantly higher growth was observed in *A. franciscana* (11.66 mm) fed a diet containing 10% algae + 1.25% bacteria (T6). The total body length of *Artemia* on day 21 was lower than those recorded in the same species by Lavens and Sorgeloos (1996), and Bahmanpour et al. (2009). However, growth rates in the current study (on day 11) were mostly higher than those reported by Naegel (1999) for *A. franciscana* (4.93, 5.02, and 4.64 mm) cultured using a commercially inert diet and *Chaetoceros* sp. for 11 days. Several environmental (e.g., temperature and salinity) and nutritional factors (food quantity and quality) can affect the growth and survival rates of *Artemia* (Lavens and Sorgeloos, 1996; Browne and Wanigasekera, 2000; Baxevanis et al., 2004). The experimental diet of this study had higher lipid content than the control diet, which could be the reason for their higher growth.

In the present study, *Artemia* survival rate was strongly influenced by the diet type. The *Artemia* fed 25% algae + 75% yeast (control diet) showed lower survival (less than 58%) at the end of day-23. in comparison to most of the experimental diets. Dwivedi et al. (1980) and Basil et al. (1989) reported that separate uses of organic manures or agricultural waste in the diet of *Artemia* reduced nauplii survival rates to less than 30-50% till an adult stage, but in the present study 58-70% survival was achieved. The survival values detected here are even higher than those reported by other workers, due to different cultural environments, initial stocking density, and feeding conditions. For example, the survival rates after 11 days of culture (91%) for *A. franciscana* fed experimental diet 6 in the current study was relatively higher than reported (72, 79, and 73.5%) by Naegel (1999), who fed *A. franciscana* with Nestum (a baby food), enriched Nestum, and the microalgae *Chaetoceros* sp., respectively, for 11 days. Ownagh et al. (2015) found a 70.3% survival rate for *A. urmiana* and 68.5% for the parthenogenetic *Artemia* when used wheat bran, Anh et al. (2009) reported a 52-54% survival rate by feeding *Artemia* with swine manure supplemented with soybean powder and rice bran and similarly Maldonado-Montiel et al. (2003) reported 50% survival in *Artemia* using poultry manure in ponds. The high survival rate in the current study proved that feed quality and feed the feed content have a significant role in the production of *Artemia* biomass.

In this study, the experimental dry diets contained significantly lower protein (14.99- 16.65%), and higher lipid levels (37.84-39.04%) than the control diet. But the protein was high in the *A. franciscana* fed all treatments, proving the capacity of *Artemia* in converting the plant protein to animal protein. The

lipid content in the *Artemia* fed diets containing different lipid levels was 7.66-17.15% showing a significantly increasing trend with elevations in the dietary levels of lipid reflecting the dietary fat content. These data are in accordance with the previous studies reporting the effects of diet types on the biochemical compositions of *Artemia*. For example, Ronsivalli and Simpson (1987) by rearing *Artemia* for 15 days with rice bran and whey powder reported protein contents of 50.1 and 61.4, lipid values of 9.5 and 7.4%, ash records of 9.9 and 9.2%, and carbohydrates amounts of 24.1 and 17.2%, respectively. Additionally, Naegel (1999) detected 56.5, 43, and 41% protein levels and lipid contents of 2.95, 16.5, and 20.3% in *A. franciscana* grown with the alga *Chaetoceros*, Nestum (powdered baby food), and enriched Nestum, respectively. Total carotenoids in the body of *Artemia* showed a significant decreasing trend by the control group. It seems that supplementation of Astaxanthin (0.001%) in dry diets could potentially help in increasing the body carotenoids. Existing of *D. salina* in the control group as an important rich source of beta-carotene (12-14% of the dry weight) (Ben-Amotz et al. 2009) could be influenced *Artemia* carotenoids. Vahdat et al. (2018) reported that feeding *A. franciscana* with 100% algae and 75% algae+25% vermicompost increased carotenoids of 45.90 μg mg^{-1} to 47.73 μg mg^{-1} at the adult stage. It is noteworthy that *Artemia*, like other crustaceans, receives carotenoid resources through dietary intake, most of which is mobilized to the gonads and egg production.

Digestive enzymes: The digestive enzymes activity is affected by many factors such as age and food quality. The knowledge about these factors may provide a guideline to use an appropriate live or dry feed that results in enhanced growth and survival of *Artemia*. The *Bacillus* spp. is one of the most popular probiotics used in aquaculture. This bacterium is part of the natural flora of *Artemia* culture medium and can secrete a number of extracellular enzymes (Moriarty, 1998) like proteases (bacitracin and subtilin) (Maget-Dana and Peypoux 1994; Sanders et al. 2003). These bacteria can stimulate the digestive system and improve digestion and, ultimately growth performance of the host. In particular, *B. subtilis* and *B. licheniformis* (as BioPlus 2BÒ product) have been successfully applied as probiotics for rainbow trout (Raida et al., 2003; Bagheri et al. 2008, Merrifield et al. 2010). It is the first time that this product is used in *Artemia*. The *B. subtilis* and *B. licheniformis* are capable of digesting proteins and carbohydrates. Based on Ahmadnia Motlagh et al. (2012), it seems that the probiotic bacteria used in the diet need at least 10 days to stimulate the digestive system and enzymes secretion (protease and amylase). The knowledge about the effect of different feed ingredients on digestive enzyme activity will help formulate a feed with a higher efficiency of digestive enzymes (Deguara et al., 2003). It is not yet clear whether the increase in the enzyme activity was due to the stimulation of the digestive system or the bacteria activity in the digestive tract. Possibly, probiotic bacteria could increase the utilization of carbohydrate that exists in the diet by *Artemia*. The existence of the extracellular digestive enzymes produced by bacteria have been demonstrated in Chinese shrimp (*P. chinensis*) (Wang and Xu 2006), *Rutilus rutilus* (Skrodenyt_e-Arbaciauskien, 2007), *Sparus aurata* (Suzer et al. 2008), and *Penaeus vannamei* (Wang 2007).

The digestive enzyme activities are affected by life stage, amount and the chemical composition of food, and nutritional requirements. The presence of high levels of bacteria and algae (1.25% + 10%, T6) in the diets for 20 days significantly increased protease activity in *Artemia* compared to other groups. The treatments fed diets containing *B. subtilis* and *B. coagulans* and algae exhibited higher but insignificant survival, growth, and body protein than control. On the contrary, amylase activity in the control group was increased. A study conducted by Avella et al. (2010) showed a mixture of three *Bacillus* strains, viz. *B. subtilis*, *B. licheniformis*, and *B. pumilus* in the diet of gilthead sea bream (*Sparus aurata*) larvae revealed significantly increased standard length and body weight. Moreover, in

another study on common carp, *Bacillus* sp. used as a probiotic led to an increase in fat, starch, and protein of the diets (Wang and Xu, 2006). *Bacillus* bacteria could increase protease, amylase, and lipase activity in *P. vannamei* (Wang, 2007). Ahmadnia Motlagh et al. (2012) reported that protease and amylase in *A. urmiana* at day 15 enhanced when probiotics (*B. subtilis* and *B. licheniformis* with a ratio of 1:1) increased from 10^4 to 10^6 CFU/g feed, while lipase activity did not show a significant difference compared to control. The protease activity significantly increased with dietary algae and bacteria levels in the present study. Higher levels of algae (10%) and bacteria (1.25%) in the diet of *Artemia* resulted in increased alkaline protease activity that could be due to the induction of probiotics for enzyme secretion. Kolkovski (2001) suggested that the protease activity may be influenced by the quality and quantity of algae and bacteria in the feed.

Activity of oxidative stress enzymes and challenge tests: The present study showed that immune defenses present alterations during the three stages of the *A. franciscana* life cycle. They are greatly affected by both pathogens and dry diet. The innate immune responses in invertebrates have been characterized as potent but non-specific (Hauton, 2012). However, it has recently been shown that the innate immune system of *Artemia* presents a trained immunity, namely memory and partial discrimination (Norouzitallab et al., 2016). Although the major immune defense mechanism in invertebrates is the antioxidant system (Rudneva, 1999), the generation of reactive oxygen species (ROS), which facilitate the degradation of foreign particles, can also result from the prophenoloxidase system (Söderhäll and Cerenius, 1998). An imbalanced increase in ROS may result in oxidative stress, and the protective mechanisms against oxidative damage, in this case are antioxidant enzymes such as SOD or GRed (Philipp et al., 2011). The pattern observed in our study for lipid peroxidation is not different in the three stages of *Artemia* trend, presenting a maximum peroxidation value at 48 h nauplii followed by a decline. The increase in lipid peroxidation in positive control placed the *Artemia* under environmental stressors, especially oxygen. These available oxygen levels lead to elevated metabolic rates and ROS-mediated reactions. Increased metabolic activity and elevated oxygen consumption have been reported to represent an additional burden on the antioxidant system (Fanjul-Moles and Gonsebatt, 2011).

Carotenoids that are known to protect nauplii against lipid peroxidation (Rudneva, 1999) are metabolized to all trans-canthaxanthin by developing *Artemia* nauplii (Nelis et al., 1988). The degree of protection offered by carotenoids and their metabolites corresponds to their stability, with cantaxanthin being more stable than b-carotene (Jorgensen and Skibsted, 1993). Hence existing Astaxanthin in the dry feed could be the reason for decreased lipid peroxidation in our study for control diet and negative control groups. Regarding the tolerance of *Artemia* to probiotics, survival was significantly altered by experimental dry feed compared to control values, but it was reduced in the positive control treatment. The increased survival of *Artemia* individuals in the experiment following probiotic administration of *B. coagulans* and *B. subtilis* in the present study indicates a beneficiary effect of the two probiotics and it can be concluded that *Artemia* is well-tolerated them. In addition to improvement in survival and growth of organisms, probiotics exert modulation of the host's immune system (Aly et al., 2008; Zorriehzahra et al., 2016). These results are in accordance with the previous findings regarding the effect of probiotics in white shrimp (Wang and Gu 2010).

A decrease in lipid peroxidation, expressed in MDA production, has previously been reported for *Litopaeneus stylirostris* following administration of the probiotic *Pediococcus acidilactici*, as well as for *Artemia* nauplii following administration of an olive fruit extract (Viciano et al., 2015) and it was considered as the protection offered to the host organism. Lipid peroxidation, which is indicative of the existence of oxidative damage to lipids, was

lower following the administration of probiotics with *B. coagulans* and *B. subtilis* being the most effective. Our results on lipid peroxidation enhance the beneficial profile of the two probiotics tested in the diet. However, in the probiotics treated group challenged with the pathogen in the current study, increased survival and enzyme activity suggest higher protection against the pathogen. Selected bacterial strains originating from well-performing *Artemia* cultures have been reported to protect *Artemia* against the pathogen *V. proteolyticus* in survival and cell ultrastructure (Verschuere et al., 2000). Moreover, protection of *Artemia* nauplii following challenge has been documented for Bacillus sp. LT3 in terms of survival and activation of prophenoloxidase (Niu et al., 2014), and for yeast either as cell wall strains (Rojas-García et al., 2008) or as cooked unicellular diets (Rojas-García et al., 2009). The induction of immune responses by probiotics has been well-documented in prawns (Parmar et al., 2012), and sea cucumber (Ma et al., 2013). The increased antioxidant enzyme activity in our study following the challenge in the probiotic treated groups suggests that the protective effect of the two probiotics tested is exerted through the activation of immune responses of *Artemia* individuals. It appears that probiotic bacteria act by stimulating ROS generation, rendering *Artemia* well equipped to fight effectively against the pathogen upon infection. This response might be characterized as non-specific training of the innate immune system of *Artemia* nauplii. Therefore, it can be concluded that each of the probiotic bacteria used in our study acts in a discrete protective manner.

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References
Abatzopoulos J.T., El-Bermawi N., Vasdekis C., Baxevanis A.D., Sorgeloos P. (2003). Effects of salinity and temperature on reproductive and life span characteristics of clonal *Artemia*. (International Study of *Artemia*. LXVI). Hydrobiologia, 492(1-3): 191-199.

Ahmadnia Motlagh H., Farhangi M., Rafiee G., Noori F. (2012). Modulating gut microbiota and digestive enzyme activities of *Artemia urniana* by administration of different levels of *Bacillus subtilis* and *Bacillus licheniformis*. Aquaculture International, 20(4): 693-705.

Aly S.M., Abdel-Galil Ahmed Y., Abdel-Aziz Ghareeb A., Mohamed M.F. (2008). Studies on Bacillus subtilis and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunology, 25(1-2): 128-36.

Anderson D.P. (1992). Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. Annual review of fish diseases, 2: 281e307.

Anh N.T.N., Van Hoa N., Van Stappen G., Sorgeloos P. (2009). Effect of different supplemental feeds on proximate composition and *Artemia* biomass production in salt ponds. Aquaculture, 286: 217-225.

AOAC. (Association of Official Analytical Chemists). (2002). Official methods of analysis of official analytical chemists international (16th Ed.). Arlington, VA, USA: Association of Official Analytical Chemists.

Avella M.A., Gioacchini G., Decamp O., Makridis P., Bracciatelli C., Carnevali O. (2010). Application of multi-species of *Bacillus* in sea bream larviculture. Aquaculture, 305: 12-19.

Bagheri T., Hedayati S.A., Yavari V., Alizade M., Farzanfar A. (2008). Growth, survival and gut microbial load of Rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turkish Journal of Fish Aquatic Science, 8: 43-48.

Bahmanpour L., Hosseini L., Heydari R., Zare S., Manaffar R. (2009). Comparative study on the biometry and stress tolerance of the introduced and original populations of *Artemia franciscana* (Kellogg, 1906) males. *Artemia* 2009, December 13-14, Urmia-Iran. pp: 42-45.

Barlow D., Michael A.S. (1980). The propulsion and use of water current for swimming and feeding in larval and adult *Artemia*. In: G. Persoone, P. Sorgeloos, O.A. Roels, E.Jaspers (Eds.). The Brine Shrimp *Artemia*.

Vol. 1. Morphology, Genetics, Radiobiology, Toxicology. Universa Press, Wetteren. pp: 61-73.

Basil A.J., Kannan A., Sathasiva P., Mathuram G., Selvarani D. (1989). Culture of *Artemia* using biogas slurry (cow dung), agricultural wastes and synthetic *Artemia* meal. Book of Abstracts, p. 59 in Aquaculture 89 conference Los Angeles, California, USA. pp: 12-16.

Baxevanis A.D., El-Bermawi N., Abatzopoulos T.J., Sorgeloos P. (2004). International Study on *Artemia*. LXVIII. Salinity effects on maturation, reproductive and life span characteristics of four Egyptian *Artemia* populations. Hydrobiologia, 513: 87-100.

Ben-Amotz A., Polle J.E.W., Subba Rao D.V. (2009). The alga Dunaliella: biodiversity, physiology, genomics and biotechnology. Science Publishers, Enfield. pp: 173-187.

Britton G. (1995). UV/Visible spectroscopy. In: G. Britton, S. Liaaen-Jensen, H. Pfander (eds.), Carotenoids, spectroscopy. Birkhauser. pp: 13-62.

Browne R.A., Wanigasekera G. (2000). Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. Journal of Experimental Marine Biology and Ecology, 244: 1-29.

Chong A.S.C., Hashim R., Lee C.Y., Ali B.A. (2002). Partial characterization and activities of proteases form the digestive tract of discus fish (*Symphysodon aequifasciata*). Aquaculture, 203: 321-333.

Coutteau P., Lavens P., Sorgeloos P. (1990). Baker's yeast as a potential substitute for live algae in aquaculture diets: *Artemia* as a case study. World Aquaculture Society, 21(1): 1-8.

Coutteau P., Sorgeloos P. (1989). Feeding of the brine shrimp *Artemia* on yeast: effect of mechanical disturbance, animal density, water quality and light intensity. In: European Aquaculture Society Spec. Publ. N° 10. Bredene, Belgium. 344 p.

D'Agostino A. (1980). The vital requirements of *Artemia*: physiology and nutrition. In: Persoone G., Sorgeloos P., Roels O., Jaspers R. (eds.), the brine shrimp *Artemia*. Physiology, biochemistry, molecular biology, vol. 2. Universa Press, Wetteren, Belgium. pp: 55-82.

Das K.M., Tripathi S.D. (1991). Studies on the digestive enzymes of grass carp, *Ctenopharyngodon idella* (Val.). Aquaculture, 92: 21-32.

Deguara S., Jauncey K., Agius C. (2003). Enzyme activities and pH variations in the digestive tract of gilthead sea bream. Journal of Fish Biology, 62: 1033-1043.

Dobbeleir J., Adam N., Bossuyt E., Bruggeman E., Sorgeloos P. (1980). New aspects of the use of inert diets for high density culturing of brine shrimp. In: The brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers (Eds). Universa Press, Wetteren, Belgium. pp: 165-174.

Dwivedi S.N., Ansari S.K.R., Ahemad M.G. (1980). Mass culture of brine shrimp under controlled conditions in cement pool in Bombay, India. In: Persoone G., Sorgeloos Roles P., Jaspers E. (Eds.), The Brine Shrimp *Artemia*, Vol. 3, Ecology, Culturing, Use in Aquaculture. Universa Press, Wettern. pp: 175-184.

Evjemo J.O., Vadstein O., Olsen Y. (1999). Feeding and assimilation kinetics of *Artemia* franciscana given different concentrations of algae (*T. iso*). Journal of Experimental Marine Biology and Ecology, 242(2): 273-296.

Fanjul-Moles M.L., Gonsebatt M. (2011). Oxidative Stress and Antioxidant Systems in Crustacean Life Cycles. Oxidative Stress in Aquatic Ecosystems. D. Abele, J.P. Vázquez-Medina, T. Zenteno-Savín (Eds.), John Wiley & Sons, Ltd, Chichester, UK.

Fuller M.F. (2004). The encyclopedia of farm animal nutrition. CABI Publishing Series. 606 p.

García-Carreño F.L., Haard N.F. (1993). Characterization of proteinase classes in *Langostilla Pleuroncodes* planipes and Crayfish *Pacifastacus astacus* extracts. Journal of Food Biochemistry, 17: 97-113.

Hauton C. (2012). The scope of the crustacean immune system for disease control. Journal of Invertebrate Pathology, 110: 251-260

Havennar R., Ten Brink B., Huisint J.H.J. (1992). Selection of strains for probiotic use. In: R. Fuller (Eds.). Probiotics, the scientific basis. Chapman and Hall, London, pp: 209-224.

Hedge J.E., Hofreiter B.T. (1962). In: Carbohydrate Chemistry, 17. In: R.L. Whistler, J.N. Be Miller (Eds.), Academic Press, New York. pp: 17-22.

Iijima N., Tanaka S., Ota Y. (1998). Purification and characterization of bile salt activated lipase from the hepatopancreas of red sea bream, *Pagrus major*. Fish Physiology and Biochemistry, 18: 59-69.

Intriago P., Jones D.A. (1993). Bacteria as food for *Artemia*. Aquaculture 113 (1-2): 115 127.

Jorgensen K., Skibsted L. (1993). Carotenoid scavenging radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. Z. Lebensm. Unters Forsch, 196: 423-429.

Kolkovski S. (2001). Digestive enzymes in fish larvae and juveniles implications and applications to formulated diets. Aquaculture, 200:181-201.

Lavens P., Leger P., Sorgeloos P. (1989). Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. In: De Pauw N., Jaspers E., Ackefors H., Wilkins N. (Eds), Aquaculture and Biotechnology in Progress. European Aquaculture Society, Bredene, Belgium. pp: 731-739.

Lavens P., Sorgeloos P. (1996). Manual on the Production and Use of Live Food for Aquaculture. University of Ghent, Ghent, Belgium. Published in Rome by FAO.

Ma Y., Liu Z., Yang Z., Li M., Liu J., Song J. (2013). Effects of dietary live yeast *Hanseniaspora opuntiae* C21 on the immune and disease resistance against Vibrio splendidus infection in juvenile sea cucumber Apostichopus japonicas. Fish and Shellfish Immunology, 34: 66-73.

Maget-Dana R., Peypoux F. (1994). Iturins, a special class of pore-forming lipopeptides: biological and physicochemical properties. Toxicology, 87: 151-174.

Magnelli P., Cipollo J., Abeijon C. (2001). A refined method for the determination of *Saccharomyces cerevisiae* cell wall composition andb-1,6-glucan fine structure. Analytical Biochemistry, 301:136e50

Maldonado-Montiel T.D.N.J., Rodriguez-Canche L.G., Olvera Novoa M.A. (2003). Evaluation of *Artemia* biomass production in San Crisanto, Yucatan, Mexico, with the use of poultry manure as organic fertilizer. Aquaculture, 219: 573-584.

McDonald P., Edwards R.A., Greenhalgh J.F.D. (2002). Animal Nutrition. 6th Edition. Longman, London and New York. 543 p.

Merrifield D.L., Dimitroglou A., Bradley G., Baker R.T.M., Davies S.J. (2010). Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquaculture Nutrition, 16: 504-510.

Munilla-Moran R., Stark J.R., Barbour A. (1990). The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus L.*). Aquaculture, 88: 337-350.

Naegel L.C.A. (1999). Controlled production of *Artemia* biomass using an inert commercial diet, compared with the microalgae *Chaetoceros*. Journal of Aquaculture Engineering, 21: 49-59.

Navarro J.C. Amat F. (1992). Effect of algal diets on the fatty acid composition of brine shrimp, *Artemia* sp. cysts. Aquaculture, 101: 223-227.

Nelis H.J.C.F., Lavens P., Van Steenberge M.M.Z., Sorgeloos P., Criel G.R., De Leenheer A.P. (1988). Qualitative and quantitative changes in the carotenoids during development of the brine shrimp *Artemia*. Journal of Lipid Research, 29: 491-499.

Niu Y., Defoirdt T., Baruah K., Van de Wiele T., Dong S., Bossier P. (2014). *Bacillus* sp. LT3 improves the survival of gnotobiotic brine shrimp (*Artemia* franciscana) larvae challenged with *Vibrio campbellii* by enhancing the innate immune response and by decreasing the activity of shrimp-associated vibrios. Veterinary Microbiology, 173(34): 279-88.

Norouzitallab P., Baruah K., Biswas P., Vanrompay D., Bossier P. (2016). Probing the phenomenon of trained immunity in invertebrates during a transgenerational study, using brine shrimp *Artemia* as a model system. Nature Scientific Reports, 6: 21166.

Ownagh E., Agh N., Noori F. (2015). Comparison of the growth, survival and nutritional value of *Artemia* using various agricultural by-products and unicellular algae *Dunaliella salina*, Iranian Journal of Fisheries Sciences, 14(2): 358-368.

Parmar P.V., Murthy H.S., Tejpal C.S., Naveen Kumar B.T. (2012). Effect of brewer's yeast on immune response of giant freshwater prawn, *Macrobrachium rosenbergii*, and its resistance to white muscle disease. Aquaculture International, 20: 951-964.

Philipp E.E.R., Lipinski S., Rast J., Rosenstiel P. (2011). Immune Defense of Marine Invertebrates: The Role of Reactive Oxygen and Nitrogen Species in Oxidative Stress in Aquatic Ecosystems. (eds Abele D., Vázquez-Medina J.P., Zenteno-Savín T.), John Wiley & Sons, Ltd, Chichester, UK. pp: 236- 246.

Piccioni M. (1965). Dictionnaire des aliments pour les animaux. Edagricole. 640 p.

Provasoli L., Kagehide S. (1959). Axenic cultivation of the brine shrimp *Artemia salina*. Biological Bulletin, 117: 347-355.

Raida M.K., Larsen J.L., Nielsen M.E., Buchmann K. (2003). Enhanced resistance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against Yersinia ruckeri challenge following oral administration of

Bacillus subtilis and *B. licheniformis* (BioPlus 2B). Journal of Fish Disease, 26: 495-498.

Reeve M.R. (1963). The filter-feeding of in *Artemia*. II. In suspensions of various particles. Journal of Experimental Biology, 40: 207-214.

Ringo E., Sinclair P.D., Birkbeck H., Barbour A. (1992). Production of eicosapentaenoic acid 20:5 n-3 by *Vibrio pelagius* isolated from turbot *Scophthalmus maximus* L. larvae. Applied Environment Microbiology, 58: 3777-3778.

Rojas-García C.R., Hasanuzzaman A.F.M., Sorgeloos P., Bossier P. (2008). Cell wall deficient Saccharomyces cerevisiae strains as microbial diet for *Artemia* larvae: protective effects against vibriosis and participation of phenoloxidase. Journal of Experimental Marine Biology and Ecology, 360(1): 1-8.

Rojas-García C.R., Sorgeloos P., Bossier P. (2009). Phenoloxidase and trypsin in germ-free larvae of *Artemia* fed with cooked unicellular diets: Examining the alimentary and protective effects of putative beneficial bacterium, yeast and microalgae against vibriosis. Journal of Experimental Marine Biology and Ecology, 381(2): 90-97.

Ronsivalli P.G., Simpson K.L. (1987). The brine shrimp *Artemia* as a protein source for humans. In: Sorgeloos P., Bengtson D.A., Decleir W., Jaspers E (Eds.). *Artemia* Research and its Applications. Ecology, Culturing, Use in Aquaculture, vol. 3. Universa Press, Wetteren, Belgium. pp: 503-514.

Rudneva I.I. (1999). Antioxidant system of Black Sea animals in early development. Comp. Biochem. Physiol. Part C, 122: 265-271.

Sanders M.E., Morelli L., Tompkins T.A. (2003). Sporeformers as human probiotics: Bacillus, *Sporolacto bacillus*, and *Brevi bacillus*. Comprehensive Reviews in Food Science and Food Safety, 12: 101-110.

Seixas P., Rey-Méndez M., Valente L.M.P., Otero A. (2010). High DHA content in *Artemia* is ineffective to improve *Octopus vulgarispara* larvae rearing. Aquaculture, 300(1): 156-162.

Skrodenyt_e-Arbaciauskiene V. (2007). Enzymatic activity of intestinal bacteria in roach *Rutilus rutilus* L. Fish Sciences, 73: 964-966.

Söderhäll K., Cerenius L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. Current Opinion in Immunology, 10(1): 23-8.

Sorgeloos P. (1982). Live animal food for larval rearing in aquaculture: the brine shrimp *Artemia*. Review paper presented at the World Conference on Aquaculture. Venice, Italy, 21-25 September, 1981.

Sorgeloos P., Baeza-Mesa M., Bossuyt E. Bruggeman E., Dobbeleir J., Versichele D., Lavina E., Bernardino A. (1980). Culture of *Artemia* on Rice Bran: The Conversion of a Wast-Product into Highly Nutritive Animal Protein, Aquaculture, 21: 393-396.

Suzer C., Coban D., Kamaci H.O., Saka S., Firat K., Otgucuoglu O., Kucuksari H. (2008). Lactobacillus spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth. Aquaculture, 280: 140-145.

Takahashi Y., Kondo M., Itami T., Honda T., Inagawa H., Nishizawa T. (2000). Enhancement of disease resistance against penaeid acute viraemia and induction of virus-inactivation in haemolymph of kuruma shrimp,*Penaeus japonicus*, by administration of Pantoea agglomeranslipopolysaccharide (LPS). Fish and Shellfish Immunology, 10: 555-563.

Touraki M., Karamanlidou G., Karavida P., Chrysi K. (2012). Evaluation of the probiotics Bacillus subtilis and *Lactobacillus plantarum* bioencapsulated in *Artemia* nauplii against vibriosis in European sea bass larvae (*Dicentrarchus labrax*, L.). World Journal of Microbiology and Biotechnology, 28(6): 2425-33.

Vahdat S., Esmaaeili Fereidouni A., Khalesi M.K. (2018). Long-term effects of vermicompost manure leachate (powder) inclusions on growth and survival, biochemical composition, total carotenoids, and broodstock reproductive performance of *Artemia franciscana* (Kellogg, 1906). Aquaculture International, 26: 569-588.

Vanhaecke P., Sorgeloos P. (1989). International study on *Artemia*. XLVII. The effect of temperature on cyst hatching, larval survival and biomass production for different geographical strains of brine shrimp *Artemia* spp. Annuals of Society of Royal Zoology, Belgium, 118: 7-23.

Verschuere L., Hanglamong H., Criel G., Sorgeloos P., Verstraete W. (2000). Selected Bacterial Strains Protect *Artemia* spp. from the Pathogenic Effects of Vibrio proteolyticus CW8T2. Applied and Environmental Microbiology, 66(3): 1139-1146.

Verschuere L., Rombaut G., Sorgeloos P., Verstraete W. (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology

Microbiology and Molecular Biology Reviews, 64(4), 655-671.

Viciano E., Monroig O., Barata C., Peña C., Navarro J.C. (2015). Antioxidant activity and lipid peroxidation in *Artemia* nauplii enriched with DHA-rich oil emulsion and the effect of adding an external antioxidant based on hydroxytyrosol. Aquaculture Research, 48(6): 1006-1019.

Wang Y., Gu Q. (2010). Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. Marine Biology Research, 6(3): 327-332.

Wang Y.B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei.* Aquaculture, 269: 259-264.

Wang Y.B., Xu Z. (2006). Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology, 127:283-292.

Wear R.G., Haslett S.J., Alexander N.L. (1986). Effects of temperature and salinity on the biology of *Artemia fransiscana* Kellogg from Lake Grassmere, New Zealand. 2. Maturation, fecundity, and generation times, Journal of Experimental Marine Biology and Ecology, 98 (1-2): 167-183.

Worthington C. (1991). Worthigton Enzyme Manual Related Biochemical Freehold. New Jersey Worthington C. (1991). Worthigton Enzyme Manual Related Biochemical Freehold. New Jersey.

Wurtsbaugh W.A., Gliwicz Z.M. (2001). Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah, Hydrobiologia, 466: 119-132.

Yu B.P. (1994). Cellular defenses against damage from reactive oxygen species. Physiological Reviews, 74: 139-162.

Zhukova N.V., Imbs A.B., Yi L.F. (1998). Diet-induced changes in lipid and fatty acid composition of *Artemia salina*. Comp. Comparative Biochemistry and Physiology B, 120: 499-506.

Zmora O., Avital E., Gordin H.T. (2002). Result of an attempt for mass production of *Artemia* in extensive ponds. Aquaculture, 213: 395-400.

Zmora O., Shpigel M. (2006). Intensive mass production of *Artemia* in recirculated system. Aquaculture, 255: 488-494.

Zorriehzahra M.J., Delshad S.T., Adel M., Tiwari R., Karthik K., Dhama K., Lazado C.C. (2016). Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Veterinary Quarterly, 36(4): 228-241.