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Evaluation of growth and survival rate of *Artemia parthenogenetica* feed with micro algae *(Isochrysis galbana* and *Chlorella vulgaris*) and bakery yeast *(Saccharomyces cerevisiae)*

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Abstract. This study was done to evaluate growth and survival rate of Maharloo lake artemia (*Artemia parthenogenetica)* (Bowen & Sterling, 1978) which feed with two species of microalgae (*Isochrysis galbana* and *Chlorella vulgaris*) and bakery yeast (*Saccharomyces cerevisiae*) with different nutritious ingredients for 15 days. We evaluated them in 3rd, 7th, 11th and 15th days of cultivation period for 4 times. This experiment was done in completely randomized design with 4 treatments (3 treatments and 1 control) and each treatment has 3 replicates. *Artemia parthenogenetica* nauplii were feed with three different types of food that includes *Isochrysis galbana* microalgae (T1), *Chlorella vulgaris* (T2) and *Saccharomyces cerevisiae* yeast (T4). Control had feed with blend of these three matters. After 15 days the highest survival rate was observed in control (84.00) and the lowest one was related to the T4 (59.58) which feed with *Saccharomyces cerevisiae* yeast ($p<0.05$). The highest growth rate was observed in T4, T3, followed by T1 and T2 respectively. Achievement results showed significant differences between control and other treatments ($p<0.05$). This study proved that treatments which feed with blend of two micro algae's species and bakery yeast have higher survival ability than the other treatments.

Key word: *Artemia parthenogenetica, Chlorella vulgaris, Isochrysis galbana, Saccharomyces cerevisiae.*

چکیده: این تحقیق به منظور بررسی رشد و میزان بقاء آرتمیا پارتنوژنز (Bowen and Sterling, 1978) دریاچه مهارلو تغذیه شده با دو نوع جلبک تک سلولی (*Isochrysis galbana* و *Chlorella vulgaris*) و مخمر ساکارومایسیس سرویزیا با ارزش غذایی متفاوت، به مدت 15 روز به ترتیب (روزهای: 3، 7، 11و 15) انجام گردید. این آزمایش در غالب طرح کاملا تصادفی با چهار تیمار (سه تیمار آزمایشی و یک تیمار شاهد) وهر کدام با سه تکرار صورت پذیرفت. ناپلی آرتمیا پارتنوژنتیکا در سه تیمار آزمایشی به ترتیب توسط ؛ جلبک ایزوکرایسیس گالبانا (T_1)، جلبک کلرلا ولگاریس (T_2) و مخمر ساکارومایسس سرویزیا (T_3) تغذیه شدند. تیمار شاهد به صورت توام توسط جلبک ایزوکرایسیس گالبانا، جلبک کلرلا ولگاریس و مخمر ساکارومایسس سرویزیا مورد تغذیه قرار گرفت. پس از 15 روز بالاترین درصد بازمندگی در تیمار شاهد (84.00) و کمترین آن مربوط به تیمار آزمایشی تغذیه شده با مخمر ساکارو مایسیس سرویزیا (59.58) بود($P<0/05$). در این آزمایش بالاترین نرخ رشد به ترتیب در تیمار شاهد، T_3، T_1و T_2 مشاهده گردید. نتایج به دست آمده از این آزمایش نشان داد که اختلاف معنی داری بین تیمار شاهد و تیمارهای آزمایشی وجود داشت ($P<0/05$). به طور کلی این آزمایش نشان داد که قدرت زیست و ماندگاری آرتمیا پارتنوژنز تغذیه شده به صورت توام با دو نوع جلبک (ایزوکرایسیس گالبانا، کلرلا ولگاریس) و مخمرساکارومایسیس سرویزیا نسبت به تیمارهای آزمایشی T_1، T_2و T_3 بسیار بالا است.

Introduction. *Artemia parthenogenetica*, the brine shrimp, are the most widely used aquaculture live food organism for marine larvae, primarily because they are very convenient to use and are readily available. The culture of larvae of many species of fish and crustaceans is highly dependent upon the availability of live food, whether plant or animal. The newly hatched nauplii of *Artemia* have generally served as an excellent source of food for larvae of many species of fish and crustaceans. However, live *Artemia* nauplii are obtained through hatching of cysts that are collected from the natural environment and subject to periodic, unpredictable shortages that cannot supply the demand. High reproduction ability and easy cultivation in experimental environments has made *Artemia* as significant live food (Coutteau 1996). For live food cultivation, experts advised some methods. These methods are containing an easy instruction for reducing the price of food preparation. *Artemia* nauplii are the best available live food that wildly use as shellfish and marine fish larvae's food (Lavens & Sorgeloos 1986). Artemia can be play role of vector in bioencapsulation form to transfer different material same as

nutritious blend (Watanabe et al 1983), antibiotics (Dixon et al 1995) and any type of vaccines (Campbell et al 1993) to target body. For improvement of nutritional features using live food especially *Artemia* is an essential matter. One of the strategies for improvement of nutritional efficiency is feeding *Artemia* with microalgae such as *Chaetocerus*, *Chlorella* and *Nannochloropsis* (Lavens & Sorgeloos 1991) and bakery yeast (*Saccharomyces cerevisiae)*. *Chlorella vulgaris* is rich in vitamin E (Tocopherol) and vitamin B_3 (Niacin) (Vazhappilly & Chen 1998). This microalgae has fatty acids (6.7%) and protein (43-44%). *Isochrysis galbana* contains (40-45%) protein and (24-26%) fatty acids (Liu & Lin 2001).

Yeast is a live and useful microorganism that can be cultured in different environments (Gatesoupe et al 2005). Industrial yeasts usually use in aquaculture as probiotic or as nutritional matters in aquatic nutrition. As an example, we can mention here the bakery yeast (*Saccharomyces cerevisiae*) (Stones & Mills 2004). Last studies showed that use of bakery yeast can be useful for security responses and improvement of growth in different aquatic species, especially in *Artemia* (Siwicki et al 1994; Olivia-Teles & Goncalves 2001). Use of microalgaes can be improved fatty acid of artemia, beside use of bakery yeast can be improved PUFA's in artemia body (Volkman et al 1989; Vazhappilly & Chen 1998). In this study we have evaluated growth rate and survival rate of *Artemia parthenogenetica* that feed with two algae species (*Chlorella vulgaris* and *Isochrysis galbana*) and bakery yeast (*Saccharomyces cerevisiae*).

Material and Methods. For micro algal cultivation we used batch growing system. They were cultured in Artemia Research Center (Uremia-Iran) as indicated in Table 1. At the end of cultivation they were compressed with centrifuge and then cells were counted with special lame. Their density was 18×10^6 cell mL^{-1} (see also Triantaophllidis et al 1998).

Table 1

Microalgae species and their cultivation features in laboratory in Walen cultivation environment

Algae	Salinity (ppt)	pH	Temperature °C	Light (Lux)
Isochrysis galbana	25	7-9	30 -35	2500
Chlorella vulgaris	25	7-9	30 - 35	2500

The *Artemia* cysts that used in this study originated from Maharloo Lake (Fars province, Iran). Their content was encapsulated using chemical way (Sorgeloos et al 1977). They were placed into conical glass with 1 L capacity. Water of incubation was sea water with 35 ppt salinity. One gram of *Artemia parthenogenetica* cyst was incubated in a one liter conical incubator with salinity of 35 ppt, at 26°C, with strong aeration and illumination with 2500 lux intensity for 24 hours (according to Gomez-Gil et al 1998). After 24 hours cysts were hatch and nauplii were separated from cysts skin's with positive phototropism effect. After filtration and concentration of nauplii, 400 of them were transferred to the conical glasses that prepared for experiment with 70 ppt salinity. On third day of study survival rate of nauplii was calculated. This process was done with 150 µm mesh. After nauplii filtration and counting microalgae that would be use as food were counted with Neubauer lame. *Isochrysis galbana* cells were counted in center of lame and *Chlorella vulgaris* cells were counted in around of lame. For counting of algae's cells, 5 mL of each microalga were added in experimental pipes and they were fixed with 1 or 2 drops of Formalin (5%). Before counting of microalgae cells we have to wash and dry Neubauer lame. After concentration of algae to 18×10^6 cells mL^{-1} they used as food for T1 and T2 treatment lots. For bakery yeast preparation, first we added 4 grams of yeast into the 600 mL of 50 ppt salt water and then kept it under aeration condition for 20 minutes. We

feed T3 with 0.8 mL of this yeast suspension. Control treatment was feed with blend of micro algae's and bakery yeast. *Artemia* survival rate and length examine was control respectively in 3rd, 7th, 11th and 15th day of experiment (Triantaphllidis et al 1998). At the end of study, percent of live nauplii were compared with initial nauplii. Growth rate was tested with length measurement (length of body from head to last pectoral band). A percentage of 30% from each treatment were randomly chosen for this process. Length of *Artemia* was measured with microscope that had micrometer in the third day and after that they were measured with Digitizer. All data were analyzed by SPSS program, Duncan exam and one way variance.

Table 2

The control and experimental treatments feeding

Nutrition type	**Number of *Artemia* per tank**	**Experimental treatments**
Saccharomyces cerevisiae & *Isochrysis galbana* & *Chlorella vulgaris*	400 individuals	Control
Isochrysis galbana	400 individuals	T_1
Chlorella vulgaris	400 individuals	T_2
Saccharomyces cerevisiae	400 individuals	T_3

Result. Results of survival rate of *Artemia parthenogenetica* were prepared by their feeding material separately in Table 3.

Table 3

Survival percent of *Artemia parthenogenetica* feed with four different types of microorganisms

Day / Treatments	**3**	**7**	**11**	**15**
Control	96.92± 0.38 [a]	93.00±0.75 [a]	89.17± 0.76 [a]	84.00±0.66 [a]
1	96.83± 0.14 [a]	93.08± 1.26 [a]	86.00± 0.90 [b]	80.50±1.15 [b]
2	87.83± 1.01 [b]	83.67±0.63 [b]	78.50± 1.25 [c]	68.17±1.13 [c]
3	84 .25± 0.75 [c]	78.17± 0.63 [c]	73.08±0.88 [d]	59.58 ± 1.53 [d]

After information evaluation difference survival rate and growth rate were observed in different treatments of animals that feed with different types of food. There was significant difference between control treatment that feed with blend of microalgae and bakery yeast and T2, or T3, that feed with different microalgae species alone ($P<0.05$). This result was observed in third and seventh experimental day. After seventh day there was significant difference between control and all other treatment lots ($P<0.05$). The best survival percent was related to control (84.00) that feed with blend of algae and bakery yeast. The lowest survival percent was related to T3 (59.58) that feed just with bakery yeast. There was no significant difference between T1 that feed with *Isochrysis galbana* and T2 feed with *Chlorella vulgaris* ($P>0.05$). Different nutrition has serious effect on survival rate and growth. In seventh and third days there was no significant difference between T1 that feed with *Isochrysis galbana* and control in survival rate ($P>0.05$) but after seventh day we observed significant difference between them ($P<0.05$).

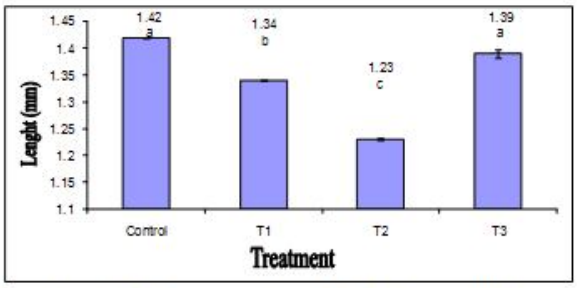

Fig. 1. Length of *A. parthenogenetica* in the third experimental day

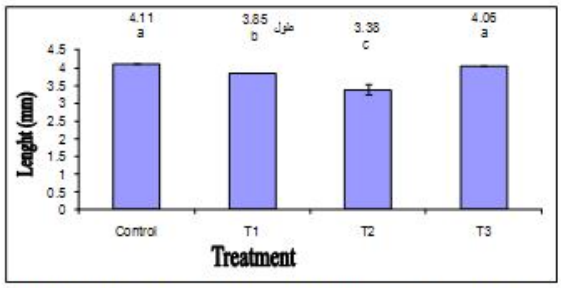

Fig. 2. Length of *A. parthenogenetica* in the seventh experimental day

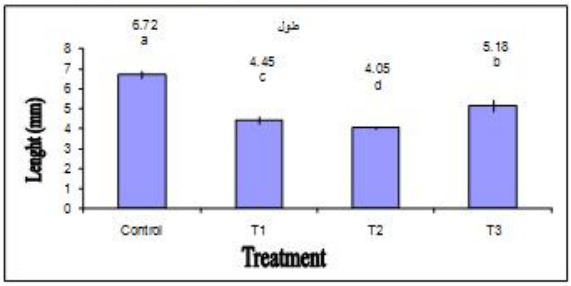

Fig. 3. Length of *A. parthenogenetica* in the eleventh experimental day

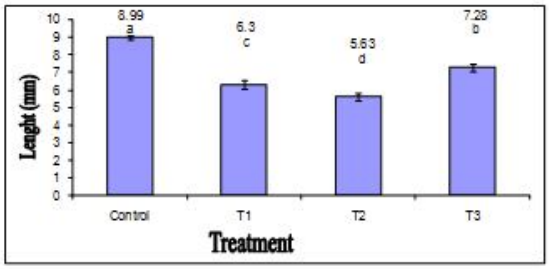

Fig. 4. Length of *A. parthenogenetica* in the fifteenth experimental day

Length examine of *Artemia parthenogenetica* on 3rd day until 15th day were explained in Figs 1-4. By this results there was no significant difference between T3 and control on 3rd and 7th day ($P>0.05$). But after reviewing analyze on 11th and 15th days significant difference was observed ($P<0.05$). On 11th day the highest growth rate was related to control treatment (6.72 mm) and the lowest was related to T2 (4.05 mm). On 15th day there were significant difference between control treatment (8.99 mm) and T1 (6.30 mm), T2 (5.63 mm) and T3 (7.28 mm). The best feeding operation that causes the highest growth and survival rate was observed in control treatment that feed with blend of algae and bakery yeast.

Discussion. Some kinds of microalgae and bakery yeast have nutritional matters that cause improvement of growth rate and survival rate. In different references we found different density of algae that use for feeding of *Artemia*. In this study, density of microalgae was 18×10^6 cell mL^{-1} (Abdul-Elah et al 2001). In other studies about algae and their use for rotifer feeding, density of *Isochrysis galbana* and *Chlorella vulgaris* were 700×10^3 - 700×10^6 cell mL^{-1}. Evjemo et al (2000) reported that *Artemia franciscana* cultured for 11 days at 26 to 28°C, 34 ppt salinity and density of 10 mg L^{-1} has the best growth and survival rate. Our study showed that a different diet which has different nutritional price has different effect of growth and survival rate. *Isochrysis galbana* has high nutritional price due to improvement of growth rate in aquatic animals (Avendano & Riquelme 1999). Phytoplankton that contains microalgae has high level of nutrient (Hatton & Wilson 2007). Recent studies showed that use of microalgae such as *Chlorella*, *Nannochloropsis* and *Chaetocerus* due to improvement of PUFA fatty acids on consumers body (Lavens & Sorgeloos 1991). At the end of experiment the best length examine was related to control treatment (8.99 mm) and after that related to T3 (7.28 mm), T1 (6.30 mm) and T2 (5.63 mm) respectively. This study has shown that growth rate of *Artemia parthenogenetica* which feed with different diets had different result. At 15th day the highest survival percent was related to control (84.00) and the lowest was related to T3 (59.58). Sahandi et al (2010) reported that rotifer treatment that feed with bakery yeast had developed well but after period of time decreased. They explained that this mortality was related to fecal that released by rotifers. In recent studies researchers pointed to the fatty acids that existed in microalgae and this cause improvement of PUFA in body of artemia and any organism that consume artemia (Chakraborty et al 2007). In our study we evaluated effect of different diets on growth and survival rate of *Artemia parthenogenetica*. For this goal we experienced four treatments, and one of them was

control and feed with blend of two microalgae and bakery yeast. This study has take 15 days long and at the end control has shown the best growth and survival rate.

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