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Comparison of the growth, survival and nutritional value of *Artemia* using various agricultural by-products and unicellular algae *Dunaliella salina*

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Abstract

Because of limitations of production of unicellular green algae (especially in large volumes), this study aimed to culture *Artemia* using three sources of cheap agricultural by-products that were coupled with small amounts of unicellular algae *Dunaliella salina.* The results of growth and survival, biomass production, Individual wet weight, wet and ash percent, FCR and SGR and nutritional value of experiments groups were compared with that of the control group that was reared on a diet completely consisting of green algae. At the end of day 15, although best results in the case of growth and survival and biomass production were obtained in the control, the results of all evaluated parameters experimental treatments were comparable to the control.

Keywords: *Artemia urmiana*, Parthenogenetic *Artemia*, Biomass, Growth and survival, Nutritional value

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Introduction

Although *Artemia* nauplii is the most used stage of *Artemia* in aquaculture, there is an increasing demand for its juvenile and adult stages (called *Artemia* biomass) (Naegel, 1999) to induce ovarian maturation of shrimp (Naessens *et al*., 1997) and as a good food source for ornamental fish (Zmora *et al*., 2002).

A. urmiana was first reported in Lake Urmia by Günter in 1900. Most recently Agh *et al*., 2007 confirmed the presence of a parthenogenetic population of *Artemia* coexisting with *A. urmiana* in Lake Urmia. Also a parthenogenetic population of *Artemia* was reported from small lagoons in the vicinity of Lake Urmia by Agh and Noori (1997). The real success in the mass culture of *Artemia* lies in the identification of a good but cheap substitute food source. Different live and dried unicellular algae like *Dunaliella* (Vanhaecke and Sorgeloos, 1989; Coutteau *et al*., 1992) are commonly used as food for *Artemia*. However the cost and laborious task of producing unicellular algae are considered as major limitations in the mass culture of *Artemia* using this source (Naegel, 1999; Hoa *et al*., 2007). Substitutes like wheat bran, rice bran and soybean meal (Dobbeleir *et al*., 1980; Sorgeloos *et al*., 1980) for microalgae have been used successfully. However low growth and survival was obtained when these foods were used as the sole diet (Dobbeleir *et al*., 1980). Considering big difference in the price of soybean than wheat bran and the high demand for soybean in human nutrition, the aim of this study was to produce *Artemia* using a pre-optimized concentration/combination from three sources of agricultural by-products and small quantities of green algae. At the end of the experiment the effects of this food replacement were assayed on growth and survival, biomass production and nutritional value of two strains of Iranian native *Artemia*.

Materials and Methods

Culture condition

This study was conducted at *Artemia* and Aquatic Animal's Research Institute of Urmia University of Iran. Cysts of both strains of *A. urmiana* and parthenogenetic *Artemia* (strain from lagoons around Lake Urmia) were hatched according to Sorgeloos *et al*. (1986). 6000 newly hatched nauplii from each *Artemia* strain, were separately transferred into glass bottles containing 6 liters of diluted Urmia lake water set at 80 ppt, and cultured for 15 days at 28±0.5 °C under light/dark condition of 12/12 hours. On days 8, and 11 water renewals were performed.

Feeding treatments

Manually prepared suspensions (Sorgeloos *et al*., 1980) of wheat bran, soybean and their 50/50 mixture (based on their dry weight), combined with small amounts of unicellular algae *D. salina*, (using an optimized concentration/combination that was obtained by a preliminary test) with three replications for each, were our experimental treatments. A standard feeding regimen using *D. salina* was used as the control group (Coutteau *et al*., 1992). The feeding schedule used in this study for all experimental treatments is summarized in Table 1. Daily increase in feeding rate (for both inert and live food) was adopted from standard feeding table for *Artemia*

(Coutteau *et al*., 1992) until the end of the experiment, as summarized in Table 2. Since *Artemia* were fed under standard laboratory conditions using a diet completely relying on single-celled algae or a combination of 25% and 75% for single-celled algae and coated yeast (Lanzy PZ) respectively, in this study, not only did we try to completely replace the yeast with agricultural wastes, but the percentage of used algae in each treatments was decreased.

Table1: Experimental treatments along with feeding amounts of each food source for 20 nauplii on the first day. (% repl. indicates the percent replacement of *algae* in comparison to the control, algae concentration is 18 000 000 cell/ml).

treatments	**wheat bran+*D.salina***			**soybean+*D.salina***			**wheat bran/ soybean +*D.salina***		
Strain	Wh.b. (mg)	*Algae* (ml)	% Repl.	Soya. (mg)	*Algae* (ml)	% Repl.	Wh.b./Soya. (mg)	*Algae* (ml)	% Repl.
A. urmiana	0.416	0.015	91	0.276	0.01	94	0.276	0.01	94
Artemia	0.554	0.020	88	0.416	0.015	91	0.554	0.02	88

Table 2: Food additive ratios used in different treatments of each food source (Coutteau *et al*., 1992).

	Day							
	2-3-4	5-6	7	8	9	10-11	12-13	14-15
Feeding increase	1.97	1.51	1.3	1.28	1.6	1.17	1.25	1.2

At the end of the experiment, number of surviving animals and their total length in each replication of each treatment, was determined by sub sampling of water column. The average length of 30–40 animals from each replication fixed in Lugol's solution was determined by drawing them from the top of the head to the end of the telson (Amat, 1980) by using a light microscope equipped with a phototube and micrometer. Drawings were later digitized using a digitizer connected to a computer.

Biomass accumulated during the culture period, was weighed in each replicate of treatments, separately.

Feed conversion ratio (FCR) for experimental treatments and specific growth rate (SGR) were calculated (Lavens and Sorgeloos 1991):

FCR= Food (mg dry weight inert diet) / *Artemia* biomass (mg wet weight)

SGR= (Ln W2- Ln W1) *100 /culture period

W2: Artemia wet weight at the end of the culture period

W1: Artemia nauplii wet weight at day 1

The proximate composition of cultured *Artemia* was obtained as follows: (a) wet weight: accurate numbers of harvested *Artemia* were washed carefully with tap water to eliminate the food particles, and then weighed after draining. (b) Wet percent, ash percent: these samples were dried in an oven at 60˚C and ashed at 500˚C in a furnace for 5 h. (c) Protein, lipid, and fatty acid profile of used inert foods and

produced *Artemia* biomass were determined according to the methods recommended by A.O.A.C. (1984).

After testing for normality (Shapiro-Wilk), the data were analyzed to determine differences in the treatments by one-way ANOVA, using Duncan's test.

Results

Table 3 shows the growth and survival of both strains of *Artemia* on different experimental treatments and control. Average length of 0.5 mm and 0.455 mm for newly hatched nauplii of *A.urmiana* and parthenogenetic *Artemia* respectively, showed an increase of about 14 times in total length and reached a size of 7.76 mm in treatment of mixed wheat bran/soybean during a 15 day culture period, that showed no significant differences with its control (7.82 mm) (Table. 3). A survival of 86.3, 70.3, 58.6 and 69.53 percent for *A.urmiana* and 76.5, 68.5, 67.6 and 66.8 percent for parthenogenetic *Artemia* was obtained in the control, and experimental groups fed wheat bran, soybean and a mix of wheat bran/soybean respectively, were obtained at the end of the experiment. Although the control group showed higher survival rate, no statistical differences were detected with experimental treatments in majority of the cases ($p>0.05$).

Table 3: Mean growth and survival (±SD) of *Artemia* at day 15 in different treatments ($p<0.05$).

	Artemia urmiana		**Parthenogenetic *Artemia***	
Treatments	Survival	Growth	Survival	Growth
Control	86.3 ± 8.08^{a}	7.82 ± 0.27^{a}	76.5 ± 3.77^{ab}	7.08 ± 0.22^{b}
Wheat bran	70.3 ± 19.63^{ab}	7.1 ± 0.22^{b}	68.5 ± 11.30^{ab}	6.04 ± 0.29^{c}
Soybean	58.6 ± 7.31^{b}	7.26 ± 0.22^{b}	67.6 ± 6.04^{ab}	6.46 ± 0.21^{c}
Wheat bran/Soybean	69.53 ± 8.35^{ab}	7.76 ± 0.17^{a}	66.8 ± 3.68^{b}	6.23 ± 0.23^{c}

*similar letters in same parameters show no significant differences ($p>0.05$).

Biomass increased from 60 mg (initial total weight of 6000 nauplii) to 7018, 5579, 4571 and 5305 mg for *A. urmiana* and 6544, 5036, 5459 and 6490 mg for parthenogenetic *Artemia* in the control group and the wheat bran, soybean and mixture of wheat bran/soybean treatments, respectively (Fig. 1). Although highest biomass production was recorded for control grope in each strain, these values showed no significant differences with biomass produced for parthenogenetic *Artemia* in the mixed wheat bran/soybean treatment. Also the results of other treatments (except for soybean treatment of *A. urmiana* and wheat bran treatments of parthenogenetic *Artemia*) were satisfactory in comparison to the control.

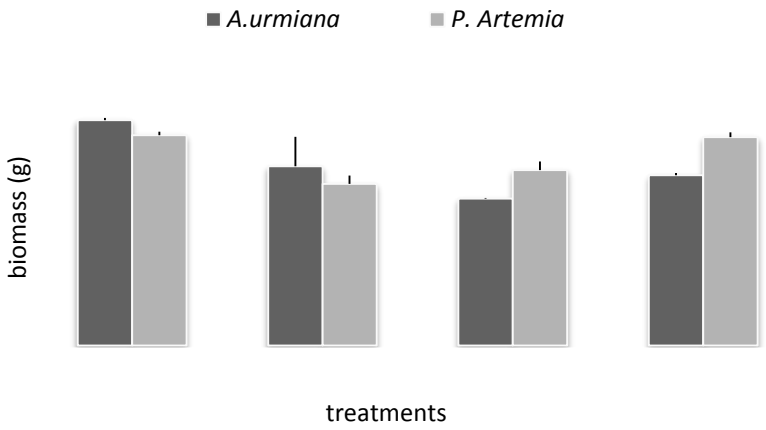

Figure 1: Biomass production (means±SD) of *Artemia urmiana* and parthenogenetic *Artemia*.

Results obtained for individual wet weight, FCR and SGR in different treatments of both strains of *Artemia*, are summarized in Table 4. As it was revealed, highest average individual wet weight of 1.61 mg was recorded in parthenogenetic *Artemia* grown on mixture of wheat bran/soybean. This value had no significant differences with that of the control in both strains of *Artemia* and also with that of parthenogenetic *Artemia* in the soybean treatment (p>0.05). Among experimental treatments of these two strains of *Artemia*, no significant difference was observed for feed conversion ratio (p>0.05).

Table 4: Mean±SD of individual wet weight, FCR, SGR of *Artemia* on day 15 in different treatments.

	A. urmiana			**Parthenogenetic *Artemia***		
Treatments	Ind. Wet weight (mg)	FCR	SGR	Ind. Wet weight (mg)	FCR	SGR
Control	1.361±0.10^{ab}	-	33.1±0.19^{ab}	1.429±0.11^{ab}	-	33±0.23^{ab}
Wheat bran	1.320±0.01^{b}	0.21±0.001^{a}	32.3±1.90^{ab}	1.254±0.27^{b}	0.23±0.05^{a}	31.3±1.00^{b}
Soybean	1.314±0.16^{b}	0.22±0.028^{a}	33.6±1.95^{a}	1.356±0.21^{ab}	0.21±0.03^{a}	31.4±1.36^{ab}
Wheat bran/Soybean	1.262±0.13^{b}	0.23±0.023^{a}	33.1±0.55^{ab}	1.619±0.02^{a}	0.17±0.002^{a}	33.3±0.26^{ab}

*similar letters in same parameters show no significant differences (p>0.05).

Highest SGR was recorded for soybean treatment of *A. urmiana*, although this value showed no significant differences with other treatments in both strains, except with wheat bran treatment of parthenogenetic *Artemia*.

Mean wet and ash percent of produced *Artemia* biomass in different treatments are summarized in Table 5. As it was revealed, there were no significant differences between treatments in most of the cases.

Table 5: Mean±SD of wet and ash percent of *Artemia* in different treatments on day 15.

Treatments	*A. urmiana*		Parthenogenetic *Artemia*	
	Wet percent	Ash percent	Wet percent	Ash percent
Control	88.2±0.2[a]	9.9±0.99[a]	88.2± 013[b]	10.1±1.09[b]
Wheat bran	88.9±1.74[a]	12.21±1.31[a]	87.8±0.28[b]	12.4±0.79[a]
Soybean	87.1±1.01[a]	10.29±2.34[a]	89.9±0.22[a]	13.3±0.47[a]
Wh.b. /Soybean	88.7±0.41[a]	10.13±1.7[a]	89.4±0.04 [a]	10±1.35[b]

*similar letters in same parameters show no significant differences (p>0.05).

Table 6 shows the nutritional data of *Artemia* reared on different experimental treatments compared with *Artemia* grown on unicellular algae *D. salina*. The proximal analysis revealed that highest protein content (%) was recorded for soybean treatment in both strains, that was significantly higher than those for the control (Table 5) (p<0.05). Significant differences were observed in lipid content between treatments of both strain, with highest lipid content of 25.02 percent recorded for wheat bran treatment of *A. urmiana* that was significantly higher than all experimental treatments and the control group. However no differences were found in lipid content between control treatments of *A. urmiana* and parthenogenetic *Artemia* (p>0.05).

Table 6: Proximate analysis (% dry matter), wet and ash percent of both strains of *Artemia* in experimental treatments, and with *Dunaliella salina* on day 15 (mean±SD).

	A. urmiana				**Parthenogenetic *Artemia***			
parameters	Control	Wheat bran	Soybean	Wh.b. / soya.	Control	Wheat bran	Soybean	Wh.b. / soya.
%Wet	88.2±0.35[ab]	88.9±3.02[ab]	87.1±1.75[b]	88.7±0.71[ab]	88.2±0.23[ab]	87.8±0.5[ab]	89.9±0.38[a]	89.4±0.07[ab]
%Ash	9.9±0.99[b]	12.21±1.3[ab]	10.29±2.3[b]	10.1±1.7[b]	10.11±1.09[b]	12.4±0.8[ab]	13.3±0.47[a]	10.02±1.35[b]
%Protein	45.7±1.1[c]	42.9±0.9[d]	48.8±0.9[b]	42.7±1.0[d]	46.6±0.8[c]	49.3±1.4[ab]	51.1±1.9[a]	46±1.4[c]
%Fat	7.51±0.08[e]	25.02±1.1[a]	13.61±0.7[c]	15.34±0.2[b]	8.11±0.14[e]	5.33±0.10[f]	11.84±0.65[d]	11.05±0.39[d]

*similar letters in same parameters show no significant differences (p>0.05).

Effects of different diets (wheat bran, soybean, mixed of wheat bran/soybean and control) on fatty acid profile of two strains of *Artemia* on day 15 are summarized in Table 6. As it was shown, acid linoleneiec (18:3n-3) and linoleic (18:2n-6) were found in all treatments of both strains, except in soybean treatment of *Artemia urmiana* which was similar to that of the control (Table 7). As it is clear from the table no values for EPA and DHA were reported in all treatments for both strains.

Table 7: Fatty acid profile of *Artemia* cultured on different treatments on day 15 (mg / gr. wet weight of *Artemia*).

Treatments	**Control**		**Wheat bran**		**Soybean**		**Wheat bran/Soybean**	
Fatty acid	A.u.	A.p.	A.u.	A.p.	A.u.	A.p.	A.u.	A.p.
C 14:0	0.411	1.364	0.983	0.521	4.902	0.239	0.971	0.611
C 14:1n5	0.071	0.956	0.502	0.672	1.144	0.275	0.240	0.456
C 16:0	3.900	11.607	5.379	4.220	23.370	3.858	6.142	3.674
C 16:1n7	0.460	1.811	1.848	1.280	10.295	0.864	2.316	0.997
C 18:0	1.619	4.266	1.620	2.076	6.149	2.643	2.295	1.543
C 18:1n9	1.805	4.879	3.839	4.527	11.109	4.707	5.555	2.790
C 18:1n7	1.012	3.496	1.827	2.126	6.975	2.712	2.781	1.283
C 18:2n6	1.994	9.709	4.158	7.587	17.53	7.252	7.849	4.672
C 18:3n3	2.415	3.577	1.655	1.541	-	1.531	2.476	0.954

A.u.: *Artemia urmiana* , A.P.: parthenogenetic *Artemia*

Discussion

For each kind of microalgae or inert food, a specific feeding regime for *Artemia* has to be developed to ensure adequate feed levels (Naegel, 1999). Mason (1963) demonstrated that the amount of feed available per animal is the most important variable affecting the growth of *Artemia*. The feeding regime developed in this study for the inert feed of cheap agricultural by-products resulted in good growth and satisfactory survival rates. Although best results in terms of growth, survival and biomass production were obtained in the control, results of experimental treatments in terms of these features in some cases and in terms of other evaluated parameters including individual wet weight, wet percent, FCR and SGR in most of the cases, were comparable to that of the control. However, the time needed for renewing the water medium and cleaning the culture systems of unconsumed feed was significantly higher and more difficult in the experimental treatments than in those fed microalgae. The survival rate after 15 days of culture obtained in this study (70.3% for *A. urmiana* and 68.5% for parthenogenetic *Artemia* using wheat bran) was comparable to the data reported by Naegel (1999). He obtained survival rates of 72%, 79% and 73.5% for *A.franciscana* reared for 11 days using a commercial inert diet of Nestum (a baby food), enriched Nestum and microalgae *Chaetoceros* sp. (at a density of 2 organisms/ml in a 10 liter bottle) respectively, as feed. Although highest growth rates of 7.82 and 7.76 mm were recorded for *A. urmiana* in the control group and in the experimental group fed a mix of wheat bran/soybean, growth values recorded in all experimental treatments were higher than 4.93, 5.02 and 4.64 mm growth of *A. franciscana* cultured for 11 days using a commercially inert diet and *Chaetoceros* sp. (Naegel, 1999).

Agh *et al*. (2008 b) obtained a survival and growth rate of 74.2% and 8.5 mm, respectively for *A. urmiana* and 72.8 % and 7.1 mm, respectively for parthenogenetic *Artemia* under standardized laboratory conditions. In other studies while Agh *et al*. (2008a) reported a survival of 75% and 85% for *A. urmiana* and this strain of parthenogenetic *Artemia*, their growth rate was 7-8 mm at the end of day 15. In both experiments they used a unicellular algae and coated yeast (lansy pz). These results were slightly higher than those obtained in the present study.

During 15 days of culture in a volume of 6 liter, 6.48 mg of biomass could be produced by parthenogenetic *Artemia* using about 1160 mg dry weight of mixed wheat bran/soybean as feed, that showed a FCR of 0.17. This biomass was much better than 3097 and 4883 mg biomass in 10 liter produced for *A. franciscana* at the density of 2 organisms/ml using an inert diet (Naegel, 1999). The biomass of 1.8 gr/liter of dried *Spirulina* at a stocking rate of 6 nauplii/ml was obtained in 15 days by Espinoza- Fuentes *et al*. (1997). Since their density was much higher than this trial, the biomass obtained in this trial (1.08 gr/l.) was comparable to their results and the food used was much cheaper than the *Spirulina*. Although it is risky to extrapolate production data from a 6-l, short term laboratory experiment to an annual production in a 1000-l tank, our system will have the potential for *Artemia* biomass

production of more than 1 kg/m^3 of *Artemia* in only 15 days (parthenogenetic *Artemia* on mix of wheat bran/soybean). Moreover these results can be much better by increasing the stocking density. Teresita *et al*. (2003) reported a food conversion ratio of 0.25 in *Artemia* reared using rice bran and green algae *Tetraselmis suecica* under laboratory conditions in 1.5-liter bottles. Zmora and Shpigel (2006) obtained a food conversion ratio of 0.17 to 0.25 in a recirculated system and a FCR of 0.75 in earthen ponds with a diet combined of green algae, troll a yeast and soybean powder (without green algae). The values obtained for FCR in this study with non-live food sources (only on inert diet), were highly acceptable and are in the category of best achieved FCR by different researchers so far. This can be related to the digestion and absorption performance of non-live food particles (Zmora and Shpigel 2006) that in this study coupled with small amounts of green algae.

Naegel (1999) reported an individual wet weight of 1.63 mg for *A. franciscana* reared on an inert diet of Nestum. Individual wet weight of 1.61 mg in parthenogenetic *Artemia* fed a mix of wheat bran/soybean was comparable with the results of these researchers. Teresita and Leticia (2004) reported an ash content of 15.4, 19.1, 8.7, 10.77 and 33.9 percent based on their dry weight belonging to the groups of *Artemia* reared on rice bran and *T. suecica*, dried *Spirulina*, wet *Spirulina*, rice bran at the end of day 15 and wild *Artemia* grown in nature, respectively. These results of ash content, especially of groups grown on rice bran, are similar to those obtained in this study.

In this study both strains of *Artemia* fed on soybean had highest protein content. These results were in accordance to those of Manaffar *et al*. (2001) who showed highest protein content (66.84%) for *A. urmiana* reared on soya powder till day 7, although this value was lower than those obtained by these researchers. Agh and Hosseini Ghatre (2002) recorded a protein content of 52.25 % for adult *A. urmiana* fed on rice bran, that were similar to 51.1 % protein obtained in this study for parthenogenetic *Artemia* fed by soybean. Naegel, 1999 obtained a protein content of 56.4%, 42.87 % and 41.16 % for *A.franciscana* fed on Chaetoceros, Nestum and enrichment Nestum, respectively. Teresita and Leticia (2004) reported an amount of 53.1 % protein for *Artemia* reared on rice bran and *T.suecica*.

Khayami and Heidari (1995) reported a total fat content of 4.93 percent for wild *Artemia* biomass harvested from Lake Urmia. Similarly, Agh and Hosseini Ghatre (2002) reported a total fat content of 15.62 and 14.28 percent of dry weight for post metanauplii and adult stages of *Artemia*, respectively, reared on rice bran. Naegel (1999) reported a fat content of 16.45, 20.33 and 2.95 percent in adult *A. franciscana*, reared on Nestum (human's baby food), and enriched Nestum with fish oil and unicellular *algae*, respectively. In the present study, the total fat content among different treatments showed significant differences (p<0.05), but in most cases these results were comparable with results of other researchers.

Fatty acid profile of *Artemia* strongly reflects its nutritional value (Millamena *et al*., 1988). Watanab *et al*. (1987) showed

that freshwater fish require mainly 18:2 (n-6) and 18:3 (n-3) or both,. Both of these fatty acids were high in all treatments of this study. Based on findings of Agh and Hosseini Ghatre (2002), *A.urmiana* was very poor in EPA and DHA, and also it was confirmed in this study, that all subjects were lacking in these two important fatty acids. Due to the high amounts of PUFA (with 18 carbon chain) present in *A. urmiana*, and according to Watanabe *et al*. (1987), Agh and Hosseini Ghatre (1381) considered this strain of *Artemia* species suitable for feeding freshwater fish. In view of this parameter, our findings were consistent with those of these researchers, and the *Artemia* produced will be suitable for freshwater fish.

The reason of compensation of deficiency of fatty acid profile of food resources in the *Artemia* biomass produced can be related to the role of green algae that was added in small amounts to each diet as a food supplementation. Also it was confirmed that *Dunaliella* is rich in n-3 series of fatty acids, especially 18:3 (n-3) (Millamena *et al*., 1988).

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