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Effects of enriched *Artemia* with *Saccharomyces cerevisiae* and *Chaetoceros gracilis* on growth performance, stress resistance and fatty acid profile of *Litopenaeus vannamei* postlarvae

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Abstract
The present study was aimed to evaluate the effects of *Artemia* enrichment with baker's yeast *Saccharomyces cerevisiae* (Y) and *Chaetoceros gracilis* (C) on growth performance, stress resistance as well as fatty acid profile of *Litopenaeus vannamei* post larvae (PL) for 15 days. Newly hatched *Artemia franciscana* nauplii (N) served as a control group. Survival did not change among different experimental groups. PL in C and N groups had the highest (767.0± 117.0µg) and the lowest (367.0± 44.0 µg) dry weight, respectively, and PL in Y group showed intermediate value (567.0± 117.0µg). PL in Y and N groups had the highest (52.3 ± 2.9%) and the lowest (31.3 ± 2.8%) survival rate, when exposed to the fresh water stress test, respectively. The concentrations of eicosapentaenoic acid and docosahexaenoic acid were higher in *Artemia* enriched with *C. gracilis* than other groups. Moreover, the n-3 to n-6 PUFA ratio was significantly higher in PL fed C group than other treatments. In conclusion, feeding enriched *Artemia* with *C. gracilis* or *S. cerevisiae* can improve growth performance and stress resistance in *L. vannamei* PL.

Keywords: *Artemia,* baker's yeast, fatty acid profile, microalgae, white leg shrimp

1. Introduction
Various species of yeast, either in live form to feed live food organisms including probiotic live yeast, autolyzed yeast and yeast fractions (yeast cell walls or yeast extracts) are being developed as functional feed additives, or as a source for more purified products in aquaculture (Ferreira *et al.,* 2010) [11]. In addition, whole cell yeast or products containing different yeast cell wall fractions used as immunostimulants in fish and crustacean diets (Ringø *et al.,* 2012) [29]. In this regard, the beneficial effects of brewer's yeast (*Saccharomyces cerevisiae*), which contains various immunostimulating compounds such asβ-glucans, nucleic acids, chitin, mannan oligosaccharides and other cell wall components, have been reported on growth performance, stress and disease resistance in various fish and crustacean species (Ringø*et al.,* 2012) [29]. Moreover, it has also been used as probiotic enrichment agent for *Artemia*, because of its rapid growth, ease in its culture, rich source of enzymes, RNA and free nucleotides, B-complex vitamins, amino acids and appropriate cell diameter (Fazeli and Azari-Takami, 2006; He *et al.,* 2011) [10, 14].
Because of the smaller shelf life of the widely available commercial n–3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) supplements for enriching live food, there is a growing interest in marine microalgae, which contain high levels of these essential fatty acids. Furthermore, microalgae are used as a food source in hatcheries for larval stages of most species of interest for commercial aquaculture, and some strains of microalgae are recognized as excellent sources of proteins, carbohydrates, lipids, vitamins, essential fatty acids (EFA), pigments and sterols to be used as food and feed additives (Krienitze and Wirth, 2006) [18]. In addition, microalgae have an important role in aquaculture and also in the enrichment of zooplankton for feeding fish larvae. In this context, *Chaetoceros* sp. are reported as the best live feed for *Artemia* because of high levels of n-3 LC-PUFA especially eicosapentaenoic acid (EPA, 20:5n-3) (Lora-Vilchis*et al.,* 2004) [23]. *Artemia* nauplii constitute the principal animal

protein source used in commercial shrimp larviculture, because of their suitable biochemical composition and size, easy acceptance by the larvae and convenient storage as cysts (Sorgeloos *et al.,* 1998) [33]. However, *Artemia* nauplii are an incomplete diet for larvae of marine finfish and crustaceans, because of their paucity of EFA as well as nucleotides. Marine microalgae and baker's yeast are reported as good sources of EFA and nucleotides, respectively (Chakraborty *et al.,* 2007; He *et al.,* 2011) [5, 14]. Thus, the objective of the present study was to carry out the efficacy of enriched *Artemia* with *S. cerevisiae* or *C. gracilis* on growth, stress resistance and fatty acid profile of *L. vannamei* postlarvae.

2. Materials and Methods

2.1 Experimental setup

This study was carried out in a private shrimp hatchery (Kamal Meygo, Choebdeh, Abadan, Iran) for 15 days. Larvae of *L. vannamei* were obtained from domesticated broodstocks, which were induced by eyestalk ablation to spawn in captivity. Larvae were reared in 10-ton rectangular concrete tanksat 28–30 °C, salinity 30 ppt and an initial density of 200nauplii l^{-1}. From protozoea I to mysis I, larvae were fed solely *Chaetoceros gracilis* at concentrations of 10×10^4. Upon reaching mysis I, larvae were individually counted and transferred to the experimental units. Nine rectangular 25-l tanks filled with 20 l of sand-filtered and UV treated seawater were used, and each tank stocked with 100 mysis I l^{-1}, with a daily water exchange of 50 %.Tanks were supplied with constant aeration maintaining oxygen near saturation levels (6 ppm).Average values for water temperature, salinity, dissolved oxygen, pH and alkalinity were 28.0 ± 1.5 °C, 30.0 ± 0.2 ‰, 6.0± 0.3 mg l^{-1},8.1 ± 0.2 and 136.0 ± 12.5 mg l^{-1} respectively, and photoperiod was 12L:12D (light: darkness).

2.2 Artemia

Three treatments with three replicates each were established: (1) Newly hatched *Artemia franciscana* nauplii (N); (2) *Artemia* metanauplii enriched with *Chaetoceros gracilis*; and (3) *Artemia* metanauplii enriched with *Saccharomyces cerevisiae*. The second instar stage *Artemia* nauplii (*A. franciscana*— Salt lake aquafeed, premium grade, USA) were separated from the hatching container by using a 120-µm sieve and transferred to 10 l enrichment containers at a density of 200 naupliiml^{-1} of sea water at room temperature (28°C).Strong aeration was provided to the rearing containers to keep the oxygenat optimum level. The nauplii were enriched for 24 h with *Chaetoceros gracilis*(30×10^4ml^{-1}) according to Karthik *et al*. (2016) [18] and *Saccharomyces cerevisiae*(1.25mg for 1000 *Artemia* nauplii) according to Ahmadnia *et al*. (2012) [2]. The enriched *Artemia* was harvested and rinsed with water over a 120-µm sieve to remove any remaining emulsion. Feeding was carried out once a day (11:00) and *Artemia* nauplii was offered at an initial density of 2ml^{-1}, which increased gradually to 10 ml^{-1}at the end of the experiment. Daily before water renewal, the number of remaining *Artemia* was estimated in each experimental unit and the amount of *Artemia* was maintained or increased as needed.

2.3 Sampling and stress tests

At the end of the feeding trial, survival was estimated by individually counting the number of PL from each experimental unit. For body weight, all the post larvae were collected, rinsed with freshwater and blotted dry and wet weights (mg post larvae^{-1}) were measured by weighing post larvae with an electronic microbalance. Dry weight was determined using an electro balance (±0.01 mg) by placing 20 post larvae on a pre-weighed microscope slide. The slides were then placed into a laboratory oven at 60°C for24 h and then reweighed to determine dry weights of post larvae. At the end of the trial, three replicate groups of 100 PL_{12} from each of the experimental groups were submitted to salinity and formalin stress tests. For the salinity stress test, PL were transferred to 2000-ml plastic containers, which filled with fresh water (0 ppt) or brackish water (15 ppt).For the formalin stress test PL were transferred to 2000-ml plastic containers filled with seawater that contained 100 ppm formalin. Mortality was monitored at 5-min intervals during 2 h. Shrimp presenting no movement of pleopods and no reaction to mechanical stimuli were considered dead.

2.4 Fatty acid analysis

For fatty acid profile assessment, fatty acid methyl esters were prepared by acidic methanolysis of lipid extracts using sulfuric acid in methanol (Christie, 1993) [7]. The fatty acid composition of *Artemia* (n = 1) and shrimp post larvae (n = 3) were determined by an auto sampler gas chromatography (GC, Agilent technologies 7890 N, USA), equipped with aflame ionization detector (FID) and a cyan opropyl–phenyl capillary column (DB-225MS, 30 m × 0.250 mm ID × 0.25µm Film thickness, USA according to Agh *et al.* (2014) [3].

2.5 Statistical analyses

Data were analyzed using SPSS ver.15.0 (Chicago, Illinois, USA). All the data are presented as mean ± standard error of the mean calculated from three biological replicates. Arcsine transformations were conducted on all data expressed as percentages. One way ANOVA was performed at a significance level of 0.05 following confirmation of normality and homogeneity of variance. Tukey's procedure was used for multiple comparisons when statistical differences were found among groups by the one-way ANOVA.

3. Results and Discussion

The results of the current study showed that survival did not change among different experimental groups ranging from 30.6% in PL fed the newly hatched *Artemia* to 36.4% in PL fed microalgae (Fig. 1a). Moreover, PL wet weight did not affect in different experimental groups ranging from 5.8 ± 0.4 to 6.7 ± 0.1 mg in Y and N groups, respectively (Fig. 1b). However, PL in C and N groups had the highest (767 ± 117 µg) and the lowest (367 ± 44.0 µg) dry weight, respectively, and PL in Y group showed intermediate value (567 ± 117 µg) (Fig. 1c). In this regard, several researches have shown that, *L. vannamei* larvae grew faster when co-cultured with microalgae because of high quality of protein and amino acid profile, vitamins, cholesterol and carotenoids (Nunes *et al.*,2011; Sanchez *et al.,* 2012; Iba *et al.,* 2014) [15, 27, 31].On the other hand, it has been reported that the beneficial effects of microalgae on growth performance might be related to their immune enhancer effects(Hayashi and Katoh, 1994) [13] as well as anti-inflammatory (Jensen *et al*., 2001) [16] and antiviral (Hayashi and Hayashi, 1996) [12] properties of microalgae rather than their nutrients. Moreover, Mustafa and Nakagawa (1995) [26] have reported that using microalgae as feed additive led to an increase in growth performance in fish, which associated with improved physiological conditions such as protein assimilation, lipid metabolism, liver function,

and stress response. Furthermore, in the current study, *Artemia* enriched with S. *cerevisiae* improved PL growth performance which might be attributed to elevated health status, provision of nucleotides, elevated digestibility and stimulation of enzymatic secretion as reported in different fish and crustacean species fed diets supplemented with baker's yeast (Thanardkit *et al*., 2002; Li and Gatlin, 2005; Abdel-Tawwab *et al*., 2008; Chotikachinda *et al*., 2008; Chiu *et al.,* 2010) [1, 6, 21, 34].

Regarding stress resistance tests, survival rate of PL in different experimental groups was over 90% and did not changed after exposing to formalin (Fig. 2a) and brackish water (Fig. 2b) stress tests. However, PL in Y and N groups had the highest (52.3 ± 2.9%) and the lowest (31.3 ± 2.8%) survival rate, when exposed to the fresh water stress test, respectively (Fig. 2c). This result indicated better performance and improved resistance against salinity stress may as a consequence of nucleotide components in yeas, which has been reported to increase stress and disease resistance indifferent crustacean species such as *L. vannamei* (Li & Gatlin 2006; Andrino *et al.,* 2012) [22], freshwater prawn (*Macrobrachium rosenbergii*; Shankar*et al.,* 2012) [32] and arrow clawed crayfish (*Astacus leptodactylus leptodactylus*; Safari *et al.,* 2014) [30]. In this context, it has been reported that dietary oligonucleotides, mannan-oligosaccharide and β-glucan extracted from *S. cerevisiae* can increase tolerance to stresses and disease resistance in different fish and crustacean species, which in line with the results of this study (Thanardkit *et al.,* 2002; Li *et al.,* 2004; Daniel *et al.,* 2006; Xiang *et al.,* 2011) [8, 20, 34, 35].

The fatty acid composition of the metanauplii enriched with *C. gracilis* or *S. cerevisiae* to some extent similar to newly hatched *Artemia* (Table 1). However, the concentrations of EPA, docosahexaenoic acid (22:6n-3, DHA) and n-3 LC-PUFA were higher in *Artemia* enriched with *C. gracilis* than other groups. In this regard, it was reported that microalgae belonging to (*Chaetoceros* spp.) have high percentages of EPA (Renaud *et al.,* 1994; Chakraborty *et al.,* 2007; Ju *et al.,* 2009) [5, 17, 28]. Moreover, the n-3 to n-6 PUFA ratio was significantly higher in PL fed C group than other treatments, which reflected the fatty acid composition of *Artemia* enriched with *C. gracilis*. PL in Y group had higher whole body lipid content than other experimental groups (Table 2). The concentrations of n-6 PUFA mainly linoleic acid (18:2n-6, LA) in C group was lower than other experimental groups. Linolenic acid (18:3n-3, LNA) concentration in PL fed *Artemia* enriched with the *S. cerevisiae* was higher than other groups. However, the concentrations of EPA, DHA and n-3 LC-PUFA were lower in Y group than other dietary treatments, which reflected the fatty acid composition of *Artemia* enriched with *S. cerevisiae*. Baker's yeast is rich in medium chain monounsaturated fatty acids specially palmitoleic and oleic acids and deficient of LC-PUFA (Murakami *et al.,* 1996) [25].

In summary, feeding enriched *Artemia* with *C. gracilis* or *S. cerevisiae* can improve growth performance and stress resistance in *L. vannamei* PL. In addition, n–3 / n–6 ratio increased in PL fed *Artemia* enriched with *C. gracilis* that might be led to higher growth performance of C group than other experimental treatments.

Table 1: Fatty acid profile (%) of *Artemia* enriched with *Chetocerous* (C) and *Saccharomyces cerevisiae*(Y) (n = 1).

Fatty acid profile	***N***	***C***	***Y***
Lipid content (%)	0.9	0.4	1.3
14:0	0.1	1.1	0.6
16:0	9.4	10.7	9.2
18:0	8.4	7.4	6.3
20:0	4.1	4.1	5.3
22:0	0.9	0.2	0.7
24:0	0.2	0.2	0.2
SFA	23.1	23.7	22.3
14:1n-5	0.1	0.6	0.2
16:1n-7	1.6	3.2	3.4
18:1n-7	8.6	8.3	7.4
18:1n-9	20.9	18.9	18.9
20:1n-9	0.7	0.7	0.5
MUFA	31.9	31.7	29.9
18:2n-6, LA	5.8	6.1	6.0
20:2n-6	0.4	0.3	0.4
20:4n-6	0.8	1.1	1.2
n–6 PUFA	7.0	7.5	7.6
18:3n-3, LNA	24.3	24.6	27.5
20:3n-3	0.8	0.9	0.8
20:5n-3, EPA	2.3	4.3	2.7
22:5n-3	-	-	0.4
22:6n-3, DHA	0.1	0.5	0.1
n–3 PUFA	27.5	30.3	31.5
Total	89.5	93.2	91.3
n-3 LC-PUFA	3.2	5.7	3.6
n-3 / n-6	3.9	4.0	4.1
EPA / ARA	2.9	3.9	2.3
DHA / ARA	0.1	0.5	0.1

Table 2: Fatty acid profile (%) of *L. vannamei* post larvae fed with *Artemia* enriched with *Chetocerous* (C) and *Saccharomyces cerevisiae* (Y) (n = 3).

		Diets		
Fatty acid profile	***Mysis***	**N**	**C**	**Y**
Lipid content (%)	0.4 ± 0.0	0.9 ± 0.1^{b}	1.0 ± 0.0^{b}	1.6 ± 0.2^{a}
14:0	1.2 ± 0.1	0.6 ± 0.0^{b}	0.9 ± 0.0^{a}	0.4 ± 0.0^{c}
16:0	17.4 ± 0.3	14.8 ± 0.4^{a}	12.4 ± 0.5^{b}	12.5 ± 0.2^{b}
18:0	8.0 ± 0.3	11.6 ± 0.2^{a}	9.6 ± 0.1^{b}	11.3 ± 0.2^{a}
20:0	1.3 ± 0.1	0.6 ± 0.0^{c}	2.6 ± 0.2^{a}	1.5 ± 0.0^{b}
22:0	0.2 ± 0.0	0.7 ± 0.0^{c}	2.8 ± 0.1^{a}	0.9 ± 0.0^{b}
24:0	0.3 ± 0.0	0.5 ± 0.0^{b}	0.8 ± 0.1^{a}	0.3 ± 0.0^{c}
SFA	28.4 ± 0.5	28.8 ± 0.5^{a}	29.1 ± 0.3^{a}	26.9 ± 1.1^{b}
14:1n-5	0.7 ± 0.0	0.4 ± 0.0^{c}	2.8 ± 0.1^{a}	0.7 ± 0.0^{b}
16:1n-7	2.4 ± 0.0	0.8 ± 0.0^{c}	2.8 ± 0.1^{a}	1.3 ± 0.1^{b}
18:1n-7	5.7 ± 0.1	7.3 ± 0.3	6.1 ± 0.6	7.0 ± 0.2
18:1n-9	17.6 ± 0.1	14.9 ± 0.8	12.8 ± 0.2	13.9 ± 0.5
MUFA	26.4 ± 0.6	23.4 ± 0.7	24.5 ± 0.9	22.9 ± 0.3
18:2n-6, LA	9.4 ± 0.1	5.3 ± 0.1^{a}	4.2 ± 0.2^{b}	5.0 ± 0.1^{a}
20:2n-6	0.7 ± 0.0	0.7 ± 0.0^{c}	5.2 ± 0.5^{b}	6.4 ± 0.2^{a}
20:4n-6, ARA	1.4 ± 0.1	7.4 ± 0.3^{a}	1.5 ± 0.1^{b}	2.1 ± 0.1^{b}
n–6 PUFA	11.5 ± 0.8	13.4 ± 0.5^{a}	10.9 ± 0.5^{b}	13.5 ± 0.8^{a}
18:3n-3, LNA	14.3 ± 0.5	12.8 ± 0.5^{b}	10.9 ± 0.4^{b}	15.5 ± 0.4^{a}
20:3n-3	0.6 ± 0.0	0.5 ± 0.1^{b}	0.5 ± 0.0^{b}	1.1 ± 0.1^{a}
20:5n-3, EPA	8.9 ± 0.5	13.2 ± 0.1^{a}	13.0 ± 0.5^{a}	11.2 ± 0.3^{b}
22:6n-3, DHA	7.4 ± 0.1	5.3 ± 0.1^{a}	4.9 ± 0.1^{a}	2.1 ± 0.1^{b}
n–3 PUFA	31.2 ± 0.9	31.8 ± 0.8	29.3 ± 0.7	28.8 ± 1.3
Total	97.5 ± 1.1	97.4 ± 1.3	93.8 ± 1.7	92.1 ± 0.6
n-3 LC-PUFA	16.9 ± 0.1	19.0 ± 0.6^{a}	18.4 ± 0.8^{a}	14.4 ± 0.7^{b}
n-3 / n-6	2.7 ± 0.1	1.4 ± 0.1^{c}	2.7 ± 0.1^{a}	2.1 ± 0.1^{b}
EPA / ARA	6.4 ± 0.1	1.8 ± 0.0^{c}	8.7 ± 0.3^{a}	5.3 ± 0.1^{b}
DHA / ARA	5.3 ± 0.5	0.7 ± 0.1^{b}	3.3 ± 0.0^{a}	1.0 ± 0.1^{b}

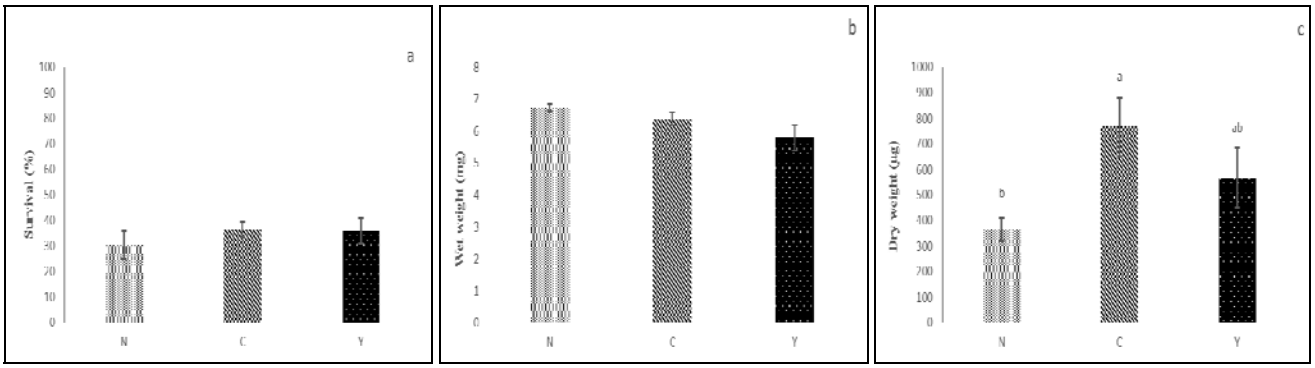

Fig 1: Survival (a), wet weight (b) and dry weight (c) of PL in different experimental groups.

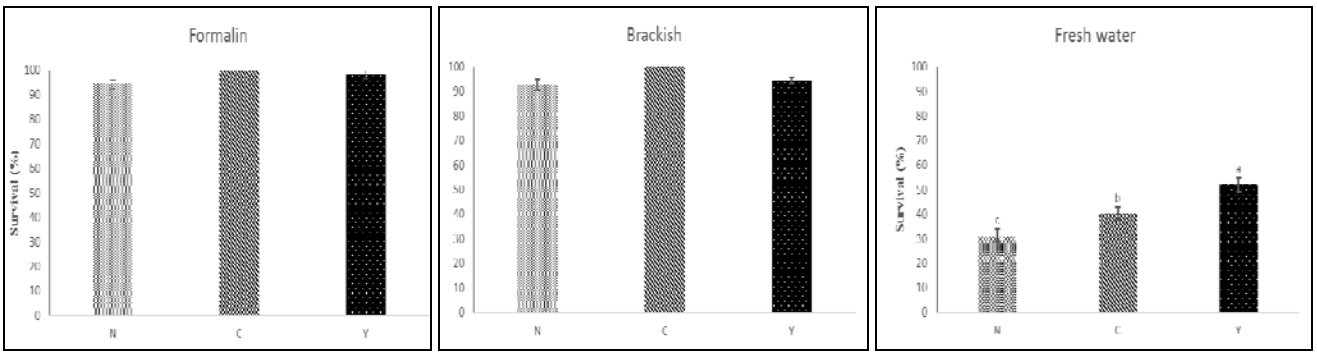

Fig 2: Survival rate (%) after stress tests with formalin (a), brackish water (b) and freshwater (c) of PL in different experimental groups

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