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Effects of Commercial Enrichment Products on Chemical Constitutions of Rotifer *Brachionus plicatilis* (O.F. Muller 1786)

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Abstract: This study involved to demonstrate changes in chemical composition of Rotifer *Brachionus plicatilis* (O.F. Muller 1786) fed on three commercial enrichment products. The rotifers were cultured on a mixture of ω3 algae and ω3 yeast 60 products. The enrichments of rotifers were made using Red Pepper Paste (ZA), AlgaMac 3050 (ZB) and Spresso (ZC) products. The analysed nutritional compositions before and after enrichment process significantly ($p<0.05$) changed for crude fat from 6.18±0.24 to 14.26±1.22% and for crude protein from 48.24±0.57 to 59.21±0.32%, respectively. Crude ash contents were significantly ($p<0.05$) changed from 6.65±0.46 to 15.39±0.39% while no significant difference were seen between the control group of K (concentrated rotifer cultures) and nutritionally enriched groups (ZA, AB and ZC). The ratio of protein: lipid in all experimental groups ranged from 3.47±0.18 to 9.54±0.34. Those values were nearly similar for enriched rotifer groups but statistically ($p<0.05$) being lower that the values of the control-group (K). Of commercial enrichment products AlgaMac 3050 and Spresso were seen to provide effective and efficient results in terms of analysed nutritional compositions.

Key words: Rotifers, *Brachionus plicatilis*, enrichment products, chemical compositions, crude protein, Turkey

INTRODUCTION

In order to improve larvae production commercial enrichment products are widely practised to improve nutritional value of rotifers (McKinnon *et al.* 2000; Glencross, 2009; Tocher, 2010; Hache and Plante, 2011). Rotifers and Artemia are generally used as first foods in marine finfish aquaculture (Palmtag *et al*., 2006). The rotifer species are commonly reported to be used in commercial hatcheries (Lubzens *et al*., 2001; McKinnon *et al*., 2000). It is shown that the lipid contents of rotifers, generally utilised from the suspended nutrient particles by filtering were positively affected by the lipid contents of consumed foods and their nutritional value can be changed by the chemical compositions based on dry weight (Lubzens and Zmora, 2003; Conceicao *et al*., 2009).

With short and long-term enrichment methods the lipid contents of rotifiers are seen to be qualitatively and quantitavely improved with the products rich in lipid content (Lubzens and Zmora, 2003; Rainuzzo *et al*., 1997). For instance the analysed energy value of rotifers fed with Baker's yeast can be increased with the enriched diets for 6 h (Lubzens and Zmora, 2003) and the ash content is determined to be ranged from 60-120 ng (Yufera and Pascual, 1989). The dry matter of rotifers contains approximately 28-63% protein and 9-28% lipids (Lubzens and Zmora, 2003; Conceicao *et al*., 2009). However, the values over 28% lipid contents were also reported (Lubzens *et al*., 1989). Westelmajer (2008) demonstrated that the lipid contents based on dry weight did only insignificantly differ in rotifers enriched with the product of Selco.

Caric *et al*. (1993) reported that the rotifers fed on alive *Nannchloropsis* sp. had a 11.1-17.2% of crude fat, a 34.3-35.3% of crude protein and a 5.0-8.3% of crude ash on a dry weight whereas those fed on yeast had a 10.3-19.4% of crude fat, a 25.5-34.0% of crude protein and a 5.8-7.9% of crude ash of dry weight. Non-enriched rotifers are reported to have a 36.06±0.52% of crude protein content Fernandez-Reiriz *et al*. (1993). According to Blair (2005) the rotifers enriched with various emulsions had a lipid content of 22-29% based on dry matter whereas the control rotifers had only a 12-13% of lipid content of dry weight and those fed on INVE CS3000 and *Nannochloropsis* sp. had a crude protein content of 57.1±0.4%. Fernandez-Reiriz *et al*. (1993) enriched the rotifers for 6 h with various products and reported increased protein and lipid contents in all experimental groups and also found that the calori value of protein, carbohydrate and lipid can reache to highest value after a 6 h period.

Oeie *et al*. (1997) reported significant differences in lipid and protein contents of rotifers fed either on

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protein-based enrichment or lipid-based enrichment. The nonenriched rotifers fed on Baker'yeast were reported to have a protein content of 63.57±0.36% based on a air-dry weight by Naz (2008). The dry weight of rotifers was reported to contain a 63-67% of crude protein and a 4-6% of crude ash (Lubzens *et al*., 1989).

The study by Oeie and Olsen (1997) revelaed that short-term food enrichment of poorly fed rotifers with balanced diet rich in protein resulted in increased protein and lipid content per rotifer. The protein content per dry weight showed only minor changes whereas lipid content per dry weight increased. In contrary, short term enrichment with a lipid rich diet resulted in increased lipid content of dry weight per individual rotifer whereas the protein content per individuals remained constant and the protein content of dry weight showed a slight decrease. Significant differences were reported in total lipid contents of rotifers enriched for 18 h with four different lipid sources (Rodriguez *et al*., 1997). Total lipid contents can be changed by the concentration level of product used and by its application period (Metusalach, 2002).

According to Hernandez-Cruz *et al*. (1999) the best growth for larvae can be managed by the commercial product of Powersh-W and when fed to rotifers total lipid content increases. In addition the enrichment products rich in lipid content resulted in high lipid content of rotifers (MacDonald, 2004). Algamac (2000) product was tested against other enrichment products on rotifers and *Artemia* sp. by Garcia (2006) and Garcia *et al*. (2008a, b). Highest lipid contents were obtained by Algamac (2000). Palmtag *et al*. (2006) demonstrated that the crude fat values vary from 15.6±1.8 to 20.4±0.8% by different enrichment products. Enrichment of rotifers is seen to be depended upon the dosage and duration of period since, the enrichment is seen to reduce protein content while lipid content increases and protein:lipid ratio was always low as compared to prior to the enrichment process (Park *et al*., 2006). Similar results with 24 h enrichment process were reported by Naz (2008). Kotani *et al*. (2009) did also observe any significant changes in nutritive value of rotifers after the enrichment process as opposed to significant changes during the application process.

Enrichment for protein content is largely dependend upon feeding stragety and generally on culturing conditions. Long-period lipid enrichment processes lead to a stable protein value while protein: lipid ratio decreasing. This is a specific matter and mostly related to the specifity of rotifer culturing (Dhert *et al*., 2001; Agh and Sorgeloos, 2005). Enrichment by using algea improves fatty acit compositions in rotifers depending on nutritive value of algae used (Lubzens and Zmora, 2003; Agh and Sorgeloos, 2005). In contrary since, these micro-algae do not support the enrichment of zooplanctons it is now common to use commercial enriching products such as DHA Selco (McKinnon *et al*., 2000). New enrichment products such as algal pastes and frozen algea were developed for rotifer feeding (Park *et al*., 2006).

Caric *et al*. (1993) reported high crude ash contents and lowest values for carbohydrates and lipids in rotifers fed on phytoplancton monocultures, natural nanoplanctons and yeast. The rotifers fed on control diet of INVE CS3000 and *Nannochloropsis* sp. had a 12.6±03% of crude fat of dry weight (Blair, 2005); those fed on Chlorella+yeast had a 20-22% of crude fat and those fed on yeast had a value of 22.19±0.28% (Lubzens *et al*., 1989; Naz, 2008).

Naz (2008) reported that the nutritional compositions and energy value of such living feeds are not as stable as in compund feeds and the compositional figures of such feeds are not always reliable. In Turkey alive enrichment products are generally being practised for a period ranging from 6-24 h.

This study was conducted to test three different commercial enrichment products in rotifers at commercial scale. The recommended dosages and application period by the producers are applied in this study. After and before enrichments the nutritive value for crude protein, crude fat, crude ash and protein: Lipid ratio were calculated. The objective was therefore to compare the nutritive values of rotifers fed on different sources of commercial enrichment products and to discuss the results in the light of literature review.

MATERIALS AND METHODS

Rotifers cultures: Rotifers (*B. plicatilis* S-strain, Egemar Su Urunleri Gida ve Sanayi ve Ticaret AS, Aydin, Turkey) were reared for 72 h in 2000-L conical tanks (V-type) and continuous artificial illumination with oxygen supply. The concentrated rotifers cultures (K) were fed with frozen microalgae of ω3 Algae (BernAqua NV Hagelberg 3 B-2250 Olen Belgium) (K-1) and powdered (*S. cerevisiae*) ω3 yeast 60 (BernAqua NV Hagelberg 3 B-2250 Olen Belgium) (K-2). During this period a group (T) was fed on a mixture containing 25% of K-1 and 75% of K-2 every 4 h day^{-1}. This mixture was then fed twice a day on a dose of 0.6 g for 0-23 h (T0), 0.5 g for 24-47 h (T1) and 0.4 g million^{-1} rotifers for 48-71 h (T2). Initially 350 rotifers mL^{-1} was cultured and during experimental period the rotifers counts were made in the mornings and afternoons. When the counst reached to a 500 rotifers mL^{-1} at the end of T0 and 1000 rotifers mL^{-1} at the end of T2 the harvesting was started at the time of 72 h (T3).

The rotifers were transferred to a 350 L conical tank containing seawater. The above mentioned feed mixture was ten fed on a basis of 0.5 g million^{-1} rotifers for 12 h. These are the rotifers subjected to enrichment (ZK). After stocking rotifies counting was made 3 times day^{-1}.

Rotifers enrichment: Enrichment (Z) was performed on the cultured rotifiers using three commercial products according to the recommended doses and periods. The study was done at a commercial scale. Rotifers were stocked in 400 L conical tanks (V-type) and continuous artificial illumination in a seawater filtred through UV and biologic filters with 25% salinity, a temparature of 26-26.50°C, a pH of 7.4±0.5 and a 19.0±1 mg L^{-1} O_2.

The enrichment treatments were as follows: the rotifers enriched with Red Pepper Paste (BernAqua NV Hagelberg 3 B-2250 Olen Belgium) (ZA) were stocked as 500 mL^{-1} (196 million/400 L) and fed on a dose of 180 g m^{-3} for 6 h and then were harvasted.

The rotifers enriched with AlgaMac 3050 (Aquafauna Bio-Marine Inc. P.O. Box 5 Hawthorne, California USA) (ZB) were stocked as 1000 mL^{-1} (194 million per 150 L) and fed on a dose of 0.3 g m^{-3} for 8 h and then were then harvasted.

The rotifers enriched with Spresso (Inve Aquaculture nv Hoogveld 91 9200 Dendermonde Belgium) (ZC) were stocked as 1000 mL^{-1} (195 million per 150 L) and fed on a dose of 175 g m^{-3} for 12 h and then were then harvasted. Furthermore, the group K rotifies (0 time) were stocked at 600 L around 597 millions and then were starved (Z0) in 600 L around 604 millions.

Rotifers were enriched twice a day at 9 am and 9 pm based on the manufacturer's recommendations and on the technical feasibility of a semi-commercial hatchery operation.

Rotifers were harvested after 24 h on a 60 μm filter and gently rinsed with filtered seawater for 5 min before sampling for nutritional analysis. All samples were made in triplicate. In addition the samples were obtained from the rotifer group fed on a mixture of feed containing K-1+K-2 at 6 h (ZKA), at 8 h (ZKB) and at 12 h (ZKC), similar time scales of the groups of ZA, ZB and ZC, respectively. All treatments mentioned above K (control), K0 (starved control), ZA, ZKA (positive control), Z0A (negative control-starved), ZB, ZKB, Z0B, ZC, ZKC and Z0C were tested together.

Chemical and statistical analysis: The determination of crude fat was done by the method of Bligh and Dyer (1959) and the crude protein was performed according to Standard method of Kjeldahl (N×6.25) AOAC (2000a) and crude ash by AOAC (2000b). The ratio of protein: lipid was calculated.

Analysis of variance using Windows SPSS 15.0 program was employed to see any significant effects of treatments and the differences between the group means were separated at a probability of 0.05 by Tukey's test.

RESULTS

The determination of crude fat, crude protein and crude ash contents and protein: lipid ratios of bological samples was shown in Table 1. No significant differences ($p>0.05$) were observed between the encrichment poducts in crude fat contents whereas the highest crude fat values were obtained from the starved rotifer groups of Z0B ile Z0C. Except the groups of ZA and ZOB the crude protein values did not differ significantly ($p>0.05$) between the experimental groups. The crude ash values showed a great extend of change depending upon the increased period of starvation. The ratios of protein: lipid were seen to be almost lower in enriched rotifer groups than other remaining groups.

According to the experimental results highest crude protein value was obtained from ZKB rotifer group (59.21±0.32%) and the lowest from ZA rotifer group fed on Red Pepper Paste (48.24±0.57%). The rotifers of ZA and Z0B groups had similar crude protein content ($p>0.05$) which are significantly different from the other groups ($p<0.05$). The rotifer groups of ZB and ZC had similar crude protein content ($p>0.05$) but differed from that of ZA group ($p<0.05$). The crude protein content was reduced by starvation in Z0B group for 8 h but increased in the rotifer group Z0C for 12 h. Highest crude fat content was obtained from the group of Z0B (14.26±1.22%) and the lowest value from the group of ZKA (6.18±0.24%).

The crude fat values of the groups of ZA, ZB and ZC rotifers were higher than those of groups of K, ZKA and

Table 1: Mean and standard deviations of the percentages of crude fat, crude protein and crude ash and the ratios of protein:lipid in rotifers fed on different enrichment products

Experimental groups	Crude fat	Crude protein	Crude ash	Protein/Lipid
K	9.56±0.91^{abc}	57.19±0.46^{b}	10.25±0.3^{cde}	6.08±0.54^{bcd}
ZA	13.98±0.89^{d}	48.24±0.57^{a}	9.26±0.35^{cd}	3.47±0.18^{a}
ZKA	6.18±0.24^{a}	58.78±0.20^{b}	7.32±0.42^{ab}	9.54±0.34^{e}
Z0A	8.80±1.10^{abc}	57.30±0.73^{b}	11.42±0.25^{e}	6.70±0.77^{cd}
ZB	13.74±0.09^{d}	56.83±0.80^{b}	10.21±0.33^{cde}	4.14±0.03^{a}
ZKB	8.42±0.57^{abc}	59.21±0.32^{b}	6.65±0.46^{a}	7.09±0.45^{d}
Z0B	14.26±1.22^{d}	49.92±1.50^{a}	10.43±0.46^{cde}	3.49±0.16^{a}
ZC	11.95±0.23^{bcd}	58.27±0.33^{b}	10.84±0.06^{de}	4.88±0.07^{ab}
ZKC	11.28±0.33^{bcd}	59.04±0.31^{b}	8.85±0.01^{cd}	5.24±0.13^{abc}
Z0C	12.78±0.38^{cd}	57.49±0.49^{b}	15.39±0.39^{f}	4.50±0.10^{ab}

K indicates intensivelly cultured rotifiers fed on a mixture of feed containing K-1+K-2, ZKA, ZKB and ZKC refers to the K group of rotifers which were harvested at the same times (6, 8 and 12 h) after treatments of three commercial enrichment products (ZA, ZB and ZC) and 0 refers to the K group of rotifers which were subjected to starvation for the same periods (6, 8 and 12 h, respectively) as the rotifers fed on three commercial enrichment products (ZA, ZB and ZC). Different letter in the same coloumn indicates significant differences ($p<0.05$)

ZKB ($p<0.05$). Similar fat contents were observed between the groups of ZC, ZA, ZB, K and ZKC ($p>0.05$). The crude fat content of ZOA was similar to the groups of K, ZKA and ZA ($p>0.05$) but increasing starvation period increased crude fat content in the rotifer groups of ZOB and ZOC ($p<0.05$).

Highest (15.39±0.39%) and lowest (6.65±0.46%) crude ash contents were obtained from ZOC and ZKB group, respectively. The crude ash content increases with the period of starvation. The differences in crude ash content were insignificant between the rotifer groups of ZA, ZB, ZC and K ($p>0.05$). Highest (9.54±0.34) and lowest (3.47±0.18) protein: lipid ratios were obtained from the groups of ZKA and ZA, respectively. This ranges from 3.47±0.18 to 4.88±0.07 in the group of encriched rotifers. Except the group of ZA and ZB the protein: lipid ratio of K group was similar to that of ZC group ($p>0.05$). Protein: lipid ratios were seen to be reduced depending on the experimental period in the cultured groups of ZKA, ZKB and ZKC and the starved groups of ZOA, ZOB and ZOC. The later group had significant differences in protein: lipid ratios.

DISCUSSION

Having reviwed the literature it can be noted that the abbreviations for commercial products used in this study are preferred to be used for the same products manufactured either by the same or different producers reported in many studies discussed hereafter. The crude fat values obtained from the present study were found to be similar to the values reported by Caric *et al.* (1993) and Blair (2005) but these were lower than the values reported by Lubzens *et al.* (1989) and Naz (2008). The crude fat contents based on dry weight reported for ZB and ZC group rotifers by Fernandez-Reiriz *et al.* (1993) were found to be similar to the values obtained for the same groups of rotifers from the present experiments. According to the research of Roo *et al.* (2009) the rotifers fed on ZA enrichment product had the highest crude fat contents as compared to other commercial products. The crude fat contents reported for ZC group by Roo *et al.* (2009) and Roo *et al.* (2010a, b) were higher than that those of ZA ve ZC rotifers obtained from the experiments. Similar results were reported by Palmtag *et al.* (2006) for the rotifers of ZB group in terms of crude fat contents as compared to ZB fed rotifers in the experiments. Such differences reported by various researchers are likely to be dependen upon the nutritional contents of products used and their doses and application periods.

The crude protein values reported by Caric *et al.* (1993) and Fernandez-Reiriz *et al.* (1993) for all experimental groups were lower than that in the experimental groups while the results of crude protein values except for the group of ZA rotifers were similar to those reported by Blair (2005). Similarly such differences may have been due to the differences in nutritional contents of products and varying rearing conditions at different studies. The crude protein values reported for ZB and ZC fed rotifers by Fernandez-Reiriz *et al.* (1993) and the values reported for the ZC fed rotifers by Naz (2008) were lower than those obtained for the groups of ZB and ZC rotifers in the experiments. The crude protein content reproted for ZA gorup by Roo *et al.* (2009) was higher than those obtained from the experiments. However, Roo *et al.* (2009, 2010a, b) reported lower crude protein values for ZC fed rotifers as compared to the findings.

The findings on crude ash contents in the experiments were higher than those reported by Lubzens *et al.* (1989) and Caric *et al.* (1993) but these were lower than those reported in the stuyd of Blair (2005).

The crude ash content (7.53±0.13%) calculated on the basis of air dry weight by Naz (2008) for the rotifers fed on yeast of *S. cerevisiae* was seen to be lower than the values obtained from the present experiments whereas the value (10.84±0.06% based on dry weight) reported by Naz (2008) for the rotifers was lower than the value reported by the experiments. The crude ash content of 1.48±0.50% reported by Roo *et al.* (2009) for group of ZA was lower than the values obtained for all experimental groups in the present case. Similarly the same result was reported by Roo *et al.* (2010a, b) for the ZC fed rotifers. Roo *et al.* (2009, 2010a, b) found that none enriched rotifers fed on only yeast had lowered crude ash contents.

The research of Garcia *et al.* (2008a, b) where the same commercial products were used for enrichment of rotifers revealed significant differences in nutritional compositions. According to Izquierdo (2004) the protein content of living feed organisms is genetically stable while the lipd contents of such cultured mass products are qualitatively and quantitatively affected by culturing methods. This is generally in a good agreement with the results of Park *et al.* (2006) where total lipit contents in all encrichment treatments increases and furthermore, the ratio of protein: lipid was lower after the enrichment as compared to prior to enrichment application. Thus, the findings on nutritional compositions of rotifers subjected to enrichment applications were paralel to the above mentioned results.

Various studies demonstrated that the nutritional compositions of rotifers are greatly influnced by the nutritional contents of products used for enrichment and their dosages and application periods. For instance despite the fact that the product of Red Pepper Paste was applied for 6 h enrichment in the experiments Hache and

Plante (2011) applied the same product for 24 h. The products of Red Pepper Paste, Algamac 3050 and Spresso used in this experiment contained a crude fat content of 13.5, 56.2 and 32%, respectively although, such differences in their nutritional contents did not lead to any significant differences in the experiments ($p>0.05$). Therefore, it can be speculated that the differed crude fat contents of commercial products may not always result in differed crude fat contents of rotifers. But the researchers believed that the product inducing a lowered fat content in rotifers is efficently utilised by the rotifers in terms of the nutrient of fat. The reason why the rotifers fed on Red Pepper Paste product (ZA) had reduced crude protein content could be attributed to the fact that the rotifers could have an adaptation impairment towards to the changes in nutrient environment made by the introduction of Red Pepper Paste rich in crude protein content and may also be due to the differed application dosages and its periods. However, the crude protein content of rotifers fed on Spresso product (ZB) which has lower protein content (3.0%) than Red Pepper Paste product (ZA) was highest as compared to all commercial products. In addition similar crude protein contents were observed for the rotifers groups of Algamac 3050 (ZC) and Spresso product (ZB), the former with a protein content of 17.6% ($p>0.05$). The one may then speculate that the product of (Red Pepper Paste) is in the form of a paste and the products of Spresso and Algamac 3050 are in the form of emulsion, leading to significant changes to ocur in the chemical compositions of rotifers which can utilise differently from these products.

The high values of crude ash contents of cultured rotifers (K) may be due to high content of ash in the mixture of K1+K2 nutrients which were fed to these rotifers. In addition no significant differences were observed in the contents of crude ash of rotifers enriched by commercial products ($p>0.05$).

CONCLUSION

This study reveladed that the nutritional value of rotifers fed on Algamac 3050 and Spresso was similar while the crude protein content of those rotifers was higher than that of Red Pepper Paste group ($p<0.05$). Furthermore, Algamac 3050 and Spresso provide good results for enrichment of rotifers than Red Pepper Paste.

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