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Research Article

Time-dependent changes of alkaline, neutral, and acid protease activities, molecular weight distributions, and proximate compositions of live feeds

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Abstract

In this study, alkaline, neutral, and acid enzyme activities, molecular weight distributions, and proximate compositions of live feeds were determined in a time-dependent manner. Samples were taken from three different live feeds and *Artemia* cysts. R refers to rotifer, A-0 refers to non-enriched *Artemia* / *A.* nauplii, and A-1 refers to enriched *Artemia* / *A.* metanauplii. Sampling was done at 0^{th}, 6^{th}, 12^{th}, and 18^{th} h (T) and before enrichment as R (R-0) and A1 (A1-0). Depending on time, while enriched rotifers have high acid enzyme values at pH 3 and 4, neutral and alkaline enzyme values of enriched *Artemia*s were higher ($p<0.05$). Time-dependent decreases were detected in the enzyme activities of enriched *Artemia* and rotifers. Live feeds enriched for up to 12 hours should be used with a molecular weight fraction of 2,532 Da≥ ($p<0.05$). When marine finfish such as European sea bass and gilthead sea bream are started feeding, their digestive enzymes have alkaline and then acid characteristics. On the other hand, in this study, it was determined that rotifer enzymes had higher acid protease activities and *A.* metanauplii had higher alkaline protease enzyme activities. Considering this situation, *A.* nauplii should be given together with rotifers and *A.* metanuplii should be started as soon as possible. In addition, considering the enzyme activity values of the rotifers used as the first feed, the use of rotifers that are not enriched in terms of the contribution of live feeds to the larval digestive system should also be taken into account.

Keywords: Protease, Molecular weight, Proximate, Rotifer, *Artemia*, Live feed

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Introduction

Although rotifer and *Artemia* taxa are not widely found in natural food areas, they are widely used in the feeding of marine finfish and crustacean larvae due to their ease of culture (Lubzens *et al*., 2001; Kanazawa, 2003; Izquierdo, 2004; Palmtag *et al*., 2006; McKinnon *et al*., 2009; Oie *et al*., 2011; Gonçalves *et al*., 2014; Zaleha Kassim *et al*., 2014; Erdogan and Ertan, 2015). *B. plicatilis* can be expressed as a species complex according to genetic differences and *Artemia* can be expressed as a species complex according to their reproductive isolation, and there may be differences in nutritional values and enzyme activities after enrichment (Leger *et al*., 1986; Lu and Warner, 1991; Van Stappen 1996; Hagiwara *et al*., 2007; Bear *et al*., 2008; Vasileiadou *et al*., 2009). The fact that the protease activities of *Artemia* cysts are higher in parthenogenetic strains than in bisexual strains (Lu and Warner, 1991) affect the larval rearing of marine hatcheries. In addition to this biological assessment, environmental and global climate changes such as El Niño also affect *Artemia* production (GSLEP, 2017; Le *et al*., 2018; Lindegren *et al*., 2018; Fuenzalida, 2019) as well as nutritional values and enzyme activities.

In the early stages of the larvae, the digestive system is not fully functional, although low gastric and digestive enzyme activities are present prior to exogenous feding (Person-Le Ruyet, 1989; Hoehne-Reitan *et al*., 2001a,b; Zambonino Infante and Cahu, 2001; Lazo et al., 2011). Alkaline proteases are important in the first day of feeding, while acid proteases are important in digestion towards the end of the larval stage (Lazo *et al*., 2011). As the weeks progress, enzyme activities begin with dietary stimuli, independent of ontogenesis, depending on the food ingested (Hoehne-Reitan *et al*., 2001a,b; Kolkovski, 2001; Lazo *et al*., 2011). The maturation process of the digestive system can be damaged if the larvae are fed with diets that are not suitable for their needs (Zambonino Infante *et al*., 2008). In this regard, live feed is an important essential food for newly hatched and immature digestive systems and enzyme-deficient finfish and shrimp larvae (Zaleha Kassim *et al*., 2014). Determining the molecular weight distributions of the nutrients used in aquaculture as well as the proximate nutritional compositions of rotifer and *Artemia* provided important approaches to the evaluation of nutritional results depending on the enrichment and enriching product (Westelmajer, 2008; Beyhan, 2011; Radhakrishnan *et al*., 2020). Molecular weight distributions were calculated to determine soluble digestive products, peptide distributions and leaching status of microdiet, protein sources of diets and use of feed ingredients, and status of live feeds (Grabner, 1985; Alarcon *et al*., 1997; Langdon, 2003; Carvalho *et al*., 2004; Lemos *et al*., 2004; Kvåle *et al*., 2006; Langdon *et al*., 2007; Onal and Langdon, 2009; Diken, 2018; Yenmiş and Naz, 2018). Besides, in many studies, protease activities of rotifers and *Artemia* were determined in pH 8.5 substrate (Naz, 2008; Naz *et al*., 2011;

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Naz and Yufera, 2012; Hakozu, 2014; Diken *et al*., 2016a, b, 2019). It has been reported that no further studies have taken place on changes in protease enzyme activity over time and hatchery practices of live feeds on different pH substrates. In this study, changes in alkaline, neutral, and acid protease enzyme activities, molecular weight distributions, and proximate nutritional compositions of rotifers and *Artemia* in commercial live feed cultures were determined depending on storage at +4ºC from before and after enrichment to use. Evaluation of these factors affecting the larval weaning success of larvae together will contribute to the literature and practices.

Materials and methods

Culture of live feeds

The live feed culture was carried out at the EGEMAR Aquaculture Food Industry and Commercial Incorporated Company (Aydın, TÜRKİYE). The live feed culture and sampling protocol are shown in Table 1. For this purpose, samples were taken from three different live feed (R refers to rotifer, *Brachionus plicatilis*; A0 refers to non-enriched *Artemia*/*A.* nauplii; A1/1, and A1/2 refers to enrihed *Artemia*/*A.* metanauplii) and *Artemia* cysts. The live feeds' sampling was made before enrichment (before enrichment; 0, rotifer; R-0 and *A.* metanauplii; A1-0), and at 0th (A0's after hatching cysts harvest and A1's after enrichment harvest), and stored at +4°C at the 6th, 12th, and 18th h(T=hours; T0, T6, T12, and T18) after enrichment (R, A1), and A0's after harvest. The live feeds were shocked in ice water, then washed with +4°C tap water and rinsed with distilled water. Samples were stored in an Air Liquide GT 40 liquid nitrogen tank at -196°C. After the field study, samples brought to the laboratory were kept in the Daihan SimpleFreez U400 freezer at -80°C until analysis. Therefore, this study does not need ethical approval as it included invertebrate groups.

Rotifer, *B. plicatilis* (R) culture (Table 1); Rotifers were fed with Algamac Protein Plus (Aquafaune Bio-Marine Inc. Hawthorne USA) at a rate of 0.4-0.6 g L million^{-1}. The stocking rate of rotifers is 462 prey mL^{-1} and the harvesting rate is 1,158 prey mL^{-1}. The sample was taken from the rotifers after being harvested and washed at 72nd hour before enrichment at 74.5th hour (R-0). Rotifers with an enrichment stock ratio of 972 prey mL^{-1} were pre-fed with Ebio FB (Ege Biyoteknoloji A.Ş. İzmir-Türkiye) at 0th and 7th hours. They were enriched with Spresso (Aquafauna Bio-Marine Inc. Hawthorne USA) at a rate of 160 g/m^3 at the 13th and 16th hours. The first sample was taken from enriched rotifers harvested and washed at the 19th hour after enrichment in 21.5th hours (R-T0) and stocked at 70 million prey L^{-1} for larvae feeding at +4°C. Samples were collected at the 6th (R-T6), 12th (R-T12), and 18th (R-T18) hours following the enrichment.

non-enriched *Artemia/A.* nauplii (A0) culture (Table 1); After hydration and decapsulation, *Artemia* (Artemia Cysts, Vinh Chau-Bac Lieu Artemia Co. Op) cysts were stocked at a rate of 0.9 g L^{-1}.

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Hatching took place in 17 hours and the total amount was 76 million. After harvesting and washing, the first sample was made at 18.5th hours without transferring to larvae (A0-T0) and the remaining samples were stored at +4°C for 5 million prey L^{-1} for larvae feeding. A0-T6, A0-T12, and A0-T18 samples were made at the 6th, 12th, and 18th hours following the first sampling, respectively. In addition, before decapsulation (A0-E) and after decapsulation (A0-D) samples were taken from *Artemia* cysts used by decapsulation.

Enriched *Artemia/A.* metanauplii (A1) culture (Table 1); After hydration, *Artemia* (Artemia EG, Artemia SepArt EG>225,000 npl/g INVE Aquaculture Salt Lake City Utah/USA) cysts were stocked in 2 tanks at a rate of 2.1 g L^{-1}. Hatching took place in 24 hours and the total amount in 2 tanks was calculated as 3,115,000,000 nauplii. Following harvesting and washing, samples were taken before enrichment at 26.5th hour (A1-0). *Artemia* enrichment was made with two different products, Spresso (1 kg ton^{-1}) and Red Algamac-Red AMC (Aquafauna Bio-Marine Inc. 4643 W. Rosecrans Ave Hawthorne California 90250 USA) (1.4 g/per million *Artemia*) at 0th and 11.5th hour. Harvesting and washing were started at the 19.5th hour, and samples were taken from enriched *Artemia*s before transfer to larvae at the 21.5th hour (A1/1 & A1/2-T0). For the larvae feeding, *Artemia* was stocked at the rate of 10 million prey l^{-1} at +4 $^{\circ}$C and was sampled at the 6th (A1/1 & A1/2-T6), 12th (A1/1 & A1/2-T12), and 18th (A1/2) hours. *Artemia* cysts were also sampled (A1-E).

Alkaline, neutral, and acid protease enzyme activities of live feeds

Protease enzyme activities were determined by extracting live feeds. Time-dependent enzyme activities of live feeds were determined according to acid (pH 3, 4, 5, 6), neutral (pH 7), and alkaline (pH 8.5) protease groups.

Extracts of live feeds

The live feeds were rinsed in distilled water after thawing, and then extracts of live feeds were prepared by homogenization (Daihan, WiseTis HG-15D) with distilled water and centrifuged at 16,000 G for 30 minutes at +4°C (Sigma, 2-16 K). The resulting supernatants were protected in a -80°C freezer (Daihan SimpleFreez U400) for analysis (Diken, 2018).

Protein analysis

Soluble protein concentrations in live feed extracts were determined by a protein-dye solution developed by Bradford (1976) (Biorad Protein Assay, Cat. No: 5002). After performing Biorad kit procedures, spectrophotometer (Shimadzu, UV mini 1204) measurements were performed at 595 nm. The values obtained were used to express the protease results in U mg^{-1} protein.

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Table 1: Culture of live feeds and sampling of live feeds (mean ± SE).

Time=day (hour)	Explanation
Rotifer, *Bracihonus plicatilis* (R)	
Culture	
T=0(0 <24 hour) T=1(24 <48 hour) T=2(48 <72 hour)	Feeding; Algamac Protein Plus (Aquafaune Bio-Marine Inc. Hawthorne USA) V=3,200 L, 25.5 ± 0.1 °C, ‰25 mg L^{-1}, and 11.4 ± 0.3 O_2
T=3(72 hour)	Harvest (ongoing washing after harvest and first sampling before transfer to enrichment. Sampling, **R-0**; from rotifer culture/non-enriched rotifer)
Enrichment	
T=0-19 hour	Pre-feeding; Ebio FB (Ege Biyoteknoloji A.Ş. İzmir TR) Enriching; Spresso V=2,500 L, 26.0 ± 0.0 °C, ‰41 mg L^{-1}, and 10.1 ± 0.5 O_2,
T=19 hour	Harvest (**s**ampling; after harvest and washing/the first sampling of before the feeding of larvae (0. hour; **R-T0**) and following samples of enriched rotifers stocked at +4 °C after harvest (6., 12., and 18. hour; **R-T6**, **R-T12**, **R-T18**)
Non-enriched *Artemia*, *Artemia* nauplii (**A0**) Artemia Cysts, Vinh Chau-Bac Lieu *Artemia* Co. Op	
T=17 hour	Hydration (1.5 hours) and Decapsulate (chlorac - sodium hypochlorite + NaOH at 15 minute); 100 g L^{-1} *Artemia*; 11‰ and 20.5 °C water V=325 L, 29.3 ± 0.0 °C, ‰41 mg L^{-1}, and 11.5 ± 0.2 O_2 Hatching (sampling; the first sampling after harvest and washing/the first sampling of before the feeding of larvae (0. hour; **A0-T0**) and following samples of enriched rotifers stocked at +4 °C after harvest (6., 12., and 18. hour; **A0-T6**, **A0-T12**, **A0-T18**), cysts (**A0-E**) and decapsulated cysts (**A0-D**) of Artemia Cysts
Enriched *Artemia*/EG *Artemia*, *Artemia* metanauplii (**A1**) *Artemia* EG, *Artemia* SepArt EG >225.000 npl/g INVE Aquaculture Salt Lake City Utah/USA	
Culture	
T=24 hour	Hydration V=3,400 L (x2), 29.5 ± 0.0 °C, 41‰ mg L^{-1}, 11.6 ± 0.1 0_2 Hatching (ongoing washing after harvest and first sampling before transfer to enrichment. Sampling (**A1-0**); before enrichment *Artemia*)
Enrichment	
T=0–19.5 hour	**Spresso** V= 4,000 L, 26.0 ± 0.0 °C, ‰41 mg L^{-1}, 13.4 ± 0.2 0_2 **Red AMC** V=325 L, 26.0 ± 0.0 °C, ‰41 mg L^{-1}, 11.5 ± 0.5 0_2
T=19.5 hour	Harvest (sampling; after harvest and washing/the first sampling of before the feeding of larvae (0. hour; **A1/1 & A1/2-T0**) and following samples of enriched *Artemia* stocked at +4 °C after harvest (6., 12., and 18. hour; **A1/1 & A1/2-T6**, **A1/1 & A1/2-T12**, **A1/2-T18**)

Determination of protease activities of live feeds

Acid and neutral protease activities of live feed extracts were determined according to Anson (1938) and alkaline protease activities according to Walter (1984). Hemoglobin solution used as substrate in acid and neutral protease groups at a concentration of 5 g L^{-1} (Buffer: Citrate-Phosphate; the mixture

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of 0.1 M citric acid and 0.2 M dibasic sodium phosphate solutions, pH: 3, 4, 5, 6, 7) and the casein solution used as substrate in alkaline protease groups were prepared at a concentration of 10 g L^{-1} (Buffer: 50 mM Tris HCl, pH: 8.5). Buffer and larvae extracts were incubated for 30 minutes at 37°C and incubated for a further 60 minutes by adding 500 µL of hemoglobin or casein. The reaction was stopped by the addition of 0.5 mL of trichloroacetic acid (TCA at a concentration of 120 g L^{-1}). Protease activities will be given as the expression of the amount of tyrosine released per minute in µg (U mg^{-1} protein).

Proximate compositions

Lipid content of live feeds was given according to Bligh and Dyer (1959), protein amount according to the Kjeldahl method (N×6.25) AOAC (2000a) using protein pre-combustion unit (Velp UD-20), and fully automatic protein distillation unit (Velp UDK 142), and ash content according to AOAC (2000b).

HPLC gel filtration chromatography determination of molecular weight profiles of live feeds

Molecular weight profiles were determined according to Boza *et al*. (1994). Samples were stirred in a phosphate buffer (pH=8.5; 10 mg mL^{-1}) and centrifuged. The supernatants obtained were passed through a 0.22 mm syringe filter and each sample extract was analyzed by HPLC-Gel Filtration Chromatography using a TSKGel G2000 SWXL column. The mobile phase used in the analysis is 0.1 M sodium sulfate solution prepared in 0.1 M phosphate buffer. The flow rate of the column was 1 mL $minute^{-1}$ and the detector used had UV light absorption of 230 nm. Molecular weight calculations were determined according to standards (from SIGMA) bovine albumin (67,000 Dalton, Da), ribonuclease A (13,700 Da), insulin chain A (2,532 Da), tyr-tyr-tyr (508 Da), tryptophan (204 Da), tyrosine (181 Da), p-aminobenzoic acid (137 Da). Molecular weights were calculated as percentage in 4 different fractions based on the retention times of the standards: 67,000 Da≤ (protein/polypeptide), 67,000-13,700 Da (protein/polypeptide), 13,700-2,532 Da (protein/polypeptide), and 2,532 Da≥ (free amino acids+di/tri/oligopeptide).

Data analysis

Protease activities, molecular weight, and proximate composition of live feeds data were subjected to one-way ANOVA, and mean±standard error (SE) differences were made by Duncan test at $p<0.05$ content level using SPSS (version 22) software statistical package. Protease activities and proximate composition measurements were carried out in triplicates and molecular weight measurements in duplicates.

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Results

Alkaline, neutral, and acid protease enzyme activities of live feeds

The statistical evaluation of the live feed groups was first performed in itself for each live feed. The highest protease activity of live feed rotifer was determined in the pH 8.5 alkaline group before being enriched ($p<0.05$). The enzyme activities in the acid substrate of the rotifers enriched as in the time-dependent with the rotifers before the enrichment were decreased from pH 3 to pH 4, from pH 4 to pH 5, and from pH 5 to pH 6, respectively. According to the substrate pH 6, an increase in the rotifers before enrichment in neutral enzyme activity and similarity or increase in the enzyme activities of rotifers other than the 18th hour after enrichment was detected. In contrast, the enzyme activities of the rotifers at alkaline substrate pH 8.5 increased relative to pH 5, pH 6, and pH 7. The decrease in substrate pH 8.5 alkaline enzyme activity of the rotifers was higher than the decrease in substrate pH 3 and pH 4 enzyme activity ($p<0.05$). The enzyme activities of the rotifers before enrichment, compared to enzyme activity without transfer to larva after enrichment were reduced by 28.5%, 19.2%, and 55.5% at pH 3, 4, and 8.5, respectively. The enzyme activities of the rotifers before enrichment, compared to at the 18th hour stored at 4°C after enrichment, decreased by 30.7%, 41.6%, and 81.0% at pH 3, 4, and 8.5, respectively.

It was determined that enzyme activities of the A0 *Artemia* cysts decreased after decapsulation at pH 3 and pH 4. In the first samples of the enriched *Artemia*/*A.* nauplii following harvest and washing after hatching of A0 cysts, pH 3 and pH 8.5 enzyme activities were similar but lower than pH 4 ($p<0.05$). The enzyme activities of the A0 *Artemia*s pH 4, and pH 8.5 were similar ($p>0.05$) and highest in the 6th hour after stock at 4 degrees centigrade. 12th and 18th hour enzyme activities were high at pH 8.5 ($p<0.05$). In short, pH 8.5 alkaline enzyme activities of the A0 *Artemia* at the 12th and 18th hours were higher than acid and neutral enzyme activities, particularly pH 3 and pH 4 enzyme activities. It was determined that the enzyme activities of the *Artemia*s after hatching from decapsulated cysts increased in pH 3, pH 4 and pH 8.5 by 51.9%, 71.4%, and 97.4%, respectively. The 18th hour enzyme activities of the *Artemia*s hatched from decapsulated cysts were increased by 34.11%, 74.0%, and 98.4% at pH 3, pH 4, and pH 8.5, respectively. No protease enzyme activity was detected at pH 8.5 in the A1 *Artemia* cysts. Enzyme activities of the A1 *Artemia* cysts were significantly higher only at pH 3 in other protease groups of the *Artemia*s (A1-0) before enrichment after hatching. The enzyme activities of the enriched A1/1 *Artemia* as in the time-dependent with the A1 *Artemia*s before the enrichment were higher at pH 4 than pH 3, at pH 6 than pH 5, at pH 7 than pH 6, and at pH 8.5 than pH 7, respectively. It was lower at pH 5 than pH 4. In contrast, the time-dependent enzyme activities of the enriched A1/2 *Artemia*s with A1

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*Artemia*s before enrichment were higher at pH 4 than pH 3, at pH 7 than pH 6, at pH 8.5 than pH 7, respectively. It was lower in the *Artemia*s at pH 5 than pH 4. It was also lower in the *Artemia*s at pH 6 compared to pH 5, but the enzyme activities of the pH 6 *Artemia*s were higher than those of the non-enriched A1 *Artemia*s and were similar to those of the *Artemia*s at the 6th hour. The enzyme activities after the enrichment were found to be high at all sampling hours at pH 3, pH 6, pH 7, and pH 8.5 in the A1/1 *Artemia*s (p<0.05) (Table 2).

Table 2: Protease activity of live feed groups (U mg^{-1} protein, mean ± SE).

Time	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8.5
	Rotifer, *B. plicatilis* (R)					
R-0	$97.07\pm0.34^{b,A}$	$72.40\pm0.15^{d,A}$	$20.65\pm1.28^{lm,E}$	$12.63\pm0.59^{p,I}$	$21.31\pm0.4^{l,F}$	$102.76\pm1.48^{a,I}$
R-T0	$69.42\pm1.03^{e,C}$	$58.52\pm0.69^{h,C}$	$18.89\pm0.76^{mn,EFG}$	$11.90\pm0.40^{pq,IJ}$	$10.25\pm0.33^{q,H}$	$45.74\pm1.13^{i,J}$
R-T6	$78.67\pm1.24^{c,B}$	$58.44\pm0.79^{h,C}$	$17.27\pm0.27^{no,FGH}$	$2.51\pm0.20^{s,M}$	$3.71\pm0.39^{s,J}$	$26.82\pm0.59^{k,M}$
R-T12	$64.53\pm0.91^{g,D}$	$63.93\pm0.87^{g,B}$	$16.75\pm0.42^{o,GH}$	$3.23\pm0.15^{s,M}$	$6.80\pm0.53^{r,I}$	$26.90\pm0.33^{k,M}$
R-T18	$67.31\pm0.67^{f,C}$	$42.26\pm0.94^{j,E}$	$15.48\pm0.74^{o,H}$	$6.65\pm0.0^{r,L}$	$3.76\pm0.54^{s,J}$	$19.53\pm0.54^{lm,NO}$
	Non-enriched *Artemia* (*Artemia* nauplii-A0)					
A0-E	$12.57\pm0.34^{ghij,1}$	$13.79\pm1.00^{fgh,1}$	$1.26\pm0.37^{p,56}$	$2.41\pm0.41^{p,45}$	$1.00\pm0.22^{p,56}$	$0.67\pm0.00^{p,6}$
A0-D	$9.91\pm0.14^{jklm,2}$	$7.12\pm0.55^{mno,3}$	$1.09\pm0.37^{p,56}$	$0.71\pm0.19^{p,6}$	$1.06\pm0.24^{p,56}$	$0.56\pm0.56^{p,6}$
A0-T0	$20.58\pm1.08^{d,E}$	$24.93\pm2.68^{c,I}$	$7.10\pm0.90^{mno,JK}$	$15.42\pm1.00^{efg,H}$	$11.10\pm0.51^{hijk,H}$	$21.63\pm0.14^{d,N}$
A0-T6	$7.66\pm0.25^{lmno,H}$	$16.58\pm0.61^{ef,J}$	$5.27\pm0.32^{o,K}$	$8.57\pm0.28^{klmn,K}$	$11.17\pm0.80^{hijk,H}$	$17.07\pm0.56^{e,O}$
A0-T12	$14.94\pm0.94^{efg,F}$	$16.15\pm0.86^{ef,J}$	$5.83\pm0.84^{no,JK}$	$11.26\pm0.40^{hijk,IJ}$	$15.85\pm1.05^{ef,G}$	$29.69\pm0.67^{b,L}$
A0-T18	$15.04\pm1.76^{efg,F}$	$27.33\pm3.64^{bc,KI}$	$7.83\pm0.56^{lmno,J}$	$10.49\pm0.18^{ijkl,J}$	$13.60\pm0.55^{fghi,G}$	$34.82\pm0.28^{a,K}$
	Enriched *Artemia*, (*Artemia* metanauplii-A1)					
A1-E	$13.21\pm0.39^{p,1}$	$9.33\pm0.76^{q,2}$	$2.80\pm0.45^{r,4}$	$2.28\pm0.39^{r,45}$	$1.19\pm0.27^{r,56}$	*
A1-0	$2.72\pm0.66^{r,I}$	$43.86\pm0.69^{ij,E}$	$19.47\pm0.14^{o,EF}$	$26.74\pm0.81^{m,F}$	$43.76\pm1.11^{ij,E}$	$134.99\pm0.32^{d,H}$
A1/1-T0	$13.71\pm0.58^{p,FG}$	$38.51\pm0.13^{k,F}$	$21.00\pm0.38^{o,E}$	$43.88\pm0.15^{ij,A}$	$68.07\pm1.19^{e,B}$	$366.19\pm1.31^{b,F}$
A1/1-T6	$12.25\pm0.46^{p,FG}$	$46.22\pm0.12^{h,DE}$	$24.04\pm1.31^{n,D}$	$42.11\pm0.42^{j,B}$	$56.92\pm0.46^{g,D}$	$344.49\pm0.68^{c,G}$
A1/1-T12	$20.22\pm0.84^{o,E}$	$44.93\pm0.59^{hi,DE}$	$12.93\pm0.68^{p,I}$	$29.13\pm0.49^{l,DE}$	$64.21\pm0.72^{f,C}$	$429.69\pm0.97^{a,B}$
A1-E	13.21 ± 0.39^{p}	9.33 ± 0.76^{rs}	2.80 ± 0.45^{tu}	2.28 ± 0.39^{tu}	1.19 ± 0.27^{tu}	*
A1-0	2.72 ± 0.66^{tu}	43.86 ± 0.69^{j}	19.47 ± 0.14^{o}	26.74 ± 0.81^{n}	43.76 ± 1.11^{j}	134.99 ± 0.32^{e}
A1/2-T0	$19.56\pm0.39^{o,E}$	$28.95\pm0.55^{lmn,H}$	$28.95\pm0.47^{lmn,BC}$	$36.31\pm1.18^{k,C}$	$80.49\pm1.25^{f,A}$	$461.59\pm1.33^{a,A}$
A1/2-T6	$7.50\pm0.51^{s,H}$	$34.37\pm0.86^{k,G}$	$28.26\pm0.99^{mn,C}$	$28.07\pm0.35^{mn,EF}$	$57.26\pm0.77^{h,D}$	$411.54\pm0.77^{c,D}$
A1/2-T12	$11.69\pm1.63^{pr,G}$	$43.86\pm0.27^{i,E}$	$35.38\pm1.12^{k,A}$	$29.92\pm0.63^{lm,D}$	$65.81\pm1.21^{g,BC}$	$406.26\pm1.11^{d,E}$
A1/2-T18	$7.13\pm1.30^{s,H}$	$48.25\pm0.65^{i,D}$	$31.02\pm1.02^{l,B}$	$17.83\pm1.21^{o,G}$	$63.52\pm0.56^{g,C}$	$417.74\pm1.47^{b,C}$

Different superscripts show significant differences between means of protease activities (p<0.05). (R-rotifer, A0-*Artemia* nauplii, A1/1 and A1/2-*Artemia* metanauplii) are important. R-0; before enrichment is taken, R-T0, 6, 12, 18; 0, 6, 12, 18 hours after enrichment harvest, A0-E; *Artemia* nauplii cysts, -, A0-D; *Artemia* nauplii cysts after decapsulation, A0-T0, 6, 12, 18; 0, 6, 12, 18 hours harvest, A1-E; *Artemia* metanauplii cysts, A1-0; before enrichment is taken, A1/1; enriched *Artemia* metanauplii-1, A1/2; enriched *Artemia* metanauplii-2 and, A1/1-T0, 6, 12 hours after enrichment and A1/2-T0, 6, 12, 18 hours post-enrichment harvest.* There is no enzyme activity. All values belong to the values after harvest and washing.

In the A1/2 *Artemia*, pH 3, pH 5, pH 7, and pH 8.5 were found to be high at all sampling hours (p<0.05). Only at pH 6, the 6th hour enzyme activities after enrichment with the non-enriched *Artemia* were similar (p>0.05). The enzyme activities of the non-enriched A1 *Artemia*s at pH 4, pH 7, and pH 8.5 after enrichment increased by 12.2%, 35.7%, and 63.1% in the A1/1 *Artemia*s at the first sampling hour, respectively. In the A1/2 *Artemia*s, it increased by 34.0%, 45.6%, and 70.8%, respectively. On the other hand, the increase rates in the last sampling hour were 2.4%, 31.8%, and 68.6% for A1/1 and 9.1%,

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31.1%, and 67.7% for A1/2 *Artemia*s, respectively.

Statistical evaluations of live feeds were made according to protease groups in Table 3. The highest enzyme activities of live feeds at pH 3 were determined in the rotifers before enrichment ($p<0.05$). In addition, enzyme activities of enriched rotifers at pH 3 were found to be significantly higher than other live feed groups ($p<0.05$). After hatching, the enzyme activities of the A0 *A.* nauplii decreased after 6th hours due to stocking at 4 degrees centigrade and then increased over time. It was found that enzyme activities increased in the A1/1 *Artemia*s after enrichment and decreased in the A1/2 *Artemia*s after enrichment. Enzyme activities of the non-enriched/A0 *Artemia*s at the 6th hour, and the enriched A1/2 *Artemia*s at the 6th, and 18th hour had the lowest enzyme values at pH 3. The highest enzyme activities of live feeds at pH 4 were determined in the rotifers after being enriched again ($p<0.05$). After hatching, the A0 *A.* nauplii had a similar level of first enzyme activity with 18th hour enzyme activity and was higher than 6th and 12th hour enzyme activities. The A0 *A.* nauplii enzyme activities at the 6th and 12th hours had the lowest enzyme values at pH 4. The enriched A1/2 Artemia's 18thhour enzyme activity was higher than enriched rotifer ($p<0.05$). The activity of protease enzymes at pH 5 was highest in A1/2 the enriched *Artemia*s ($p<0.05$). Enzyme activities of the A1 *Artemia* and rotifers which were not enriched were similar ($p>0.05$). Enzyme activities of the enriched A1/1 *Artemia* and rotifers were similar in the first sampling after enrichment. The enzyme activities of the A1/2 *Artemia*s were higher than enriched rotifers ($p<0.05$). Protease enzyme activities at pH 6 were found to be high in the enriched *Artemia*s ($p<0.05$). After enrichment first sampling and 6th hour samples were higher in the A1/1 *Artemia*s ($p<0.05$). The enzyme activities of *Artemia*s enriched at the 12th hour were similar in the A1/1 and A1/2 *Artemia*s ($p<0.05$). The lowest enzyme activities were detected in the enriched rotifers at 6th hours and after ($p<0.05$). Similarly, protease enzyme activities at pH 7 were detected in high amounts in the enriched *Artemia*s ($p<0.05$) (Table 3).

The first samples of A1/2 *Artemia*s after enrichment had higher enzyme activities ($p<0.05$). 6th and 12th hour enzyme activities were similar in both the enriched *Artemia* groups ($p>0.05$). As at pH 6, the lowest enzyme activities were also detected in the enriched rotifers at 6th hours and after ($p<0.05$). At pH 8.5, the highest enzyme activities were detected in the enriched *Artemia*s ($p<0.05$) (Table 2) -. The highest enzyme value of live feeds was determined from 461.59±1.33 U/mg protein the enriched A1/2 *Artemia*s after the first samples. The enzyme activities of the enriched A1/1 *Artemia*s increased at the 0th and 12th hours except for the 6th hour. On the other hand, it has been found that A1/2 tended to decrease. It was determined that it increased only in the 18th hour but remained below the 0th hour. Enzyme activity decreased in the rotifers after enrichment, whereas A0

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Artemia nauplii were found to increase in the 6th hour and after.

Table 3: Time dependent enzyme activities of live feeds according to protease groups (U mg^{-1} protein, mean±SE).

pH	Live feed	T0	T6	T12	T18
3	R-0	97.07±0.34^{e}	97.07±0.34^{e}	97.07±0.34^{e}	97.07±0.34^{d}
	R	69.42±1.03^{h}	78.67±1.24^{f}	64.53±0.91^{g}	67.31±0.67^{f}
	A0	20.58±1.08^{n}	7.66±0.25^{q}	14.94±0.94^{mn}	15.04±1.76^{op}
	A1-0	2.72±0.66^{s}	2.72±0.66^{s}	2.72±0.66^{q}	2.72±0.66^{u}
	A1/1	13.71±0.58^{op}	12.25±0.46^{p}	20.22±0.84^{l}	
	A1/2	19.56±0.39^{n}	7.50±0.51^{q}	11.69±1.63^{o}	7.13±1.30^{r}
4	R-0	72.40±0.15^{g}	72.40±0.15^{g}	72.40±0.15^{f}	72.40±0.15^{e}
	R	58.52±0.69^{i}	58.44±0.79^{h}	63.93±0.87^{g}	42.26±0.94^{i}
	A0	24.93±2.68^{m}	16.58±0.61^{o}	16.15±0.86^{m}	27.33±3.64^{l}
	A1-0	43.86±0.69^{j}	43.86±0.69^{j}	43.86±0.69^{h}	43.86±0.69^{i}
	A1/1	38.51±0.13^{k}	46.22±0.12^{i}	44.93±0.59^{h}	
	A1/2	28.95±0.55^{l}	34.37±0.86^{k}	43.86±0.27^{h}	48.25±0.65^{h}
5	R-0	20.65±1.28^{n}	20.65±1.28^{n}	20.65±1.28^{l}	20.65±1.28^{mn}
	R	18.89±0.76^{n}	17.27±0.27^{o}	16.75±0.42^{m}	15.48±0.74^{op}
	A0	7.10±0.90^{r}	5.27±0.32^{r}	5.83±0.84^{p}	7.83±0.56^{rs}
	A1-0	19.47±0.14^{n}	19.47±0.14^{n}	19.47±0.14^{l}	19.47±0.14^{mn}
	A1/1	21.00±0.38^{n}	24.04±1.31^{m}	12.93±0.68^{no}	
	A1/2	28.95±0.47^{l}	28.26±0.99^{l}	35.38±1.12^{i}	31.02±1.02^{k}
6	R-0	12.63±0.59^{pq}	12.63±0.59^{p}	12.63±0.59^{no}	12.63±0.59^{pq}
	R	11.90±0.40^{pq}	2.51±0.20^{s}	3.23±0.15^{q}	6.65±0.0st
	A0	15.42±1.00^{o}	8.57±0.28^{q}	11.26±0.40^{o}	10.49±0.18^{qr}
	A1-0	26.74±0.81^{lm}	26.74±0.81^{l}	26.74±0.81^{k}	26.74±0.81^{l}
	A1/1	43.88±0.15^{j}	42.11±0.42^{j}	29.13±0.49^{jk}	
	A1/2	36.31±1.18^{k}	28.07±0.35^{l}	29.92±0.63^{j}	17.83±1.21^{no}
7	R-0	21.31±0.4^{n}	21.31±0.4^{n}	21.31±0.4^{l}	21.31±0.4^{m}
	R	10.25±0.33^{q}	3.71±0.39^{rs}	6.80±0.53^{p}	3.76±0.54^{tu}
	A0	11.10±0.51^{pq}	11.17±0.80^{p}	15.85±1.05^{m}	13.60±0.55^{pq}
	A1-0	43.76±1.11^{j}	43.76±1.11^{j}	43.76±1.11^{h}	43.76±1.11^{i}
	A1/1	68.07±1.19^{h}	56.92±0.46^{h}	64.21±0.72^{g}	
	A1/2	80.49±1.25^{f}	57.26±0.77^{h}	65.81±1.21^{g}	63.52±0.56^{g}
8.5	R-0	102.76±1.48^{d}	102.76±1.48^{d}	102.76±1.48^{d}	102.76±1.48^{c}
	R	45.74±1.13^{j}	26.82±0.59^{l}	26.90±0.33^{k}	19.53±0.54^{mn}
	A0	21.63±0.14^{n}	17.07±0.56^{o}	29.69±0.67^{j}	34.82±0.28^{j}
	A1-0	134.99±0.32^{c}	134.99±0.32^{c}	134.99±0.32^{c}	134.99±0.32^{b}
	A1/1	366.19±1.31^{b}	344.49±0.68^{b}	429.69±0.97^{a}	
	A1/2	461.59±1.33^{a}	411.54±0.77^{a}	406.26±1.11^{b}	417.74±1.47^{a}

Different superscripts show significant differences between means of protease activities (p<0.05) (R–rotifer, A0-*Artemia* nauplii, A1/1 and A1/2-*Artemia* metanauplii). R-0; before enrichment is taken, R-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest, A0-T0, 6, 12, 18; 0., 6., 12., 18. hours after harvest, A1-0; before enrichment is taken, A1/1; enriched *Artemia* metanauplii-1, A1/2; enriched *Artemia* metanauplii-2 and, A1/1-T0, 6, 12; 0., 6., 12. hours after enrichment and A1/2-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest. Dark lines are values of live feeds before enrichment. All values belong to the values after harvest and washing.

The protease enzyme activities of the rotifers before enrichment were found to be higher than after enrichment values at all substrate pHs (Table 3). There was the only similarity with the first sample group (0th) after statistical enrichment at pH 5 and pH 6 (p>0.05). In general, it was found that the enzyme activities of

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the enriched rotifers and samples stored at 4 degrees centigrade after the first harvest decreased within time. Apart from pH 3 and pH 6, the enzyme activities of the rotifers decreased at the 18th hour compared to the enzyme values of the previous hour and were the lowest ($p<0.05$). Only enzyme activities at pH 5 were similar to the previous one ($p>0.05$) (Table 2). The 12th hour enzyme activity at pH 7 increased compared to the previous one ($p<0.05$) and the 12th hour enzyme activity had the lowest value similar to the 18th hour enzyme activity ($p<0.05$). After the enrichment of the rotifers, there were not many changes in time-dependent changes in enzyme activities at pH 3, pH 4, and pH 5. Enzyme activities of the non-enriched rotifers and the A1 *Artemia*s were found to be high in pH 3, and pH 4 in the rotifers, similar to pH 5, and high in the A1 *Artemia* at pH 6, pH 7, and pH 8.5. The pH 3 and pH 4 acid enzyme activities of decapsulated and non-decapsulated A0 *Artemia* cysts and A1 *Artemia* cysts were found to be higher than other enzyme groups cyst activities ($p<0.05$) (Tables 2 and 3). The pH 3 enzyme activities of the A0 and A1 *Artemia* cysts were similar ($p>0.05$). The enzyme activity of the A0 *Artemia* cyst at pH 4 was higher than A1 *Artemia* cysts ($p<0.05$). Enzyme activities of the A0 *Artemia* cysts except at pH 6 and decapsulated A0 *Artemia* cysts except at pH 7 decreased from pH 3 to pH 8.5. Similarly, enzyme activities of the A1 *Artemia* cysts also decreased from pH 3 to pH 8.5. The decapsulation process was found to reduce the enzyme activity of cysts at pH 3, 4, and 6.

Determination of proximate compositions of live feeds

Crude ash and crude lipid values after the enriched rotifers increased compared to the rotifers before enrichment, while crude protein values decreased ($p<0.05$) (Table 4). The crude ash values of the non-enriched *Artemia* remained in similar proportions. In contrast, crude protein values decreased ($p<0.05$) while crude lipid values did not change ($p>0.05$). The increase in crude ash values of the enriched A1/1 *Artemia* was higher than that of the A1/2 *Artemia*. Crude protein values of the enriched *Artemia*s were decreased. However, crude lipid values increased. All proximate rates of decapsulated non-enriched *Artemia* were increased, while crude ash and crude lipid values were much higher. While the crude ash and crude lipid values of the non-decapsulated non-enriched *Artemia* cyst and enriched *Artemia* cyst were high, the crude protein value was higher in the non-enriched *Artemia* cyst. The highest crude ash values were determined at the 6th and 12th hours of the rotifer and the lowest crude ash values were detected in both *Artemia* cysts. The highest crude protein values were determined at the 0th hour of the enriched A1 *Artemia* and non-enriched *Artemia* (A1-0) while the lowest values were determined at the 6th hour of the enriched rotifer, at the 12th and 18th hours of the non-enriched *Artemia* (A0) and the enriched A1/2 *Artemia*. The highest crude lipid values

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were also detected at the 12th hour in A1/1 and at the 18th hour in A1/2 of the enriched *Artemia*, while the lowest values belonged to both *Artemia* cysts.

Table 4: Proximate composition of live feeds (%, mean±SE).

Live feed	Crude ash	Crude protein	Crude lipid	CP/CL
Rotifer, *B. plicatilis* (R)				
R-0	8.30±0.56^{h}	60.53±0.70^{bc}	13.21±0.38^{h}	4.59±0.17^{c}
R-T0	15.50±0.67^{bc}	46.01±1.94^{hi}	17.72±0.50^{fg}	2.61±0.18^{hi}
R-T6	17.08±0.53^{a}	44.53±1.89^{ij}	21.25±0.70^{cd}	2.10±0.13^{kl}
R-T12	17.46±0.21^{a}	48.84±0.35^{fg}	20.53±0.30^{de}	2.38±0.02^{ijk}
R-T18	14.86±0.46^{bc}	47.72±0.92^{fgh}	22.23±0.03^{c}	2.15±0.04^{kl}
Non-enriched *Artemia* (*Artemia* nauplii-A0)				
A0-E	3.62±0.18^{i}	59.33±0.20^{cd}	7.25±0.07^{i}	8.19±0.09^{a}
A0-D	10.93±0.31^{de}	60.46±0.34^{bc}	19.78±0.23^{d}	3.06±0.04^{fg}
A0-T0	10.77±0.50^{def}	57.33±1.19^{de}	20.36±0.79^{de}	2.82±0.05^{gh}
A0-T6	9.53±0.13^{fgh}	46.84±0.69^{ghi}	21.11±0.30^{cde}	2.22±0.01^{jk}
A0-T12	8.77±0.32^{gh}	45.77±0.77^{hij}	20.44±0.15^{de}	2.24±0.02^{jk}
A0-T18	9.74±0.44^{efg}	43.19±0.20^{ij}	20.41±0.47^{de}	2.12±0.04^{kl}
Enriched *Artemia* (*Artemia* metanauplii-A1)				
A1-E	4.41±0.18^{i}	56.42±0.79^{e}	7.46±0.14^{i}	7.57±0.15^{b}
A1-0	9.02±0.24^{gh}	64.35±0.30^{a}	16.50±0.41^{g}	3.90±0.08^{d}
A1/1-T0	8.87±0.30^{gh}	58.71±0.78^{cde}	17.98±0.41^{e}	3.27±0.05^{f}
A1/1-T6	11.60±0.44^{d}	49.84±0.52^{f}	20.21±0.36^{de}	2.47±0.07^{ij}
A1/1-T12	14.60±0.61^{bc}	49.05±0.96^{fg}	27.01±0.73^{a}	1.82±0.08^{m}
A1/2-T0	9.77±0.19^{efg}	62.12±0.11^{ab}	17.38±0.41^{fg}	3.58±0.08^{e}
A1/2-T6	10.66±0.80^{def}	45.77±0.08^{hij}	24.25±0.70^{b}	1.89±0.06^{lm}
A1/2-T12	14.03±0.35^{c}	44.61±0.71^{ij}	23.71±0.38^{b}	1.88±0.03^{lm}
A1/2-T18	11.61±0.41^{d}	45.07±0.43^{hij}	26.61±0.11^{a}	1.69±0.02^{m}

Different superscripts show significant differences between means of proximate composition (p<0.05) (R-rotifer, A0-*Artemia* nauplii, A1/1 and A1/2- *Artemia* metanauplii). R-0; before enrichment is taken, R-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest, A0-E; *Artemia* nauplii cysts, A0-D; *Artemia* nauplii cysts after decapsulation, A0-T0, 6, 12, 18; 0., 6., 12., 18. hours harvest, A1-E; *Artemia* metanauplii cysts, A1-0; before enrichment is taken, A1/1; enriched *Artemia* metanauplii-1, A1/2; enriched *Artemia* metanauplii-2 and, A1/1-T0, 6, 12; 0., 6., 12. hours after enrichment and A1/2-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest. CP; crude protein, CL; crude lipid.

Distribution of molecular weight of live feeds

Molecular weight values of the highest 2,532 Da≥ free amino acid+di/tri/ oligopeptides were determined in the rotifers after enrichment harvest and in the A1/2 *Artemia* (Table 5). 67,000≤ Da protein/polypeptide values in A0 cysts, 67,000-13,700 Da protein/polypeptide values in decapsulated A0 cysts, and at 0th and 18th hour in the A0 *Artemia*s and 13,700-2,532 Da protein/polypeptide values at 18th hour in the A0 and A1/2 *Artemia*s were determined. It was determined that time-dependent 2,532 Da≥ changes of the rotifers before and after enrichment were less than other live feeds. Compared to the non-enriched *Artemia* (A1-0), both enriched *Artemia*s have high molecular weights of 2,532 Da≥. It was determined that the molecular weight of the enriched *Artemia*s 2,532 Da≥ -was similar in A1/1 but decreased in A1/2. However, 2,532 Da≥ values at 0th and 12th hours were higher at A1/2. According to the before

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enrichment situation, 67,000≤ Da protein/polypeptide values were similar in the rotifer, while the enriched *Artemia* decreased. The molecular weight value of 67,000-13,700 Da was reduced in the enriched *Artemia*s and rotifers except in the 18th hour. The value of 13,700-2,532 Da increased in the enriched rotifers and the enriched A1/2 *Artemia*s at the 18th hour. The A1 *Artemia* cysts 2,532 Da≥ value was higher than A0 *Artemia* cysts. 67,000 Da≤ was higher in A0 cysts, 67,000-13,700 Da in decapsulated A0 cysts.

Table 5: Molecular weights of live feeds (%, mean ± SE).

Live feed	Molecular weight fraction (Da)				
	67,000≤ protein/polipeptit	67,000-13,700 protein/polypeptide	13,700-2,532 protein/polypeptide	2,532≥ free a.acid+di/tri/ oligopeptide	2,532≥ / 67,000≤
Rotifer, *B. plicatilis* (R)					
R-0	13.71±0.94^{kl}	16.47±0.60^{g}	1.95±0.07^{j}	67.86±1.64^{b}	4.98±0.46^{c}
R-T0	12.95±0.12^{l}	13.47±0.17^{i}	1.65±0.01^{k}	71.97±0.24^{a}	5.56±0.07^{b}
R-T6	14.84±0.36^{jk}	14.08±0.92^{hi}	1.92±0.02^{j}	69.21±0.62^{b}	4.67±0.07^{cd}
R-T12	14.98±0.51^{j}	13.88±0.06^{i}	1.95±0.01^{j}	69.24±0.61^{b}	4.63±0.20^{cd}
R-T18	14.63±0.27^{jk}	17.28±0.27^{g}	2.25±0.04^{i}	65.98±0.48^{c}	4.51±0.12^{d}
Non-enriched *Artemia* (*Artemia* nauplii-A0)					
A0-E	37.40±0.12^{a}	21.95±0.46^{cd}	2.55±0.06^{gh}	38.60±0.72^{i}	1.03±0.02^{k}
A0-D	33.19±0.18^{b}	26.57±0.13^{a}	2.52±0.01^{h}	37.99±0.17^{i}	1.14±0.01^{k}
A0-T0	21.92±0.24^{f}	27.66±0.01^{a}	3.04±0.01^{b}	47.46±0.18^{h}	2.17±0.03^{i}
A0-T6	22.03±0.14^{f}	24.72±0.23^{b}	2.85±0.02^{cd}	50.54±0.25^{g}	2.29±0.03^{i}
A0-T12	22.40±0.16^{f}	25.31±0.35^{b}	2.99±0.07^{bc}	49.45±0.50^{g}	2.21±0.04^{i}
A0-T18	30.97±0.06^{c}	27.16±0.17^{a}	3.12±0.04^{ab}	38.96±0.35^{i}	1.26±0.01^{k}
Enriched *Artemia* (*Artemia* metanauplii-A1)					
A1-E	29.30±0.96^{d}	19.31±0.50^{e}	2.59±0.09^{fgh}	49.23±1.16^{gh}	1.68±0.10^{j}
A1-0	27.43±0.27^{e}	22.33±0.09^{c}	2.78±0.00^{de}	47.56±0.32^{h}	1.73±0.03^{j}
A1/1-T0	15.58±0.41^{ij}	18.50±0.10^{ef}	2.80±0.08^{de}	63.16±0.57^{d}	4.06±0.14^{e}
A1/1-T6	16.69±0.32^{hi}	17.47±0.53^{fg}	2.72±0.09^{def}	63.38±0.25^{d}	3.80±0.06^{ef}
A1/1-T12	18.87±0.09^{g}	15.04±0.04^{h}	2.46±0.03^{h}	63.82±0.25^{d}	3.38±0.03^{g}
A1/2-T0	10.67±0.10^{m}	14.17±0.18^{hi}	2.08±0.04^{j}	73.09±0.24^{a}	6.85±0.09^{a}
A1/2-T6	17.13±0.37^{h}	19.02±0.03^{e}	2.69±0.01^{efg}	61.28±0.36^{e}	3.58±0.10^{fg}
A1/2-T12	13.41±0.13^{l}	16.57±0.20^{g}	2.53±0.02^{h}	67.53±0.29^{bc}	5.04±0.07^{c}
A1/2-T18	19.95±0.20^{g}	20.91±0.32^{d}	3.21±0.04^{a}	56.54±0.38^{f}	2.83±0.01^{h}

Different superscripts show significant differences between means of molecular weights (p<0.05) (R-rotifer, A0-*Artemia* nauplii, A1/1 and A1/2- *Artemia* metanauplii). R-0; before enrichment is taken, R-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest, A0-E; *Artemia* nauplii cysts, A0-D; *Artemia* nauplii cysts after decapsulation, A0-T0, 6, 12, 18; 0., 6., 12., 18. hours harvest, A1-E; *Artemia* metanauplii cysts, A1-0; before enrichment is taken, A1/1; enriched *Artemia* metanauplii-1, A1/2; enriched *Artemia* metanauplii-2 and, A1/1-T0, 6, 12; 0., 6., 12. hours after enrichment and A1/2-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest.

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Evaluation of alkaline, neutral, and acid protease enzyme activities, molecular weights, and proximate compositions of live feeds

As a result (Tables 2, 3, and 6); (i) the non-enriched rotifer at pH 3 and 4 (ii) the enriched *Artemia* 12th and 18th at pH 5 of A1/2, 0th and 6th at pH 6 of A1/1, and 0th in pH 7 of A1/1, and A1/2, A1/1 and non-enriched (A1-0) in pH 8.5 enzyme values were significant (p<0.05). The enriched *Artemia* A1/2 and after harvest the rotifer (T0) were important at the initial feeding in terms of molecular weights of 2,532 Da≥ (p<0.05) (Tables 5, 6).

Table 6: Evaluation of live feed preferences according to enzyme value (%, mean±SE).

pH groups	Live feed	Protease	Molecular weight (2,532≥ / 67,000≤)	Proximate (protein / lipid)
pH 3	R-0	97.07±0.34^{A}	67.86±1.64^{b}/13.71±0.94^{kl}	60.53±0.70^{bc}/13.21±0.38^{h}
pH 4	R-0	72.40±0.15^{A}	67.86±1.64^{b}/13.71±0.94^{kl}	60.53±0.70^{bc}/13.21±0.38^{h}
pH 5	A1/2-T12	35.38±1.12^{A}	67.53±0.29^{bc}/13.41±0.13^{l}	44.61±0.71^{ij}/23.71±0.38^{b}
	A1/2-T18	31.02±1.02^{B}	56.54±0.38^{f}/19.95±0.20^{g}	45.07±0.43^{hij}/26.61±0.11^{a}
pH 6	A1/1-T0	43.88±0.15^{A}	63.16±0.57^{d}/15.58±0.41^{ij}	58.71±0.78^{cde}/17.98±0.41^{e}
	A1/1-T6	42.11±0.42^{B}	63.38±0.25^{d}/16.69±0.32^{hi}	49.84±0.52^{f}/20.21±0.36^{de}
pH 7	A1/2-T0	80.49±1.25^{A}	73.09±0.24^{a}/10.67±0.10^{m}	62.12±0.11^{ab}/17.38±0.41^{fg}
pH 8.5	A1/2-T0	461.59±1.33^{A}	73.09±0.24^{a}/10.67±0.10^{m}	62.12±0.11^{ab}/17.38±0.41^{fg}
	A1/1-T12	429.69±0.97^{B}	63.82±0.25^{d}/13.41±0.13^{l}	49.05±0.96^{fg}/27.01±0.73^{a}
	A1/2-T18	417.74±1.47^{C}	56.54±0.38^{f}/19.95±0.20^{g}	45.07±0.43^{hij}/26.61±0.11^{a}
	A1/2-T6	411.54±0.77^{D}	61.28±0.36^{e}/17.13±0.37^{h}	45.77±0.08^{hij}/24.25±0.70^{b}
	A1/2-T12	406.26±1.11^{E}	67.53±0.29^{bc}/13.41±0.13^{l}	44.61±0.71^{ij}/23.71±0.38^{b}
	A1/1-T0	366.19±1.31^{F}	63.16±0.57^{d}/15.58±0.41^{ij}	58.71±0.78^{cde}/17.98±0.41^{e}
	A1/1-T6	344.49±0.68^{G}	63.38±0.25^{d}/16.69±0.32^{hi}	49.84±0.52^{f}/20.21±0.36^{de}
	A1-0	134.99±0.32^{H}	47.56±0.32^{h}/27.43±0.27^{e}	64.35±0.30^{a}/16.50±0.41^{g}
Molecular weights & proximate	R-T0		71.97±0.24^{a}/12.95±0.12^{l}	46.01±1.94^{hi}/17.72±0.50^{fg}
	A1/2-T0		73.09±0.24^{a}/10.67±0.10^{m}	62.12±0.11^{ab}/17.38±0.41^{fg}
	A0-E		38.60±0.72^{i} /37.40±0.12^{a}	59.33±0.20^{cd}/7.25±0.07^{i}
	A0-D		37.99±0.17^{i} /33.19±0.18^{b}	60.46±0.34^{bc}/19.78±0.23^{d}
	A0-T18		38.96±0.35^{i} /30.97±0.06^{c}	43.19±0.20^{ij}/20.41±0.47^{de}
	A1-E		49.23±1.16^{gh}/29.30±0.96^{d}	56.42±0.79^{e}/7.46±0.14^{i}
	A1-0		47.56±0.32^{h}/27.43±0.27^{e}	64.35±0.30^{a} /16.50±0.41^{g}
	A1/1-T12		63.82±0.25^{d}/13.41±0.13^{l}	49.05±0.96^{fg}/27.01±0.73^{a}
	A1/2-T18		56.54±0.38^{f}/19.95±0.20^{g}	45.07±0.43^{hij}/26.61±0.11^{a}

Different superscripts show significant differences between means of protease activities, molecular weights, and proximate composition (p<0.05) R-rotifer, A0-*Artemia* nauplii, A1/1 and A1/2- *Artemia* metanauplii. R-0; before enrichment is taken, R-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest, A0-E; *Artemia* nauplii cysts, A0-D; *Artemia* nauplii cysts after decapsulation, A0-T0, 6, 12, 18; 0., 6., 12., 18. hours harvest, A1-E; *Artemia* metanauplii cysts, A1-0; before enrichment is taken, A1/1; enriched *Artemia* metanauplii-1, A1/2; enriched *Artemia* metanauplii-2 and, A1/1-T0, 6, 12; 0., 6., 12. hours after enrichment and A1/2-T0, 6, 12, 18; 0., 6., 12., 18. hours after enrichment harvest.

The after enrichment (T0) values of the non-enriched *Artemia* (A1-0) and enriched *Artemia* (A1/2) in terms of crude protein values were important (p<0.05) (Tables 4, 6). The *Artemia* enriched in terms of crude lipid values at 12th (A1/1) and at 18th hours (A1/2) after enrichment were important (p<0.05).

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Discussion

The activities of protease enzymes in live feeds were generally determined in pH 8.5 substrate (Naz *et al*., 2011; Naz and Yufera, 2012; Haközü, 2014; Diken *et al.*, 2016b, 2019). In this study, alkaline, neutral, and acid total protease enzyme activities of live feeds were revealed. Warner and Shridhar (1985) reported that most *Artemia* cysts have cysteine proteases that are inactive at alkaline pH. Similarly, before and after decapsulation, the enzyme activities of *A.* nauplii and non-decapsulated *A.* metanaplii cysts in the substrate with pH 3 and 4 were higher. Although decapsulation decreased the protease enzymes of *Artemia* cysts, a significant increase was observed after hatching (A0-T0). This result was similar to the study reports reporting the effect of decapsulation on the digestibility of *Artemia* (Garcia-Ortega *et al*., 1998; Hekimoglu *et al*., 2014).

Yufera *et al*. (2000) determined that the acid protease activity of rotifers was high and the contribution of rotifer proteases to the larval gut was not significant. In the results of the study, it is seen that enriched rotifers have high acid protease values. However, unenriched rotifers (R-0) have higher alkaline protease activities. This means that rotifers to be used without enrichment will contribute more exogenous enzymes to the alkaline larval digestive system. The present study results differ from the Munilla-Moran *et al*. (1990) report, which reported that the enzyme activity of rotifers was lower than that of *Artemia* and copepods. Compared to *Artemia* groups, rotifers had higher acid protease enzyme activities in pH 3 and 4 substrates. Both the increased protease enzyme activity in alkaline protease activities of rotifers and the increase in alkaline enzyme values in *Artemia* groups were similar to the highest enzyme increase at alkaline pH expressed by Warner (1987). The differences in the enzyme activities of *Artemia* metanauplii enriched according to different enrichments in the study were similar to the result reported by Munilla-Moran *et al*. (1990) that the enzymatic activity varies depending on the nutritional status and developmental stage. According to acid protease (pH 3 and 4) activities in rotifers, and alkaline protease activities in *Artemia* groups, the increase in enzyme activities as a result of enrichment supports the result reported by Naz (2008) that the exogenous enzyme contribution of live feeds will increase after enrichment. Warner *et al*. (1995), reported that protease activities increased after hatching in *A. franciscana* depending on the incubation medium. However, the present study showed fluctuations in protease enzyme activities due to storage at 4 degrees centigrade. Enzyme values increased in one group of enriched *Artemia*s and decreased in the other group. The increase in protease activity of *A.* nauplii at the end of 25 hours (Garcia-Ortega *et al*., 1998), was similar to the trend of increase in acid (pH 3), neutral, and alkaline enzyme activities at the end of the 18th hour of the study. In contrast in this study, the excess amount

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of acidic cysteine protease of *Artemia* (Warner and Shridhar, 1985; Warner *et al*., 1995) was much lower than neutral and acid protease enzyme activities due to the low activity of acid proteases. According to Naz *et al*. (2021), different fluctuations were detected with the results of the study in the changes in alkaline, neutral and acid protease enzyme activities of *Artemia* enriched with different products. The results of this report and the present study reveal the effect of the enrichment used in live feed enrichment on the enzyme activities of live feeds.

The results of the studies of other researchers including the protease enzyme values in the pH 8.5 substrate were discussed with the total protease activity (T0) pH 8.5 substrate values of the current study. Naz (2008), found that the amount of trypsin increased during fasting, especially in *A.* metanauplii, and found that trypsin decreased during fasting in the enriched rotifer, although it was found higher than in enriched rotifers. In the present study, the total amount of protease was similarly reduced and significantly lower than enriched *Artemia*. However, contrary to Naz (2008), the fluctuations of the total protease content of the *Artemia* stored at 4 degrees centigrade in the first enrichment and the decreases in the second enrichment reveal the difference in the live feed culture of the present study. Naz *et al.* (2011), the protease activity value of non-enriched (114.26±20.19 U mg^{-1} protein), the enriched rotifers (139.42±18.38 U mg^{-1} protein), *A.* metanauplii (481.31±22.10 U mg^{-1} protein) and *A.* nauplii (253.48±6.54 U mg^{-1} protein) were found to be higher than the results of the present study. According to Naz and Yufera (2012), the protease activity value of *A.* metanaupli (414.5±0.41 U mg^{-1} protein) was found to be lower than the second enriched value of the present study and higher than the first enriched *Artemia* value. The rotifer (156.25±0.09 U mg^{-1} protein) protease activity value was higher than the protease value of the present study. Hakozu (2014), reported that protease activity values of the enriched rotifer, *A.* nauplii, and *A.* metanauplii (U mg^{-1} protein) were 17.98±2.82, 34.67±0.88, and 317.16±2.67, respectively. According to the present study, the rotifer and the enriched *Artemia* values of these results were low and the *A.* nauplii values were high. According to Diken *et al.* (2016b), the enriched *A.* metanauplii's 338.02±4.65 U mg^{-1} protein protease activity value was lower than the enriched *Artemia* of this study but higher than the enriched *A.* metanauplii value of the Hakozu (2014) report. According to the results of the protease activity values of live feeds in the present study, Diken *et al.* (2019), reported that the rotifer value (21.76±0.31 U mg^{-1} protein) was low, *A.* nauplii value (29.3 3±0.93 and 36.00±1.48 U mg^{-1} protein) was high, and *A.* metanauplii value (403.53±11.85 and 416.44±19.70 U mg^{-1} protein) was higher than the first enriched *Artemia* value and lower than the second enriched *Artemia* value. Differences in protease activities of *A.* nauplii and metanauplii and an increase in protease

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activity of live feeds due to *Artemia* enrichment were reported by Pan *et al.* (1991), depending on the age of the *Artemia* hatching 12 hours after hatching *Artemia* increased expression of digestive enzymes supported the results of the study. The expectation of the low contribution of the *Artemia* to the end of the metamorphosis of the larvae of the inactive alkaline pH cysteine protease enzyme (Warner and Shridhar, 1985; Solovyev *et al.*, 2016) was supported by the study results.

Exogenous enzyme additives will be insufficient in the feeding of early larvae that have an ontogenetically alkaline digestive system, in the use of rotifers by stocking them at 4 degrees centigrade due to their neutral and alkaline enzyme activities. According to the changes in protease enzyme activity, the use of rotifers used as the first feed without enrichment should be discussed. Considering this situation of the larvae whose digestive system has an acid character ontogenetically, the alkali enzyme contributions of *Artemia* are higher depending on the enrichment process. Due to the high alkaline protease activity of *A.* nauplii, it is important to feed *A.* nauplii and rotifer live feed, depending on the alkaline digestive system of the larvae. Care should be taken as storage at 4 degrees centigrade causes a decrease in the enzyme values of the rotifers. On the contrary, there is an increase in the enzyme values of *A.* nauplii due to storage at 4 degrees centigrade. Due to the high alkaline protease enzyme activity in enriched *Artemia*, it will be important to switch to feeding the larvae with a microdiet as soon as possible. It can be used by stocking *A.* nauplii and metanauplii at 4 degrees centigrade until the 18th hour. On the other hand, it is recommended to pay attention to the use of rotifers by stocking. While the alkaline, neutral and acid character of the digestive system is taken into account in the ontogenetic development of the larvae, this situation of live feeds should also be taken into account.

The biochemical composition of live feeds and their exogenous enzymes affect weaning success. It has been reported that the biochemical compositions of live feeds change depending on the enrichment (Westelmajer, 2008; Beyhan, 2011; Radhakrishnan *et al*. 2020; Pan *et al*., 2022). In the study, crude protein values of live feeds decreased, and crude lipid values increased depending on time. The present study results are similar to the report results, which reported that the enriched rotifer dry weight consisted of 28-63% protein and 9-28% lipid (Lubzens *et al*., 1989; Lubzens *et al*., 2001; Lubzens and Zmora, 2003; Conceição *et al*., 2010). Time-dependent crude protein reduction, crude lipid increase fluctuations, and crude ash fluctuations of rotifers were similar to Naz's (2008) report. However, in the report of Naz (2008), crude lipid values were high and crude ash values were low. Naz (2008), Yenmis and Naz (2018), and Aktas *et al*. (2019) *A.* nauplii and metanauplii crude ash values contain similar rates to the results of the study. Beyhan (2011) reported that

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Artemia had a high crude lipid value after enrichment harvesting. The fluctuations of time-dependent changes in crude lipid values of enriched *Artemia* were similar to Akkus (2015). Time-dependent crude lipid and crude ash values of unenriched and enriched *Artemia* (A0 and A1) decreased in Naz's (2008) report, unlike the present study. The crude protein value of Naz (2008) was higher than the results of the study. The time-dependent crude protein value of enriched *Artemia* (A1) decreased depending on the value before enrichment, whereas it increased in Naz's (2008). Aktas *et al*. (2019) reported that the crude protein value of unenriched *A.* nauplii was almost twice that of enriched *A.* metanauplii. However, this situation was quite different from the results of the study. Unenriched *Artemia* had a higher crude protein value than the current study, while the crude lipid value was much higher than the current study and the crude ash value was considerably lower. According to this study, while crude ash and crude lipid values of enriched *A.* metanauplii were similar, crude protein value was high.

Feeds have been reported to contain large amounts of free amino acids and oligopeptides (Lavens and Sorgeloos, 1996). In this sense, the fact that *Brachionus* have small peptides with molecular weights <1,500 Da (Ronnestad *et al.*, 2003) can be seen as an important advantage. Based on these considerations, it is an important nutritional value of live feeds to have high molecular weight distributions of 2,532 Da≥, which do not show much variation due to enrichment of the study results and storage at 4 degrees centigrade. However, it is a parameter that should be observed and paid attention to due to the low value of 2,532 Da≥ (Diken, 2018) in the other study. Similarly, the height of 67,000 Da≤ in Diken (2018) according to the results of the study is another important molecular weight class value to be monitored. The present study and Aktas *et al.* (2019), showed that enriched *A. metanauplii's* value of 2,532 Da≥ was similar to the second enrichment (A1/2-T0) of the present study and higher than the first enrichment (A1/1-T0). Aktas *et al.* (2019), had a 67,000 Da≤ value of enriched *A.* metanauplii, which is similar to the first enrichment (A1/1-T0) of the present study and higher than the second enrichment (A1/2-T0). According to the present study, the non-enriched *A.* nauplii value of 2,532 Da≥ was quite high, and the value of 67,000 Da≤ was low. A high proportion of protein in the peptide groups/ molecular weight distribution is necessary to have a balance (Carvalho *et al.*, 2003). This equilibrium ratio based on live feed molecular weights was given in microdiets of *in vitro* meagre larvae (Diken, 2018). In the current study results, the equilibrium ratio of 2,532 Da≥ / 67,000 Da≤ is important *in vitro* reference in the formulation of microdiets for these evaluations. At the same time, depending on the necessity of evaluating free fatty acids in larval feeding according to (Fyhn *et al.*, 1993),

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molecular weight distributions of 2,532 Da≥ are also an important approach.

The alkaline, neutral, and acid enzyme activities, proximate composition, and molecular weight may vary with enrichment depending on the species differences and/or geographical differences of live feeds. Live feeds' storage at 4 degrees centigrade and the amount of lipid that changes with time is the result of oxidation. However, lecithin can be considered an important factor with natural and synthetic antioxidation which may prevent oxidation used in enrichment products. However, the most important factors affecting the autoxidation of oils are the type and amount of fatty acids and storage conditions such as temperature and humidity. In sensitivity of lipoxygenases, particularly the temperature and pH range are important factors. In particular, the significant decrease in pH 7 and 8.5 with the change in lipid amount after enrichment in the rotifers was related to this result. In contrast, the similarity in enzyme activities at pH 3 and 4 is indicative of this result. And for the rotifers to work at high enzymatic activity, the pH 3 and 4 acid protease groups can also be expressed as the required pH range. In other words, when the rotifers are enriched with such enriching products, they do not exhibit high enzymatic activity at the pH 8.5 required for the larva. This is indicative of the fact that the non-enriched rotifer (R-0) exhibits higher activity than enrichment at pH 8.5. This is the case in *Artemia*. This shows the species differences between rotifer and *Artemia* of these enriching products that increase the lipid content of live feeds. As a result of enrichment with these enriching products, *Artemia* exhibits very high enzymatic activity at pH 8.5. Despite the increase in the crude lipid values of live feeds as a result of enrichment, the activity of the enzyme group in which the live feeds should study is insufficient depending on the age of the larvae.

In summary, the present study sheds light on the necessity of evaluating the substrate according to the pH group range in determining the enzyme activities of live feeds, and the importance of molecular weight determination of live feeds. These results, especially on the activities of enzymes ranging from alkalinity to acidity in the ontogenetic development of marine finfish larvae, will provide a more accurate assessment of the exogenous enzyme effects of live feed. Preferred molecular weight analyses as an alternative to SDS-PAGE offer a practical approach to determine the peptide structures and amino acid distributions of live feeds.

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References

Akkus, E., 2015. The effects of commercial enrichments on the fatty acid composition of Artemia (*Artemia* sp.) Nauplii used for larval fish . MSc Thesis. Çanakkale Onsekiz Mart University, Türkiye, 41P.

Aktas, M., Genç, M.A., Bozkurt, A., Genç, E. and Naz, M., 2019. The changes in the molecular weight profiles and biochemical compositions of potential feed ingredients for sustainable aquaculture. *Journal of Aquaculture Engineering and Fisheries Research*, 5(**1**), 1-11.

Alarcon, F.J, Díaz, M. and Moyano, F.J., 1997. Studies of digestive enzymes in characterization and practical applications. *Chiem-Cahiers Options Meditérranéennes*, 22, 113-121.

Anson, M.L., 1938. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. *The Journal of General Physiology*, 22, 79-89.

AOAC, 2000a. Official methods of analysis of AOAC International First Action 1940. AOAC Official Method 940.25 Nitrogen (Total) in Seafood, 17th ed. Association of Official Analytical Chemists. Gaithersburg, MD, USA, 2200P.

AOAC, 2000b. Official Methods of Analysis of AOAC International. In: AOAC Official Method 938.08 Ash of Seafood, 17th ed. Association of Official Analytical Chemists. Gaithersburg, MD, USA, 2200P.

Bear, A., Langdon, C., Mills, S., Schulz, C. and Hamre, K., 2008. Particle size preference, gut filling and evacuation rates of the rotifer *Brachionus* "Cayman" using polystyrene latex beads. *Aquacultur*e, 282, 75-82. https://doi.org/10.1016/j.aquaculture.2008.06.020

Beyhan, T., 2011. Bazı zenginleştirici ürünlerin Artemia (*Artemia salina* Linneaus, 1758)'nın besinsel içeriğine ve yağ asidi kompozisyonu üzerine etkileri. MSc Thesis, Süleyman Demirel Üniversitesi, Turkey, 46P.

Bligh, E.G. and Dyer, J., 1959. A rapid method of total extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 8. https://doi.org/10.1139/o59-099

Boza, J.J., Jimenez, J., Martínez, O., Suarez, M.D. and Gil, A., 1994. Nutritional value and antigenicity of two milk protein hydrolysates in rats and guinea pigs. *The Journal of Nutritional*, 124(**10**), 1978-1986. https://doi.org/10.1093/jn/124.10.1978

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72, 248-254.

Carvalho, A.P., Oliva-Teles, A. and Bergot, P., 2003. A preliminary study on the molecular weight profile of soluble protein nitrogen in live food organisms for fish larvae. *Aquaculture*, 225(**1-4**),445-449. https://doi.org/10.1016/S0044-8486(03)00308-9

[DOR: 20.1001.1.15622916.2022.21.5.10.4]

Carvalho, A.P., Sa, R., Oliva-Teles, A. and Bergot, P., 2004. Solubility and peptide profile affect the utilization of dietary protein by common carp (*Cyprinus carpio*) during early larval stages. *Aquaculture*, 234(**1-4**), 319-333. https://doi.org/10.1016/j.aquaculture.2004.01.007

Conceiçao, L.E.C., Yúfera, M., Makridis, P., Morais, S. and Dinis, M.T., 2010. Live feeds for early stages of fish rearing. *Aquaculture Research*, 41, 613-640. doi:10.1111/j.1365-2109.2009.02242.x

Diken, G., Demir, O. and Naz, M., 2016a. The potential effects of commercial feeding protocol on protease activities and cortisol stress responses of meagre (*Argyrosomus regius*). *Journal of International Scientific Publications Agriculture & Food*, 4, 460-472.

Diken, G., Demir, O. and Naz, M., 2016b. Sörvaj dönemi çipura (*Sparus aurata* Linnaeus, 1758) ve levrek (*Dicentrarchus labrax* Linnaeus, 1758) balığı larvalarının proteaz aktiviteleri üzerine besinlerin etkileri ve fizyolojik durumlarının belirlenmesi. p 4. In: *IV. Balık Besleme ve Yem Teknolojileri Sempozyumu.* 01-02 September. Çukurova University, Adana.

Diken G., 2018. Determination Using ***in vitro*** assay of inhibition values ff different feed ingredients on the protease activities of meagre, *Argyrosomosus regius* (Asso, 1801) larvae and production of species–specific microdiet. PhD. Thesis. Süleyman Demirel Universty, Türkiye, 350P.

Diken, G., Demir, O. and Naz, M., 2019. The inhibitory effects and positive contributions of live foods on protease activities of meagre, *Argyrosomus regius* (Asso 1801), larvae in vitro assay. *Fishes*, 4(**5**), 1-12.

Erdogan, O. and Ertan, O.O., 2015. Abundance and diversity of zooplankton in the Kopruçay estuary, Turkey. *Journal of Aquaculture Engineering and Fisheries Research*, 1(**1**), 19-32. https://doi.org/10.3153/JAEFR15002

Fuenzalida, J.Y., 2019. Inside the commercial production of *Artemia* biomass in Ecuador. Global Aquaculture Advocate. https://www.aquaculturealliance.org/advocate/inside-commercial-production-artemia-biomass-ecuador/ (Accessed 1 October 2019)

Fyhn, H.N., Finn, R.N., Helland, S., Ronnestad, I. and Lomsland, E., 1993. Nutritional value of phyto-and zooplankton as live food for marine fish larvae. p. 121-126. In: *Fish farming technology*. Reinertsen, H., Dahle, L.A., Jorgensen, L. and Tvinnerheim, K. (Eds). Balkema, Rotterdam.

Garcia-Ortega, A., Verreth, J.A.J., Coutteau, P., Segner, H., Huisman, E.A. and Sorgeloos, P., 1998. Biochemical and enzymatic characterization of decapsulated cysts and nauplii of the brine shrimp *Artemia* at different developmental

[DOR: 20.1001.1.15622916.2022.21.5.10.4]

stages. *Aquaculture*, 161, 501-514. https://doi.org/10.1016/S0044-8486(97)00297-4

Gonçalves J, Sardinha M, Dias J, Tokle N. and Conceição, L., 2014. A survey to characterize the state of the at on microdiets used at marine fish hatcheries. Symposiun Aquaculture Europe14 AE2014. Spain. Donostia-San Sebastian. October. 2014.

Grabner, M., 1985. An in vitro method for measuring protein digestibility of fish feed components. *Aquaculture*, 48, 97-110. https://doi.org/10.1016/0044-8486(85)90097-3

GSLEP, 2017. Great Salt Lake Ecosystem Program Manager Utah Division of Wildlife Resources. https://wildlife.utah.gov/gsl/harvest/historic_harvest_data.php (Accessed 10 April 2017).

Hagiwara, A., Suga, K., Akawaza, A., Kotani, T. and Sakakura, Y., 2007. Development of rotifer starins with useful tarits for rearing fish larvae. *Aquaculture*, 268(**1-4**), 44-52. https://doi.org/10.1016/j.aquaculture.2007.04.029

Hakozu, G., 2014. Çipura (*Sparus aurata*, Linneaus 1758) Larvalarının kortizol seviyeleri üzerine ticari besleme prosedürünün etkileri. MSc Thesis. Mustafa Kemal Üniversitesi, Türkiye, 68P.

Hekimoglu, M.A., Suzer, C., Saka, Ş. and Fırat, K., 2014. Enzymatic characteristics and growth parameters of ornamental Koi carp (*Cyprinus carpio* var. Koi) larvae fed by *Artemia nauplii* and cysts introduction. *Journal of Fisheries and Aquatic Sciences*, 14:125-133. https://doi.org/10.4194/1303-2712-v14_1_14

Hoehne-Reitan, K., Kjørsvik, E. and Gjellesvik, D.R., 2001a. Development of Bile Salt-Dependent Lipase in Larval Turbot. *Journal of Fish Biology*, 58, 737-745. https://doi.org/10.1006/jfbi.2000.1483

Hoehne-Reitan, K., Kjørsvik, E. and Reitan, K.I., 2001b. Bile salt-dependent lipase in larval turbot as influenced by density and lipid content of fed prey. *Journal of Fish Biology*, 58, 746-754. https://doi.org/10.1006/jfbi.2000.1493.

Izquierdo, M.S. 2004. Nutritional requirements for finfish larvae. p. 8-16. In: *The second hatchery feeds and technology workshop*, 30 September-1 October, Sydney.

Kanazawa, A., 2003. Nutrition of marine fish larvae. *Journal of Applied Aquaculture*, 13(**1-2**), 103-143. https://doi.org/10.1300/J028v13n01_05

Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles-implications and application to formulated diets. *Aquaculture*, 200, 181-201. https://doi.org/10.1016/S0044-8486(01)00700-1

Kvåle, A., Yufera, M., Nygård, E., Aursland, K., Harboe, T. and Hamre, K., 2006. Leaching properties of three different micropaticulate diets and preference

[DOR: 20.1001.1.15622916.2022.21.5.10.4]

of the diets in cod (*Gadus morhua* L.) larvae. *Aquaculture*, 251, 402-415. https://doi.org/10.1016/j.aquaculture.2005.06.002.

Langdon, C., 2003. Microparticle types for delivering nutrients to marine fish larvae. *Aquaculture*, 227, 259-275. https://doi.org/10.1016/S0044-8486(03)00508-8

Langdon, C., Clack, B. and Onal, U., 2007. Complex microparticles for delivery of low-molecular weight, water-soluble nutrients and pharmaceuticals to marine fish larvae. *Aquaculture*, 268, 143-148. https://doi.org/10.1016/j.aquaculture.2007.04.035

Lavens, P. and Sorgeloos, P., 1996. *Manual on the production and use of live food for aquaculture FAO Fisheries Technical Paper No: 361*. FAO, Rome, 295 P.

Lazo, J.P., Darias, M.J. and Gisbert, E., 2011. Ontogeny of the digestive tract. p. 5-46. In: *Larval fish nutrition.* Holt, J.G. (Ed). John Wiley & Sons, Chichester.

Le, T.H., Van Hoa1, N., Sorgeloos, P. and Van Stappen, G., 2018. Artemia feeds: a review of brine shrimp production in the Mekong Delta, Vietnam. *Reviews in Aquaculture*, 11(**4**), 1169-1175. https://doi.org/10.1111/raq.12285

Leger, P., Bengtson, D.A., Simpson, K.L. and Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. *Oceanography Marine Biology Annual Review*, 24, 521-623.

Lemos, D., Navarrete del Torob, A., Cordova-Muruetab, J.H. and Garcia-Carreno, F., 2004. Testing feeds and feed ıngredients for juvenile pink shrimp *Farfantepenaeus paulensis*: *in vitro* determination of protein digestibility and proteinase inhibition. *Aquaculture*, 239, 307-321. https://doi.org/10.1016/j.aquaculture.2004.05.032

Lindegren, M., Checkley, Jr.D.M., Koslow, J.A., Goericke, R. and Ohman, M.D., 2018. Climate-mediated changes in marine ecosystem regulation during El Niño. *Global Change Biology*, 24, 796-809. https://doi.org/10.1111/gcb.13993

Lu, J. and Warner, A.H., 1991. Immunodetection of thiol proteinase levels in various populations of *Artemia* cysts and during development. *Biochemistry and Cell Biology*, 69, 96-101. https://doi.org/10.1139/o91-015

Lubzens, E., Tandler, A. and Minkoff, G., 1989. Rotifers as food in aquaculture. *Hydrobiologia*, 186/187, 387-400. https://doi.org/10.1007/BF00048937

Lubzens, E., Zmora, O. and Barr, Y., 2001. Biotechnology and aquaculture of rotifers. *Hydrobiologia*, 446/447, 337-353. https://doi.org/10.1007/978-94-010-0756-6_44

Lubzens, E. and Zmora, O., 2003. Production and nutritional value of rotifers. p. 17-64. In: *Live feeds in marine aquaculture.* Stottrup, J.G.

[DOR: 20.1001.1.15622916.2022.21.5.10.4]

and McEvoy, L. (Eds). Blackwell Science, Malden.

McKinnon, D., Rimmer, M., Kolkovski, S. and Littmann, M.Y., 2009. Hatchery feeds research and development plan 2000-2005. The Australian Institute of Marine Science**.** http://www.aims.gov.au/pages/research/hatchery-feeds/r & d-plan.doc (Accessed 28 May 2009)

Munilla-Moran, R., Stark, J.R. and Barbour, A., 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). *Aquaculture*, 88 (**3-4**), 337-350.

Naz, M. and Yúfera, M., 2012. The potential inhibitory effects of the commercial diets on protease activities of larvae and live foods. *Journal of Fisheries Sciences*, 6, 224-231. https://doi.org/ 10.3153/jfscom.2012026

Naz, M., 2008. The changes in the biochemical compositions and enzymatic activities of rotifer (*Brachionus plicatilis*, Müller) and *Artemia* during the enrichment and starvation periods. *Fish Physiology Biochemistry*, 34, 391-404. https://doi.org/10.1007/s10695-007-9199-5

Naz, M., Yılmaz, E., Töre, Y., Diken, G., Tanrıverdi, Z. and Tekin, S., 2011. Canlı yemlerin proteaz aktivitelerinin belirlenmesi. 16. Ulusal Su Urunleri Sempozyumu.Turkey: Akdeniz Üniversitesi.

Naz, M., Diken, G., Yazici, M., Mazlum, Y., Sayin, S., Soyler, O., 2021. The changes in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations. *Aquatic Sciences and Engineering*, 36(**3**), 152-158. https://doi.org/10.26650/ASE2020793132

Oie, G., Reitan, K.J., Evjemo, J.O., Støttrup, J. and Olsen, Y., 2011. Live feeds. p. 307-334. In: *Larval fish nutrition.* Holt, G.J. (Ed). John Wiley & Sons, Chichester.

Onal, U. and Langdon, C., 2009. Potential delivery of water-soluble protein hydrolysates to marine suspension feeders by three different microbound particle types. *Aquaculture*, 296, 174-178. https://doi.org/10.1016/j.aquaculture.2009.07.002

Palmtag, M.R., Faulk, C.K. and Holt, G.J., 2006. Highly unsaturated fatty acid composition of rotifers (*Brachionus plicatilis*) and *Artemia* fed various enrichments. *Journal of World Aquaculture Society*, 37(**1**),126-131.

Pan, B.S., Lan, C.C. and Hung, T.Y., 1991. Changes in composition and proteolytic enzyme activities of *Artemia* during early development. *Comparative Biochemistry and Physiology Part A*, 100, 725-730. https://doi.org/10.1016/0300-9629(91)90397-U

Pan, Y.J., Dahms, H.U., Hwang, J.S. and Souissi, S., 2022. Recent trends in live feeds for marine larviculture:

[DOR: 20.1001.1.15622916.2022.21.5.10.4]

A mini review. *Frontiers in Marine Science*, 9, 1-9. https://doi.org/10.3389/fmars.2022.864165

Person-Le Ruyet, J., 1989. Early weaning of marine fish larvae onto microdiets: constraints and perspectives. n°9 Chap 59. p. 625-642. In: *Advances in Tropical Aquaculture. Aqua Cop Ifremer Actes De Colloque. 20 February-4 March,* Tahiti.

Radhakrishnan, D.K., AkbarAli, I., Schmidt, B.V., John, E.M., Sivanpillai, S. and Vasunambesan, S.T., 2020. Improvement of nutritional quality of live feed for aquaculture: An overview. *Aquaculture Research*, 51, 1-17. https://doi.org/10.1111/are.14357

Ronnestad, I., Tonheim, S.K., Fyhn, H.J., Rojas-García, C.R., Kamisaka, Y., Koven, W., Finn, R.N., Terjesen, B.F., Barr, Y. and Conceição, L.E.C., 2003. The supply of amino acids during early feeding stages of marine fish larvae: A review of recent findings. *Aquaculture*, 227, 147-164. https://doi.org/10.1016/S0044-8486(03)00500-3

Solovyev, M.M., Campoverde, C., Ozturk, S., Moreira, C., Diaz, M., Moyano, F.J., Estévez, A. and Gisbert, E., 2016. Morphological and Functional Description of the Development of the Digestive System in Meagre (*Argyrosomus regius*): An Integrative Approach. *Aquaculture*, 464, 381-391. https://doi.org/10.1016/j.aquaculture.2016.07.008

SPSS Inc. (2015). SPSS for IBM Version 23.0. IL, Chicago

Van Stappen, G., 1996. Introduction, biology and ecology of *Artemia*. p. 79-136. In: Lavens P, Sorgeloos P (eds) Manual on the production and use of live food for aquaculture. FAO, Rome.

Vasileiadou, K., Papakostas., S., Triantafyllidis., A., Kappas., I. and Abatzopoulos., T.J., 2009. A multiplex PCR method for rapid identification of *Brachionus rotifers*. *Marine Biotechnology*, 11, 53-61. https://doi.org/10.1007/s10126-008-9119-4

Walter, H-E., 1984. Proteinases: methods with haemoglobin, casein and azocoll as substrates. p. 270-277. In:*Methods of enzymatic analysis volume 5*. Bergmeyer, H.U. (Ed). Verlag Chemie, Weinheim.

Warner, A.H. and Shridhar, V., 1985. Purification and characterization of a cytosol protease from dormant cysts of the brine shrimp *Artermia*. *The Journal of Biological Chemistry*, 260, 7008-7014. https://doi.org/10.1016/S0021-9258(18)88880-9

Warner, A.H., 1987. The role of proteases and their control in *Artemia* development. p. 203-219. In: *Artemia* research and its applications. Physiology, biochemistry, molecular biology, vol. 2. Declair, W., Moens, L., Slegers, H., Sorgeloos, P. and Jaspers, E. (Eds). Universa Press, Wetteren.

Warner, A.H., Pertz, M.J., Osahan, J.K.- and Zielinski, B.S., 1995. Potential role in development of the majör cysteine protease in larvae of the brine shrimp *Artemia franciscana*. *Cell Tissue Resources*, 282, 21-31.

Westelmajer, S.K.M., 2008. Ontogeny of the corticosteroid stres response and effect of differentially enriched live feed on growth, lipid composition and acute stres tolerance of larval atlantic cod, *Gadus morhua*. MSc Thesis, Memorial University of Newfoundland, Canada, 128 P.

Yenmis, A.M. and Naz, M., 2018. The determination of the leaching ratios of microdiets containing algae used as direct and indirect in aquaculture. *Journal of Applied Animal Research*, 46(**1**), 1496-1504. https://doi.org/10.1080/09712119.2018.1546584

Yufera, M., Fernández-Diaz, C., Pascual, E., Sarasquete, M.C., Moyano, F.J., Diaz, M., Alarcom, F.J., Garcia-Gallego, M. and Para, G., 2000. Towards an inert diet for first feeding gilthead seabream *Sparus aurata* L. larvae. *Aquaculture Nutrition*, 6, 143-152.

Zaleha Kassim, Z., John, A., Chin, L., Zakaria, N.F. and Asgnari, N.H., 2014. Sustainable technique for selected live feed culture. p. 105-133. In: *Sustainable Aquaculture Techniques*. Martha Patricia Hernandez-Vergara, M.P., Perez-Rostro, C.I. (Eds). InTech Publisher-Published by AvE4EvA, Open Access. https://doi.org/10.5772/57212

Zambonino Infante, J.L. and Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. *Comparative Biochemistry Physiolgy Part-C*, 130, 477-487. https://doi.org/10.1016/S1532-0456(01)00274-5

Zambonino Infante, J.L., Gisbert, E., Sarasquete, C., Navarro, I., Gutiérrez, J. and Cahu, C.L., 2008. Ontogeny and physiology of the digestive system of marine fish larvae. p. 281-348. In: *Feeding and digestive functions of fishes*. Cyrino, J.E.P., Bureau, D. and Kapoor, B.G. (Eds). CRC Press, Boca Raton.

[DOR: 20.1001.1.15622916.2022.21.5.10.4]