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Original Article

The changes in Alkaline, Neutral and Acid Protease Activities of *Artemia* Enriched with Commercial Emulsion and Different Additive Combinations

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ABSTRACT

The biochemical compositions and the changes observed in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations were determined. Artemia nauplii (ArtN), GroBiotic-A (GA), Red Algamac (RA), Red Algamac:GroBiotic-A (50:50) (RA:GA (50:50)), Spirulina (SP), Spirulina:Red Algamac (50:50) (SP:RA (50:50)), Spirulina:GroBiotic-A (50:50) (SP:GA (50:50)) and Spirulina:Red Algamac:GroBiotic-A (33:33:33) (SP:RA:GA (33:33:33)) were tested in the study. The lowest and highest protein contents after the enrichment of Artemia were 40.74±1.02% (RA) and 55.03±1.26% (SP:RA:GA (33:33:33)), respectively. The lowest lipid contents of tested groups were found in 5.63±0.47% (GA) and 5.63±0.84% (RA:GA (50:50)). The highest lipid value after the enrichment were observed in 16.98±1.15 % (RA). The lowest and highest ash values observed after the enrichment were 4.51±0.27% (SP:GA (50:50)) and 6.07±0.35% (RA) ($p<0.05$). The lowest and highest protease activities of the pH=3, pH=4, pH=5, pH=6, pH=7 and pH=8.5 values were 18.18±0.37 U/mg protein (GA), 31.04±0.38 U/mg protein (RA), 9.1±0.32 U/mg protein (SP), 9.66±0.19 U/mg protein (SP), 16.94±0.61 U/mg protein (SP), 63.09±0.75 U/mg protein (SP) and 33.77±0.59 U/mg protein (RA:GA (50:50)), 57,54±0.34 U/mg protein (RA:GA (50:50)), 23.75±0.28 U/mg protein (GA), 40.82±0.49 U/mg protein (GA), 69.94±0.65 U/mg protein (GA), and 286.14±8.2 U/mg protein (GA) ($p<0.05$). In conclusion, GA and SP:RA:GA (33:33:33) enrichment combinations are recommended as an alternative to enrichment products. On the other hand, SP and RA should not be used alone due to the disadvantages such as the biochemical composition and proteolytic enzyme activities of *Artemia* observed in the present study.

Keywords: *Artemia*, enrichment, GroBiotic-A, biochemical compositions, enzymes

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INTRODUCTION

Fish larvae are strongly dependent on the exogenous enzymes of live foods used in the feeding protocols due to a poor development of the digestive system. The contribution of exogenous enzymes to the digestive system of larvae is an important issue in aquaculture. When the larvae begin to feed, their digestive enzyme activities are alkaline. Acid proteases towards the end of the critical larval stage are important in digestion. This information about the digestive physiology of larvae is important for the utilization of microdiets in the digestive system. According to the mentioned data, protease activities are indicators for determining the digestive ability of larvae (Diken, Demir & Naz, 2019).

Feeding protocols are of critical importance for aquaculture. The larval stages need live food such as *Artemia* due to the digestive enzymes contributed to the digestion of nutrients. Although *Artemia* is not a natural food such as copepods for marine fish larvae, it is an important food source in the larval stages of aquaculture. The alkaline enzyme activity of Artemia metanauplii (ArtMn) is higher than that of Arte-

mia naupli (ArtN). This shows that live food exogenous enzyme contributions will change according to the developmental periods of live foods and larvae. Therefore, the exogenous enzyme contributions by the live foods offered to the fish needs to be considered in combination with their dietary nutrition and/or enrichments used in their culture (Naz & Yufera, 2012).

Live food enrichment should be done to meet the nutritional requirements of fish larvae (McEvoy et al., 1998). It is known that enriching products contain high amounts of essential ingredients for larval feeding. However, these products exhibit large differences in primary nutrients, such as proteins, lipids and ash. In addition, the different forms of enrichment products could effect the biochemical composition of live food. The enrichment process of *Artemia* is one of the most important applications in hatcheries. The enrichment techniques for *Artemia* are well developed in the aquaculture sector. Efficient enrichment techniques contribute to the production of high-quality live food.

Nakagawa & Montgomery (2007) reported that *Spirulina* is one of the most frequently used algae in diets due to valuable ingredients such as high protein and fatty acid content. Several studies have been carried out using dried *Spirulina* as feed additives (Jaime-Ceballos, Villarreal, Garcia, Perez-Jar & Alfonso, 2005; Hanel, Broekman, de Graaf & Schnack, 2007; Dernekbasi, Unal, Karavucel & Aral, 2010; Ghaeni, Matinfar, Soltani, Rabbani & Vosoughi, 2011). Cho, Hur & Jo (2001) showed the effect of nutritional enrichment of live foods by w-yeast, *Spirulina* powder and Super Selco TM on survival and growth rates of rockfish larvae. Rocha, Garcia & Henriques (2003) indicated that microalgae are recognized as excellent sources of proteins, carbohydrates, lipids and vitamins. Senthil, MaruthuPandi, Kumar, Devi & Balasubramanian (2012) used algae such as Nannochloropsis sp., Chlorella sp. and Spirulina sp. and commercial A1 DHA Selco media for the enrichment of Artemia salina. Arumugam, Inbakandan, Ramasamy & Murugan (2013) tested liposome encapsulated Spirulina to enrich Artemia. Eryalcin, (2018) determined the effects of enrichments on growth, biochemical and fatty acid composition of L-type rotifer and Artemia franciscana nauplii.

Prebiotics are defined as non-digestible carbohydrates that cannot be digested by the host, but can be metabolized by normal gastrointestinal (GIT) microflora (Manning & Gibson, 2004). Although prebiotics have beneficial effects on the survival, growth and immune system of some species in aquaculture studies, there are limited studies on the effects of prebiotic encapsulation in live feed. (Yazıcı et al., 2020; Daniels et al., 2010; Hoseinifar, Zare & Miandare, 2015; Azimirad, Meshkini, Ahmadifard & Hoseinifar, 2016; Azimirad & Meshkini, 2017; Widanarni, Yuhana & Ekasari, 2018).

García-Ortega, Verreth, Coutteau, Segner, Huisman & Sorgeloos (1998) determined the biochemical and enzymatic characterization of decapsulated cysts and ArtN at different developmental stages. Naz (2008) revealed the changes observed in the biochemical compositions and the digestive enzymes such as trypsine, aminopeptidase N and alkaline phosphates at the different developmental stages of Artemia. Yenmis & Naz (2019) showed the changes in protease activities (alkaline, neutral and acid) of Artemia during starvation, enrichment and 4°C storage.

Until now, protease activities of Artemia were determined in pH 8.0-8.5 substrate (Naz, 2008; Naz et al., 2011; Naz & Yufera, 2012; Haközü, 2014; Diken, Demir & Naz, 2016a; Diken, Demir & Naz, 2016b; Diken, Demir & Naz, 2019) except for Garcia-Ortega et al. (1998) and Yenmis & Naz (2019). There is no available combined study on protease activities (alkaline, neutral and acid) and the biochemical compositions of Artemia enriched with the commercial emulsion (Red Algamac (RA)) and different additive combinations such as GroBiotic-A (GA), and Spirulina (SP). Considering this information, the aims of this study were (1) to determine the biochemical compositions such as proteins, lipids and ash of Artemia enriched with commercial emulsion and different additive combinations and also, (2) to reveal the changes in alkaline, neutral and acid protease activities of Artemia enriched with commercial emulsion and different additive combinations. The results will provide important knowledge to the aquaculture sector on the biochemical compositions and enzyme contributions of Artemia enriched with commercial emulsion and different additive enrichment products.

MATERIALS AND METHODS

Artemia hatching and enrichment process

Artemia cysts (1 g L^{-1}) (Artemia SepArt EG >250000 np/g, INVE Aquaculture Inc.) were incubated in a 150 L tank (approximately 35-36 ppt) at 30°C under continuous aeration and illumination. After 24 h, the Artemia nauplii (ArtN) were collected and washed with tap water. Enrichment was performed in a 5 L glass jar. ArtN were added to give a density of 400 nauplii mL^{-1}. The nauplii were enriched with GroBiotic-A (GA), Red Algamac (RA), Red Algamac:GroBiotic-A (50:50) (RA:GA (50:50)), Spirulina (SP), Spirulina:Red Algamac (50:50) (SP:RA (50:50)), Spirulina:GroBiotic-A (50:50) (SP:GA (50:50)) and Spirulina:Red Algamac:GroBiotic-A (33:33:33) (SP:RA:GA (33:33:33)). The proximate composition of Grobiotic A, Red Algamac and Spirulina is shown in Table 1.

The enrichment diet was added after hatching (time 0) and again 12 h. The experimental groups were performed in triplicate. Samples for the proximate composition and protease (alkali, neutral and acid) analyses were taken and washed with distilled water, transferred to cryotubes and stored immediately at -80 °C until the analysis stage.

Table 1. Proximate compositions of ingredients used for the enrichment periods of *Artemia*

	GroBiotic-A	Red Algamac	Spirulina
Ash	6%	8%	8.65%
Crude Lipid	0.1-2% min	50%	8.86%
Crude Protein	30-32%	18%	51.82%
Crude Fiber	2-3% max	-	-
Moisture	5%	5%	-
Carbohydrate	53%	19%	-

ANALYSES

Biochemical compositions

Biochemical compositions such as dry matter, ash and protein of samples taken from the experimental groups were tested according to the AOAC (2000) procedures and also lipid analyses were performed according to the chloroform-methanol extraction method described by Bligh & Dyer (1959).

Live food extracts

Artemia metanauplii (ArtMn) sampled after the enrichment period from the experimental groups were analyzed. The samples were rinsed in distilled water after thawing and then, the extracts of live foods were homogenized and centrifuged (16,000 g, 30 minute 4°C).

Determination of alkaline, neutral and acid protease activities of live foods

Protease activities were assayed at pH=3, 4, 5, 6, 7 and pH=8.5. Hemoglobin (1%) was used as the substrate in acid pH (3–6) and casein (1%) in neutral and alkaline pH (7–8.5). Protease activities were evaluated according to Walter (1984) and Anson (1938) as follows: The mixtures including extracts of live food-substrate were incubated at 37°C and then the reaction was stopped by addition of 500 µL trichloroacetic acid (TCA) (120 g/L). One unit of enzyme activity was defined as 1 µg of tyrosine release per minute. The soluble protein concentrations of live foods were determined according to Bradford (1976).

Statistical methods

All measurements were carried out in triplicate. The experimental data were subjected to one-way (ANOVA) and mean±standard error (SE) differences were calculated by Duncan test at $p<0.05$ content level by using SPSS 15.0 statistical package (SPSS, 2006).

RESULTS AND DISCUSSION

In the present study, the biochemical compositions and the changes observed in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations were determined. The biochemical compositions of *Artemia* tested in the current study are given in Table 2.

The changes observed in the ash, lipid and protein values were statistically significant ($p<0.05$). According to Table 2, ash, lipid and protein values tended to decrease at the end of enrichment. A decrease in protein value was observed at the end of the enrichment process, except for SP: RA: GA (33:33:33). The lowest and highest protein contents after the enrichment of ArtN were 40.74±1.02% (RA) and 55.03±1.26% (SP:RA:GA (33:33:33)), respectively. Lipid contents after the enrichment period of ArtN decreased ($p<0.05$). The lowest lipid contents of tested groups were found in GA (5.63±0.47%) and RA:GA (50:50) (5.63±0.84%). The highest lipid value after the enrichment were observed in RA (16.98±1.15%). The lowest and highest ash values observed after the enrichment were SP:GA (50:50) (4.51±0.27%) and RA (6.07±0.35%).

Changes in the biochemical compositions and enzyme contributions of live foods are the main factors that affect weaning success. Westelmajer (2008) and Beyhan (2011) reported that the biochemical compositions of ArtN changed depending on the enrichment process. The current study data is important because hatchery practices often involve an enrichment process of ArtN before it is given to the larvae.

Naz (2008) showed that the ash levels of ArtN and ArtMn ranged from 11.52 ±0.33% to 12.30±0.15%. Yenmis & Naz (2018) reported that ash levels of ArtN and ArtMn ranged from 6.95±0.26% to 9.66±0.02%. Aktaş, Genç, Bozkurt, Genç & Naz (2019) determined that ash levels of ArtN and ArtMn ranged from 5.84±0.05% to 9.32±0.48%. In the current study, ash contents of ArtN and ArtMn values were lower than Naz (2008) and similar to Yenmis & Naz (2019) and Aktaş et al. (2019).

Naz (2008) determined that the lipid contents of ArtN and ArtMn were 20.97±0.15% and 24.80±0.48%, respectively. Yenmis & Naz (2019) reported that lipid levels of ArtN and ArtMn ranged from 19.99±0.13% to 25.28±0.07%. Aktaş et al. (2019) determined that lipid levels of ArtN and ArtMn were 30.19±0.65 and 16.59±0.53%, respectively. In the present study the lipid contents for ArtN was higher than Naz (2008) and Yenmis & Naz (2019) and lower than Aktaş et al. (2019) while lipid content of ArtMn was lower than Naz (2008), Yenmis & Naz (2019) and lower than Aktaş et al. (2019) except for the RA group.

Naz (2008) revealed that the protein levels of ArtN and ArtMn ranged from 62.66±0.47% and 49.10±0.32%. Yenmis & Naz (2019) reported that protein levels of ArtN and ArtMn ranged from 60.61±0.04% to 49.28±0.07%. Aktaş et al. (2019) determined that protein levels of ArtN and ArtMn ranged from 61.55±0.28% and 64.4±0.78%, respectively. In the present study the protein con-

Table 2. Biochemical compositions of *Artemia* enriched with different enrichment emulsions (%).

Groups	Ash	Lipid	Protein
Artemia nauplii	7.54±0.29^{e}	27.93±1.04^{e}	58.42±2.40^{c}
GroBiotic-A	5.71±0.20^{cd}	5.63±0.47^{a}	46.70±0.55^{b}
Red Algamac	6.07±0.35^{d}	16.98±1.15^{d}	40.74±1.02^{a}
Red Algamac:GroBiotic-A(50:50)	5.30±0.12^{bc}	5.63±0.84^{a}	45.93±0.54^{b}
Spirulina	4.98±0.12^{ab}	9.99±0.41^{c}	49.78±0.62^{b}
Spirulina:Red Algamac (50:50)	6.23±0.22^{d}	7.73±0.53^{abc}	49.75±1.67^{b}
Spirulina:GroBiotic-A (50:50)	4.51±0.27^{a}	7.23±0.89^{ab}	48.97±2.49^{b}
Spirulina:Red Algamac:GroBiotic-A (33:33:33)	5.88±0.13^{cd}	9.07±0.89^{bc}	55.03±1.26^{c}

tents for ArtN were similar to Naz (2008), Yenmis & Naz (2019) and Aktaş et al. (2019) while protein contents of ArtMn were similar to Naz (2008) and Yenmis & Naz (2019) except for RA and SP-RA-GA groups and lower than Aktaş et al. (2019).

Navarro et al (1999) revealed that the digestive tract of ArtN during the enrichment period were differentiating and the differences observed in enrichment products could be effected on their ontogenesis, absorption efficiency and the nutritional value of live food. In addition, a combination of the proximate composition and form of the enrichment products, and the lipid pathways of ArtN could be responsible for the biochemical composition of live food. In this study, differences were observed in protein, ash, and lipid values due to the use of enrichment products and combinations. When the results were compared with previous studies, ash contents of ArtN and ArtMn values were lower than Naz (2008) and similar to Yenmis & Naz (2019) and Aktaş et al. (2019).

It is known that the protein and lipid contents of commercial RA, GA and SP used for ArtN enrichment are 18%, 30%, 51.82% and 50%, 0.1%, 8.86%, respectively. Ash levels are similar to other enhancers (8-9%), although RA has a higher lipid content (50%) and a lower protein content (18%) than the others. On the other hand, the protein and lipid contents of SP were found to be higher than that of GA. Thus, it is not surprising that the groups tested after enrichment had a tendency to decrease in protein and lipid contents. The post-enrichment RA group has the highest lipid value due to its high lipid composition compared to other enrichment emulsions. Biochemical data showed that proteins and lipids were used as energy sources after enriching with the emulsion used in this study. Our results were supported by Naz (2008) and Yenmis & Naz (2019).

Table 3 shows the changes observed in the different pH values such as alkaline (pH=8.5), neutral (pH=7) and acid protease activities (pH=6, pH=5, pH=4, pH=3) of ArtN and ArtMn. The changes observed in the protease activities (alkaline, neutral and acid) of the tested groups were statistically significant ($p<0.05$). The products, which used as enricher, showed alteration in the developmental periods of Artemia, depending on the pH on the protease activities. The lowest protease activities after enrichment according to pH = 3, pH = 4, pH = 5, pH = 6, pH = 7 and pH = 8.5 values were given as; 18.18±0.37 U / mg protein (GA), 31.04±0.38 U / mg protein (RA), 9.1±0.32 U / mg protein (SP), 9.66±0.19 U / mg protein (SP), 16.94±0.61 U / mg protein (SP), and 63.09±0.75 U / mg protein (SP), respectively. In addition, the highest protease activities after enrichment according to pH = 3, pH = 4, pH = 5, pH = 6, pH = 7 and pH = 8.5 values were determined as 33.77±0.59 U / mg protein (RA: GA (50:50)), 57.54±0.34 U / mg protein (RA:GA (50:50)), 23.75±0.28 U / mg protein (GA), 40.82±0.49 U / mg protein (GA), 69.94±0.65 U / mg protein (GA) and 286.14±8.2 U / mg protein (GA), respectively. The lowest and highest alkaline, neutral and acid protease activities after the enrichment were 63.09±0.75 U/mg protein (SP), 16.94±0.61 U/mg protein (SP), 86.57±0.38 U/mg protein (RA) and 286.14±8.2 U/mg protein (GA), 69.94±0.65 U/mg protein (GA), 140.14±0.38 U/mg protein (RA:GA (50:50)), respectively.

Munilla-Moran, Stark & Barbour (1990) indicated that the enzymatic activity of *Artemia* varies depending on the nutritional status and developmental stage. Differences in protease activities of ArtN and ArtMn due to *Artemia* enrichment were reported by Bonnie, Lan & Hung (1991). Naz (2008) revealed that the maximum enzyme contributions from live food to fish larvae were at the end of the enrichment process. The protease activity value of ArtN (253.48±6.54 U/mg) and ArtMn (481.31±22.10 U/mg protein) were found to be higher than the results of the present study. Protease activity values of ArtMn (414.5±0.41 U / mg protein) determined by Naz and Yúfera (2012) were higher than the current study. Haközü (2014) reported that ArtN and ArtMn protease activity values were found to be lower (34.67±0.88 U/mg protein) and higher (317.16±2.67 U / mg protein) than the current study, respectively. Diken et al. (2016a) revealed that protease activity value of ArtMn was 338.02±4.65 U/mg protein and higher than the present study. The differences observed in enzyme activities of tested groups could be related to developmental periods, the age of the live food, and also, enrichment applications. Depending on the enrichment process, *Artemia*'s enzyme contributions are more attractive in neutral (pH 7) and alkaline (pH 8.5) conditions. It is known that the digestive enzymes of larvae are

Table 3. The protease activities observed at different pH values of Artemia enriched with different enrichment emulsions.

	Protease Activities (U/mg protein)					
	Acid				Neutral	Alkaline
Groups	**pH=3**	**pH=4**	**pH=5**	**pH=6**	**pH=7**	**pH=8.5**
AN	28.77±0.13^{c}	52.04±0.40^{f}	31.61±0.68^{f}	32.82±0.42^{g}	75.92±0.20^{g}	185.22±0.53^{e}
GA	18.18±0.37^{a}	34.47±0.41^{b}	23.75±0.28^{e}	40.82±0.49^{h}	69.94±0.65^{f}	286.14±8.2^{g}
RA	26.15±0.41^{b}	31.04±0.38^{a}	12.45±0.40^{c}	16.93±0.34^{d}	28.56±0.34^{c}	115.20±0.65^{c}
RA:GA (50:50)	33.77±0.59^{e}	57.54±0.34^{g}	23.27±0.60^{e}	25.56±0.45^{e}	53.29±0.45^{e}	158.44±0.68^{d}
SP	31.95±0.66^{d}	45.65±0.37^{d}	9.10±0.32^{a}	9.66±0.19^{a}	16.94±0.61^{a}	63.09±0.75^{a}
SP: RA (50:50)	27.52±0.22^{bc}	47.77±0.22^{e}	11.06±0.07^{b}	13.95±0.67^{c}	18.27±0.46^{a}	82.97±0.53^{b}
SP:GA (50:50)	31.96±0.82^{d}	52.16±0.52^{f}	11.79±0.32^{bc}	12.61±0.44^{b}	25.63±0.14^{b}	81.52±0.15^{b}
SP:RA:GA (33:33:33)	18.43±0.53^{a}	42.14±0.73^{c}	14.98±0.07^{d}	29.14±0.23^{f}	43.83±0.46^{d}	196.97±0.97^{f}

alkaline due to a poor development of the digestive system and then, acid proteases are important in digestion at the end of the larval period.

Simpson (2000) revealed that fish acid proteases showed high activity from pH=2 and to pH=4. In the present study, the highest acid proteolytic activities were detected at pH=4 except for GA group (pH=6). Similarly, the current study findings showed that ArtN and ArtMn have significant levels of acid proteolytic activity between pH 3-6. The potential activities of proteases depend on the pH of the larval digestive system. Warner & Shridhar (1985) indicated that acid proteases are not active at alkaline pH. This process may be neutralized when acid proteases are in contact with the alkaline conditions of the larval digestive system. Therefore, the contribution of acid proteases might be meaningless due to the pH in the larval digestive system being around 8.

It is known that the importance of the contribution of proteolytic enzymes from *Artemia* is decreased due to the differences in the digestive system of larvae. Cahu, Zambonino Infante, Le Gall & Quazuguel (1995) and Kurokawa, Shiraishi & Suzuki (1998) showed that live food proteases provided only a low contribution to the protease activities observed in sea bass and sardine larvae. Simpson (2000) indicated that alkaline proteases are most active between pH=8 and pH=10. Yufera, Fernandez-Diaz, Vidaurreta, Cara & Moyano (2004) showed that sea bream larvae digest mainly by the action of alkaline proteases due to the gastrointestinal pH being alkaline. The present data indicated that the GA group exhibited good performance due to the highest alkaline proteolytic activities between tested groups, followed by SP:RA:GA (33:33:33).

Figure 1 reveals the observed changes in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations. Total alkaline and neutral protease activities obtained from the tested groups were found to be higher than those of the total of acid protease activities**.** The combination of ArtN enrichment with the RA-GA (50:50) group tended to decrease to alkaline proteolytic activities, while the enrichment with the GA group of ArtN increased to alkaline proteolytic activities. On the other hand, the enrichment with RA group of ArtN had higher alkaline proteolytic activity than those of the enrichment with SP group. The enrichment with SP group of ArtN showed the lowest alkaline proteolytic activity. The alkaline proteolytic activities of the SP-GA (50:50) and SP-RA (50:50) groups were found to be similar, but not lower than that of the SP group. The alkaline proteolytic activity of the SP-RA-GA (33:33:33) group was lower than those of the GA group, but not lower than the other experimental groups. The highest acid proteolytic activity in tested groups was detected in the RA-GA (50:50) group following the GA group, the lowest acid proteolytic activity was observed in the RA group. Acid protease/Total protease (Alkaline + Neutral + Acid proteases) ratios observed in the tested groups ranged from 54.62% (SP) to 24.76% (GA).

According to the results of the study, alkaline and neutral protease activities were higher than those of acid protease activities. ArtN and ArtMn acid protease contents should be taken into account for optimum growth and survival rate, together with functional differences in the digestive system of the larvae. Overall, the acid protease/Total protease ratios of the SP containing groups were high except for SP:RA:GA (33:33:33). The results revealed that the enrichment with SP of ArtN was not effective due to high acid proteolytic activities. Warner & Shridhar (1985) showed that over 90% of the protease activity in Artemia embryos and ArtN was associated with a cysteine protease. Our study findings confirmed the presence of a significant level of cysteine proteases of ArtN and ArtMn.

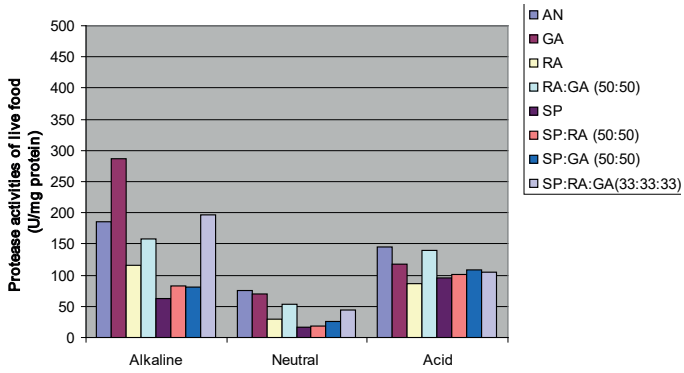

Figure 1. The changes observed in alkaline, neutral and acid protease activities of Artemia enriched with different enrichment emulsions (AN: Artemia nauplii; GA: GroBiotic-A; RA: Red Algamac; RA:GA(50:50): Red Algamac:GroBiotic-A(50:50); SP: Spirulina; SP:RA (50:50): Spirulina:Red Algamac (50:50); SP:GA (50:50): Spirulina:GroBiotic-A (50:50); SP:RA:GA (33:33:33): Spirulina:Red Algamac:GroBiotic-A (33:33:33).

During the critical periods of marine larvae, the digestive system is not yet developed, and alkaline digestion is present. For this reason, proteolytic enzymes working in the acid digestive system do not contribute significantly during feeding. However, proteolytic enzymes working in an alkaline digestive system have important contributions to digestion. The results in the present study showed that commercial emulsion and different additive enrichment groups have significant effects on acid, neutral and alkaline proteolytic enzymes.

CONCLUSIONS

In this study, which was carried out considering biochemical values and proteolytic enzyme activities, it is recommended to use the enrichment combinations GA and SP: RA: GA (33:33:33) as an alternative to enrichment products. On the other hand, SP and RA should not be used alone due to the disadvantages such as the biochemical compositions and protease activities of ArtMn observed in the present study.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical statement: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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Disclosure: -

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