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Survival Rate and Biochemical Parameters in *Mugil cephalus* (Linnaeus, 1758) Larvae Fed Garlic (*Allium sativum* L.) Extract

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Abstract

This study was carried out to investigate the effect of different concentrations of garlic (*Allium sativum* L.) extract in fish diet on survival rate, digestive enzymes and some biochemical parameters of *Mugil cephalus* larvae. Three hundred and sixty fish with mean weight 0.45 g were randomly divided into equal four groups; each one contained 3 replicates. Fish was fed with diets containing 0% (control), 0.5%, 1% and 3% concentrations of garlic extract (GE) in diet. 30 days after feeding, survival rate, different biochemical (larvae extract total protein, albumin, globulin, glucose, cholesterol and triglyceride) parameters and digestive enzyme activity (lipase, protease and amylase) were evaluated. There was a significant increase in survival rate of all groups fed with GE as compared to the control diet ($P < 0.05$). The administration of *A. sativum* in all levels significantly decreased ($P < 0.05$) the content of cholesterol, triglyceride and glucose in larvae extract. Also total protein, albumin and globulin levels had significantly increased in all groups fed GE ($P < 0.05$). The highest total protein (2.13 ± 0.12 g/dL), albumin (0.37 ± 0 g/dL), globulin (1.76 ± 0.12 g/dL), amylase (9.25 ± 0.14 U/mg protein), protease (4.20 ± 0.08 U/mg protein) and lipase (2.62 ± 0.14 U/mg protein) and the lowest serum triglyceride (22.78 ± 0.20 mg/dL), glucose (8.76 ± 0.09 mg/dL), cholesterol (3.69 ± 0.07 mg/dL) levels were observed in fish fed 3% GE in diet. Garlic inclusion in fish diet at 3% concentration is therefore beneficial for use in aquaculture to improve the general health and digestive enzyme activity of *M. cephalus* larvae.

Keywords

***Mugil cephalus*, Garlic Extract, Biochemical Indices, Digestive Enzyme Activity**

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1. Introduction

Successful rearing of larval fish is the most critical stage in the production cycle for many species. *Mugil cephalus* has a promising market potential in Europe, East and South Asia [1]. It is also an important aquaculture species in Iran. The consumer demand stimulated the development of intensive aquaculture of this species in Asian countries. The problem in rearing larval fish is food supply [2]. Therefore a readily available, easily acceptable and highly digestible diet with high nutritional value should be used as larval fish starter diet [2] [3].

Plants are natural sources of safer and cheaper chemicals. Plant products have been reported to promote various activities like anti stress, growth promotion, appetite stimulation and immunostimulation in aquaculture practices [4] [5].

Garlic (*Allium sativum*) is one of known medicinal plants in aquaculture used as a flavoring agent, traditional medicine, and a functional food to enhance physical and mental health. Garlic was studied in different forms of extracts: aqueous, ethanol and dried powder [6] [7]. It contains a variety of organosulfur compounds such as allicin, ajoene, S-allylcysteine, diallyl disulfide, S-methylcysteine sulfoxide and S-allylcysteine [8].

Garlic inclusion in fish feeds has also been reported to increase growth performance in fish [9]-[11]. Khalil *et al.* [12] mentioned that garlic contained allicin which promoted the performance of the intestinal flora, thereby improving digestion, enhancing the utilization of energy, and leading to improved growth.

Metabolic activities of blood plasma of *Clarias gariepinus* [12] and *Labeo ruhu* [8] have been improved by the inclusion of garlic in fish feed. Dietary garlic decreases blood glucose by increasing the level of serum insulin [13] and the S-allylcysteine sulfoxide present in garlic is responsible for its hypoglycaemic activity [14] [15].

Garlic is useful for *Mugil cephalus* which is one of the commercially important culture fish in Iran due to their euryhaline, fast-growing, and disease resistant [1] [16].

Until now, no trial has been conducted to study the effect of dietary garlic extract on survival and some of serum biochemical parameters of grey mullet larvae. This study was therefore designed to investigate the effect of garlic on survival, larvae serum biochemical parameters (protein, glucose, albumin, globulin, cholesterol and triglyceride) and digestive enzyme activity of *M. cephalus.*

2. Materials and Methods

2.1. Experimental Fish and Husbandry

At Mid February, larvae of *M. cephalus* (average wet weight 0.45 ± 0.11 g) captured from the coastal water of Chabahar port, were transformed in the Researches Institute of Fisheries, Chabahar, Iran and stocked in a 500-L tank for quarantine and health check After quarantine, fish were acclimatized for one week in 400-L chlorine-free tap water and fed with commercial diet. Water exchange (50%) was done daily and water quality was monitored throughout the experiment at weekly intervals. Temperature was 28.2˚C ± 0.5˚C, dissolved oxygen concentration 7.01 ± 0.87 mg/L, ammonia nitrogen concentration 0.11 ± 0.04 mg/L and pH 7.8 ± 0.4. Fish were fed ad labium with commercially available pelleted feeds (Beyza Feed Mill Company, Iran) at the rate of 3% of body weight following assessment of biomass by bulk weighting every 7 days (thirty fish for each group). Growth was calculated based on the difference in the final body weight and initial body weight, and expressed as weight gain (%). The daily ration was subdivided into two and fed at 9:00 hours and 16:00 hours.

2.2. Preparation of Garlic Extract

Two kg garlic (*A. sativum*) bulbs was obtained from the local market in Chabahar, Iran and oven dried at 60˚C, powdered by mortar and pestle and sieved Then garlic powder was left during 48 h in 99% ethanol 10 L (10% w/v) in room temperature (24˚C ± 1.2˚C) and the resulting extract was concentrated to 300 mL using rotary evaporator (IKA, Germany) giving the extract of 6.1 g of garlic powder mL^{-1}. This extract was sprayed on the diet after dilution in 300 mL of distilled water [11].

2.3. Preparation of Herbal Diets

A commercial extruded pellet of 1.6 mm size (Beyza Feed Mill, Iran) was employed as the experiment diet. The analysed composition was as follows moisture 10%, fibre 1.7%, crude protein 50%, crude fat 13.5% and crude ash 14.8%. Four diets were prepared to contain 0% garlic extract (control diet), 0.5% (diet 2.0 kg + 100 mL gar-

lic extract + 300 mL distilled water), 1.0% (diet 2.0 kg + 200 mL garlic extract + 300 mL distilled water) and 3.0% (diet 2.0 kg + 600 mL garlic extract + 300 mL distilled water) garlic extract. The mixture of garlic extracts and distilled water was sprayed on the experiment diets and dried in room temperature at 30˚C for 48 h in order to volatilize remaining ethanol. All diets were stored at −20˚C until used [11].

2.4. Experimental Design and Feeding Diet

The study was conducted over a period of 30 days to evaluate the efficiency of garlic extract in promoting growth and carcass composition of grey mullet. Grey mullet larvae (n = 360) were divided into four equal groups. Three tanks (30 fish in each tank) were used in each group and randomly assigned to 12 plastic tanks each, 20 L. Control group (1) was fed with basal diet and the remaining groups (2-4) were fed with 0.5%, 1% and 3% concentrations of garlic extract in diets respectively.

2.5. Homogenate of Larvae and Digestive Tracts

After 30 days of feeding, Larvae were starved for 24 h immediately after termination of the experiment. The number of surviving fish was recorded and used for calculating mortality. Nine fish (three fish per tank) from each group were selected at equivalent weight and anesthetized by clove oil (5 mg/L) to assay both digestive enzymes (protease, lipase and amylase) and some biochemical indices (total protein, cholesterol, glucose, albumin, triglyceride and globulin) serum biochemical parameters. For biochemical indices assay, larvae were washed two times with sterile phosphate buffer saline (PBS), pH 7.2, homogenized with 1 volume of PBS and centrifuged at 3000 g for 10 min at 4˚C. The supernatants were collected, centrifuged once at 3000 g for 5 min and stored at −20˚C. Also for enzyme assay, the digestive tracts of larvae were carefully removed, thoroughly washed with PBS weighted and homogenized with chilled saline (0.65%) and supernatant was extracted by centrifuged at 3000 g for 10 min at 4˚C.

2.6. Assay of Some Biochemical Parameters

The biochemical indices were determined with a Hitachi 917 (Hitachi, Tokyo, Japan) according to the commercially available diagnostic Experimental Protocols kits (Co. Pars Azmoon, Iran). These included total protein (g/dL larvae extract) (biuret method), albumin (g/dL larvae extract) (bromocresol green method), globulin (g/dL larvae extract) (subtracting albumin from total protein), triglyceride (mg/dL larva extract) (glycerol phosphate oxidase-paminophenazone method) and cholesterol (mg/dL larvae extract) (enzymatic endpoint method) levels. They were determined spectrophote metrically at 546 nm and 37˚C [17] [18].

2.7. Assay of Digestive Enzymes

The influence of garlic extract on amylase, protease and lipase activities in the digestive tract was estimated by the method of Bernfeld [19] and Worthington [20]. Unit amylase activity was calculated as the weight (mg) of maltose liberated for a duration of 10 min at 30˚C. Unit protease activity was expressed as the amount of tyrosine liberated in 15 min under the assay conditions. Unit lipase activity was expressed as the amount of 0.025 N NaOH required to neutralize the fatty acids liberated during 18 h of incubation at pH 6.9 and temperature 30˚C. The activities of digestive enzymes were calculated as enzyme unit per milligram protein ($U \cdot mg^{-1}$ protein).

2.8. Statistical Analysis

Data are presented as means means ± standard error (SE). All the biochemical parameters and digestive enzymes were analysed using one way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test. Significance was tested at 5% level and all statistical analysis was carried out using the SPSS Version 16.

3. Results

After 30 days of culture, survival rate in groups fed GE in diet was significantly higher ($P < 0.05$) than the control group. The highest survival rate of larvae 99% was found in fish fed 3% GE in diet while the lowest (87%) was in the control group (as shown in **Figure 1**). There was no significant difference among fish fed different

concentrations of GE in diet ($P > 0.05$).

The lowest glucose (18.76 ± 0.09 mg/dL), triglyceride (22.78 ± 0.20 mg/dL) and cholesterol (3.69 ± 0.07 mg/dL) levels were observed in fish fed 3% GE in diet compared to the rest experimental groups and the control. Also there were significant different ($P < 0.05$) among all fish fed different concentrations of GE in diet in glucose, triglyceride and cholesterol levels (as shown in **Figure 2**).

After 30 days of feeding, the total protein and globulin and albumin levels were significantly higher ($P < 0.05$) in the experimental groups than the control group (as shown in **Figure 3**). The highest globulin (1.78 ± 1.24 g/dL) level was observed in fish fed 3% GE in diet compared to the rest experimental groups (**Figure 3(c)**). Albumin and total protein levels was slightly higher in fish fed 3% GE in diet (0.37 ± 0 and 2.13 ± 0.12 g/dL respectively) compared with fish fed 1% GE in diet (0.35 ± 0 and 1.86 ± 0.01 g/dL respectively) but showed no significant difference (**Figure 3(a)**, **Figure 3(b)**).

Protease activity significantly ($P < 0.05$) increased in fish fed GE in diet (all concentrations).But for amylase and lipase activities, only at 1% and 3% GE in diet significantly ($P < 0.05$) varied. Highest amylase (9.25 ± 0.14 U/mg protein), lipase (2.62 ± 0.10 U/mg protein) and protease (4.20 ± 0.08 U/mg protein) activities were observed in at 3% GE in diet. No difference was measured in activity of protease at 1% and 3% GE in diet (**Table 1**).

4. Discussion

Garlic is an important medicinal herb extensively cultivated in many countries and has played an important dietary function as well as medicinal role for centuries. In the present study, survival rate was significantly greater in all garlic-supplemented groups when compared with the control group at the end of the experiment (30 days after feeding). However, it was slightly higher in fish fed 3% GE compared with fish fed 0.5% and 1% GE in diet, but showed no significant difference ($P > 0.05$). Although the use of garlic resulted in good survival rate, feeding the higher doses of garlic for extended periods gave better results. Using a combination of five herbs developed an Artemia-enriched herbal diet for *Penaeus monodon*, which significantly increased survival rate during stress conditions [4]. In agreement with the present results, Javadzadeh *et al*. [21] reported a significant increased survival rate (81.6%) in which were fed enriched *Artemia* nauplii with 200 mg garlic extract/L. In the present study, GE in diet enhanced total protein, globulin and albumin levels of *M. cephalus* larvae. Highest globulin level was observed in fish fed 3% GE in diet compared to the rest experimental groups. Albumin and total protein levels was slightly higher in fish fed 3% GE in diet compared with fish fed 1% GE in diet, but showed no significant difference ($P > 0.05$). Which agrees with the results of increase in serum protein, albumin and globulin levels are thought to be associated with a stronger innate immune response and higher survival of fish [22]. Our results are comparable with those of Qompsell—which are extracts of several traditional chine's medicines have been found to significantly increase serum albumin, total protein and globulin in *Cyprinus carpio*

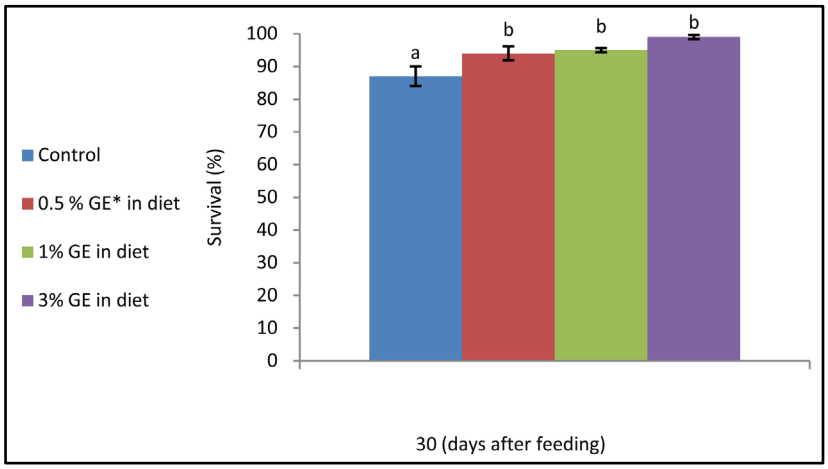
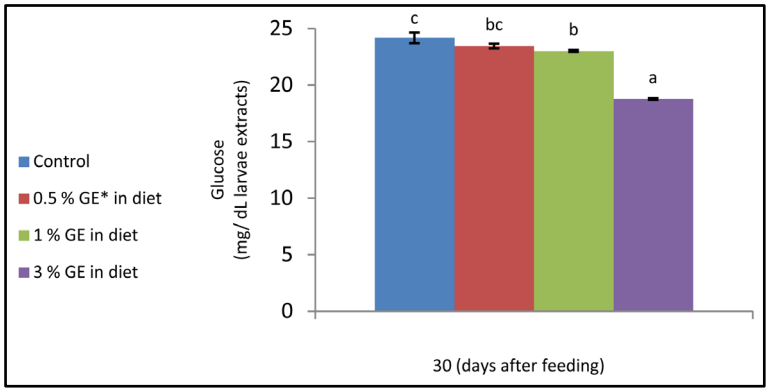
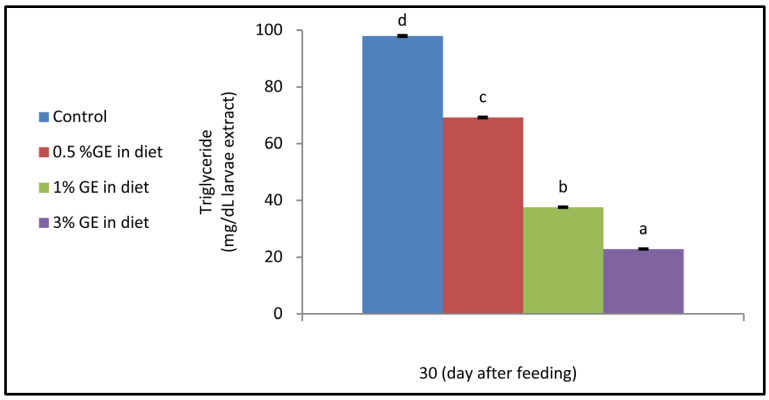


Figure 1. Survival rate in control and experimental groups. The values represent the mean ± SE. Groups with the same superscript do not differ from each other ($P > 0.05$). *Garlic extract.



(a)



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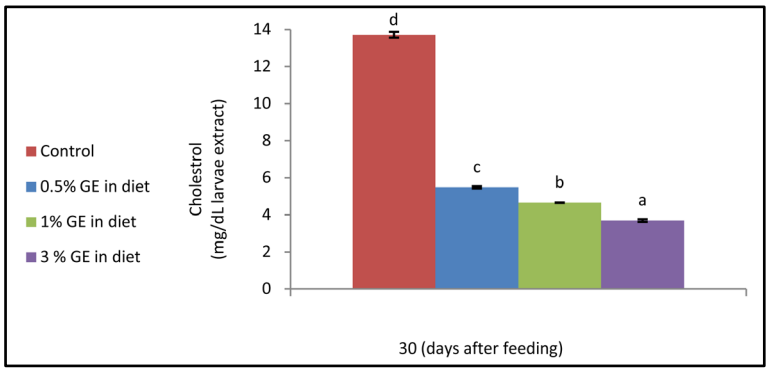
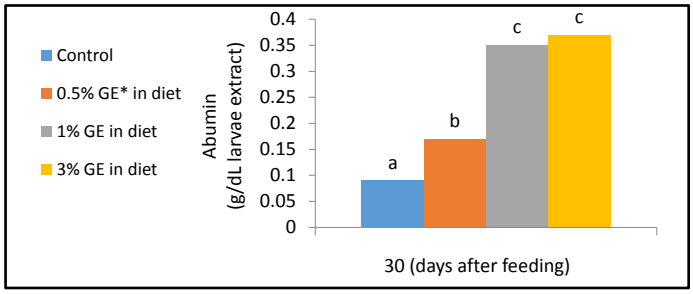


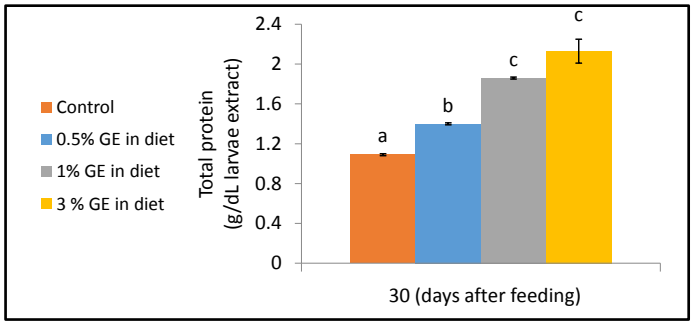
(c)

Figure 2. (a) Glucose, (b) triglyceride and (c) cholesterol levels in control and experimental groups. The values represent the mean ± SE (n = 9). Groups with the same superscript do not differ from each other ($P > 0.05$). *Garlic extract. Duncan's test.

by oral rout [23]. This finding is similar to earlier reports that total plasma protein in fish could vary from 2 - 8 $g \cdot d^{-1}$ [24]. Nwabueze [10] also reported increased levels of plasma protein in *Clarias gariepinus* and fed with different concentrations of garlic in diet. Sahu *et al.* [8] reported that the serum total protein, albumin and glucose levels in *Labeo ruhu* after 60 days feeding with *A. sativum* increased in comparison to the control diet. In the present study, plasma glucose concentration reduced significantly in fish fed on diets containing the highest level of *Allium sativum* (3% in diet). These results agree with those of Lee *et al.* (2012) showed that hypoglycaemic effect for juvenile sterlet sturgeon (A. ruthensis) fed diet with 0.5% GE was accompanied with blood plasma glucose depletion after 1 h (50.8 mg/dL) and 24 h (57.6 mg/dL) of meal. Kumar and Reddy [25] and



(a)



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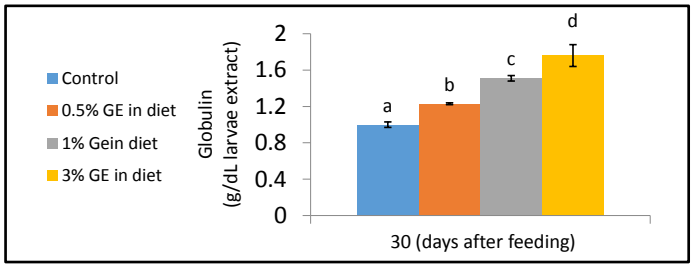

(c)

Figure 3. (a) Albumin, (b) total protein and (c) globulin levels in control and experimental groups. The values represent the mean ± SE (n = 9). Groups with the same superscript do not differ from each other ($P > 0.05$). *Garlic extract. Duncan's test.

Table 1. Digestive enzymes levels in *Mugil cephalus* larvae fed diet with or without garlic extract at the end of experiment (30 days after feeding).

Diet	Amylase (U/mg protein)	Lipase (U/mg protein)	Protease (U/mg protein)
Control	6.33 ± 0.16^{a}	1.46 ± 0.10^{a}	3.05 ± 0.11^{a}
0.5% GE*	6.50 ± 0.13^{a}	1.79 ± 0.07^{ab}	3.69 ± 0.08^{b}
1% GE	8.30 ± 0.09^{b}	2.15 ± 0.12^{b}	3.96 ± 0.07^{bc}
3% GE	9.25 ± 0.14^{c}	2.62 ± 0.10^{c}	4.20 ± 0.08^{c}

*Garlic extract. The values represent the mean ± SE (n = 9). The values where are similar in columns are identified by the same superscript ($P > 0.05$). Duncan's test values.

Thomson and Alim [26] who found that feeding mice with 45 mg garlic/kg body weight for 28 days induced significant decrease of serum glucose levels. Lower levels of plasma glucose in fish have also been reported in the assessment of physiological effects of *Allium sativum* [23] [27].

Dietary garlic decreases blood glucose by increasing the level of serum insulin [13]. According to Vazquez-Prieto *et al.* [14] and Ademiluyi *et al.* [15], the S-allyl cysteine sulfoxide present in garlic is responsible for its

hypoglycaemic activity. In the groups fed GE in diet (different concentrations) was observed a significant decrease in glucose, cholesterol and triglyceride levels. The lowest triglyceride and cholesterol levels were observed in fish fed 3% GE in diet compared to the rest experimental groups and the control ($P < 0.05$). These results are in agreement with the study by Adler and Holub [28], who verified that serum total lipid and total cholesterol decreased significantly in men treated with garlic and fish oil alone or combined. Also, Hussein *et al*. [22] found that the serum total lipid decreased significantly in albino rats after administration of garlic. Conversely, other study on *C. carpio* showed that Qompsel (extracts of several traditional Chinese medicines), enhanced the serum triglyceride and cholesterol levels slightly, but none were significantly different. The different composition and quantity of sulfur components of different garlic preparations used in various studies could account for the inconsistent findings. It highlights the need for standardization of different garlic preparations and to arrive at a valid conclusion. Other factors might include the subject recruitment, duration of study, dietary control, lifestyle and methods of lipid analyses [29].

This study indicated that garlic extract contained abundant proteins, but only a few lipids. The findings conform to those Lee *et al.* [13] who reported that garlic inhibits the synthesis of cholesterol and fatty acids in the liver; however, the exact mechanisms are not well understood. Therefore, further studies should be done to establish the relationship between the dose used, period of application.

Apart from enhancing the taste and flavor of food, spices have been widely believed to exert digestive stimulant action. Spices such as mint and garlic play a very important role in fat digestion and absorption [13]. The results of the present study indicate that *A. sativum* plays a positive role on activities of amylase, lipase and protease. Highest digestive enzyme activities in 3% GE in diet fed larvae were shown, probably, because of the high concentration of compounds such as allicin, ajoene, S-allylcysteine, etc. [14]. Similar positive observations were reported by Venkatramalingam *et al*. [30]. A significantly ($P < 0.05$) improvement of digestive enzyme activity (amylase, protease and lipase) were observed that in post larvae of *P. monodon* fed with different percentage (0%, 25%, 75% and 100%) of the herbal appetizer *Zingiber officinalis* enriched Artemia, after 30 days of culture [30], In another study, the effect of Livol (IHF-1000), as a herbal growth promoter, on the rohu, *Labeo rohita*, the Livol incorporated diet stimulated digestive enzyme activity and led to increased consumption [31]. Generally all spices shorten the feed transit time; this reduction was more prominent in the case of *A. sativum*. Also, the increases in enzyme production can result in improvements in digestibility and availability of nutrients from feedstuffs [32]. Reducing the amount of undigested material passing into the large intestine limits the amount of substrate available for proliferation of pathogenic bacteria. The enhanced proteolytic and lipolytic activities in the digestive tract of the GE in diet fed larvae could also be linked to better protein and lipid digestibilities [33].

5. Conclusion

Finally, the results of the present study indicate that *A. sativum* plays a positive role on activities of amylase, lipase and protease. Highest digestive enzyme activities in 3% GE in diet fed larvae were shown, probably, because of the high concentration of compounds such as allicin, ajoene, and S-allylcysteine. Also, the enhanced proteolytic and lipolytic activities in the digestive tract of the GE in diet fed larvae could also be linked to better protein and lipid digestibilities.

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