Iran. J. Ichthyol. (2023) 10(2): 78-92
P-ISSN: 2383-1561; E-ISSN: 2383-0964

ORIGINAL ARTICLE

Dietary supplementation of *Allium hirtifolium* essence supports gut bacteria, and health status in fuelleborn's cichlid (*Labeotropheus fuelleborni*) juveniles

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Article history:
Accepted 22 June 2023

Abstract

The present research was undertaken with the aim of investigating the effects of dietary supplementation with Persian shallot (*Allium hirtifolium*) essence in the diet on the growth, intestinal microbiota, biometric indexes, and immune response in *Labeotropheus fuelleborni* juveniles. For this purpose, four experimental diets containing 0, 0.10, 0.15, and 0.20 mL kg^{-1} of the essence were prepared and the effects of each of those diets were examined with completely randomized designs over the course of 60 days. The addition of *A. hirtifolium* to the diets of the fish was not found to have an impact on growth parameters. Furthermore, no significant differences were observed in the gastrosomatic index values with different treatments (P>0.05). The hepatosomatic index was highest with the 0.20mL kg^{-1} *A. hirtifolium*-supplemented diet (P<0.05). Survival was increased with 0.20mL kg^{-1} *A. hirtifolium* treatment in comparison to the control fish (P<0.05). *A. hirtifolium* essence did not affect the total aerobic bacteria (P>0.05), but the enteric gram-negative bacteria and lactic acid bacteria counts were found to be decreased in the experimental treatments with statistical significance (P<0.05). Values obtained for alternative complement haemolytic activity levels, lysozyme activity levels, total immunoglobulin, and dissolved protein in the skin mucus among fish that received essence were found to be higher in comparison to the control group (P<0.05), while no change was recorded for alkaline phosphatase, aspartate aminotransferase, or alanine aminotraansferase liver enzyme activities (P>0.05). Results revealed that, *Allium hirtifolium* essence is suggested to obtain survival rate, skin mucus immunity and intestinal microbiota. The maximum level to add to the feed of juvenile *L. fuelleborni* can be recommended as 0.15-0.20mL kg^{-1}.

Keywords: *Allium hirtifolium*, Aquaculture, Persian shallot, *Labeotropheus fuelleborni*, Gut bacteria.

INTRODUCTION

The gastrointestinal (GI) system constitutes a dynamic and complicated ecosystem of microbes that are essential nutritionally, physiologically, and pathologically (Hussain et al. 2021). Among the microbial groups, bacteria were reported to be the most heavily colonized group in the GI systems of fish (Llewellyn et al. 2014). Since the components of the intestinal microbiota play crucial roles in the metabolic functions, immunity, and general health of their hosts, it is important to investigate the structure and composition of these systems of microorganisms (Ni et al. 2014; Pérez et al. 2010).

Fish are considered valuable models for studying the microbiota of vertebrates due to their relatively short life cycles, large numbers of progeny, and diversity in genetic, immunological, and physiological characteristics that can be controlled with ease in experimental settings (Lescak & Milligan-Myhre 2017; Leulier et al. 2017).

The formation and natural structure of microbial compositions in GI systems may vary depending on several internal and external factors. The developmental stage of the fish (Sugita et al. 1985), gut morphology (Sera 1974; Sugita et al. 1985), environmental atmosphere (Sugita et al. 1985) and temperature (Lesel & Peringer 1981; Sugita et al. 1985), and growing conditions (Ringø et al. 2006) are factors that affect the initial colonization and subsequent settlement processes of microorganisms. In addition, stress factors can significantly affect the GI microbiota (Lesel & Peringer 1981). Feed and

feeding conditions are other factors that significantly affect fish GI microbiota compositions (Martin-Antonio et al. 2007; Ringø et al. 2006). It was shown that the intestinal flora changes rapidly depending on feeding, especially in the larval stage (Brunvold et al. 2007; Reid et al. 2009).

Ornamental fish farms have adopted intensive breeding techniques to maximize productivity, creating stressful conditions such as high stocking density and poor hygiene, which make fish more susceptible to pathogens and increase the incidence of microbial diseases (Au-Yeung et al. 2022). Antibiotics are frequently used in ornamental fish farming to control and combat diseases (Preena et al. 2019). However, uncontrolled and repetitive applications of antibiotics have facilitated the proliferation of antibiotic-resistant pathogens with the emergence of harmful consequences for both the aquatic environment and human health (Sicuro et al. 2020). Therefore, the possibility of utilizing various additives as alternatives to conventional antibiotics remains a crucial subject of research (Yilmaz 2020). Phytobiotics are natural plant-derived compounds that increase animal productivity. They are abundant in the natural environment and have no residual effects. In addition, they have antimicrobial, antioxidant, and growth-stimulating properties (Antache et al. 2013; Cristea et al. 2012).

Allium is one of the most important genera of the family Amaryllidaceae. Onion, garlic, leek, and shallot are the most important species of this genus. Among them, Persian shallot (*Allium hirtifolium*) is a particularly nutritious medicinal plant. Persian shallot is a rich source of bioactive compounds such as proteins, carbohydrates, lipids, amino acids, flavonoids, and organic sulphur compounds with extensive medicinal properties and biological activities (Ghahremani-majd et al. 2012; Mahmoodi et al. 2013; Rashidian et al. 2022a; Soorni et al. 2021). It is rich in calcium, zinc, and magnesium, and the amount of linolenic acid found in Persian shallot is higher than that of common shallot and onion (Moradi et al. 2013). *A. hirtifolium* has useful secondary biological metabolites such as diallyl disulfide (DADS), diallyl trisulfide (DATS), S-allyl cysteine (SAC), alliin, allicin, and methyl allyl (Asgari et al. 2012a; Mikaili et al. 2013). There are several reports highlighting the medicinal effects of *A. hirtifolium* as a result of its antitoxic (Omidifar et al. 2020), antioxidant (Leelarungrayub et al. 2006; Omidifar et al. 2020; Pirbalouti et al. 2015), cardioprotective (Asgari et al. 2012b), anticancer (Ghodrati Azadi et al. 2008), antifungal (Diba & Alizadeh 2018; Fateh et al. 2010), antibacterial (Karthikkumar et al. 2020), hypocholesterolemic (Satvati et al. 2017), antimicrobial (Satvati et al. 2017) and immune system-regulating effects (Jafarian et al. 2003).

Some studies have revealed the efficacy of a hydromethanolic extract of *A. hirtifolium* against 10 strains of methicillin-resistant bacteria from the genera *Staphylococcus*, *Streptococcus*, *Escherichia*, *Salmonella*, *Proteus*, and *Klebsiella* (Ismail et al. 2012). Another previous study determined that the aqueous extract of *A. hirtifolium* had a more persistent inhibitory effect on salivary bacteria count than chlorhexidine mouthwash, being effective for up to 24 hours (Amin et al. 2012).

It was previously determined that applications of *A. hirtifolium* extract at concentrations of 0.5%, 1%, 2%, and 3% in the diet of fry fish (*Oncorhynchus mykiss*) had a positive effect on growth and antioxidant and immune system (Ghafarifarsani et al. 2022). It was also reported that the addition of 5, 10, 15, 20, and 25g/kg *A. hirtifolium* extract added to the feeding regimen of rainbow trout (*O. mykiss*) improved the outcomes of growth, biochemical parameters, and innate immunity after 4 weeks of feeding and that it would be appropriate to use it in feeds for rainbow trout at a dose of 20g kg^{-1} (Rashidian et al. 2022a). In another study on rainbow trout, it was observed that the addition of Persian shallot powder to animal feeds at concentrations of 0.5% and 1% had a beneficial effect on growth performance, blood biochemistry profiles, and immune responses (Shekarabi et al. 2022).

The present study was undertaken with the aim of

Table 1. Ingredient composition and chemical analysis of the basal diet.

Ingredients	%
Fish meal	26
Meat meal	20
Baker's yeast	20
Wheat flour	25
Soy oil	3
Fish oil	3
Vitamin supplementation	1.5
Mineral supplementation	1.5
Chemical composition (% dry matter, n=3)	
Crude protein	35.05±0.14
Crude fat	8.00±0.12
Crude fibre	3.98±0.03
Ash	4.01±0.04
Nitrogen-free extract (NFE)	48.96±0.03
Gross energy (kcal kg^{-1})	3400±1.00

assessing the administration of *A. hirtifolium* essence at three different concentrations on the growth performances, biometric indexes, immune responses, and intestinal microbiota of *L. fuelleborni* juveniles under experimental conditions.

MATERIALS AND METHODS

Experimental design: *L. fuelleborni* juveniles were obtained from a fish seller in Mashhad, Iran, and were held in the laboratory for 10 days to adapt to the experimental conditions. Subsequently, 240 acclimated and healthy fish (5.56±0.11g) were distributed across 12 experimental glass tanks of 100L in volume. Each experimental group was evaluated based on three replications. Over the course of the 60 days of the experiment, the nutritional requirements of the fish were met by hand-feeding three times a day with the administration of feeds at a rate of 2% of their body weigh. Ingredient composition and chemical analysis of the basal diet are presented in Table 1.

Sardine fishmeal and fish oil from Adriatic Co©, Qeshm, Iran. Baker's yeast from Razavi Yeast Co, Mashhad, Iran. Meat meal and wheat flour kindly provided by Yadegar Company©, Mashhad, Iran. Mineral supplementation: Zn, 1500mg kg^{-1}; Mn, 7500mg kg^{-1}; Cu, 900mg kg^{-1}; Fe, 15000mg kg^{-1}; Co, 150mg kg^{-1}; Cr, 75mg kg^{-1}; Se, 75mg kg^{-1}. MaxiAct-FH kindly provided by Ariana Knowledge-Based Company, Mashhad, Iran. Vitamin supplementation: A, 8000000 IU; D, 2000000 IU; E, 200000mg; K, 10000mg; B_1, 30000mg; B_2, 40000 mg; B_3, 60000 mg; B_5, 20000mg; B_6, 20000mg; B_9, 10000mg; B_{12}, 40mg; H_2, 1200mg; C, 300000mg per 15 kilograms. Mega Mix-FH kindly provided by Ariana Knowledge-Based Company, Mashhad, Iran.

Preparation of *Allium hirtifolium* essence and experimental diets: The allicin concentration of the Persian shallot essence was determined spectrophotometrically (2.56±0.2 mg g^{-1}) based on the method proposed by (Miron et al. 2002). The principle of this method is to monitor the decrease in the absorbance at a wavelength of 324nm of 4-mercaptopyridine (4-MP) (10^{-4} M) in 50mM NA-phosphate buffer, 2 mM EDTA (pH 7.2). In this study, samples were incubated with increasing concentrations of Persian shallot essence.

Growth performances and survival rates: When the 60 days experiment was over, the experimental fish were weighed with a digital balance that had a confirmed accuracy level of 0.01g. Subsequently, the statistics for survival rates, growth parameters, hepatosomatic index (HSI) values, and gastrosomatic index (GaSI) values were obtained by applying the following formulas (Farhangi & Carter 2001):

Specific growth rate (SGR)= [(Ln final weight (g)–Ln initial weight (g))/experimental days] × 100

Survival rate (%)= (Final number of individuals/initial number of individual)×100

Hepatosomatic index (HSI)= (Liver weight (g)/total body weight (g))×100

Gastrosomatic index (GaSI)= (Gut weight (g)/total body weight (g))×100

Nonspecific immune parameters of the skin mucus and liver enzymes: In the final stage of our experiment, feed was withheld from the fish for 24 hours and three of them were randomly chosen from each aquarium to be used in the analysis. Skin mucus analysis was performed as per a method previously suggested in the literature (Taee et al. 2017). In summary, after the fish were anaesthetized by applications of clove powder (5mg L^{-1}), they were moved into polyethylene bags with 10mL of NaCl (50mmol) and the mucus was separated by shaking the bags. Extraction was then performed for 2 minutes, and mucus samples transferred to sterile tubes for centrifugation at 1500xg and 4°C for 10 minutes. Subsequently, the supernatant was held at -80°C until the time of analysis. The skin mucus alternative complement pathway haemolytic activity (ACH50) analysis was determined by the method previously described in the literature (Stolen et al. 1994). Rabbit red blood cells were then added to mucus samples and the prepared solution was incubated for 90 minutes at room temperature. Finally, 3.15mL of NaCl solution (0.85%) was added to the samples and all samples were centrifuged at 1600rpm for 10 minutes. Centrifuged samples were later evaluated with the help of a spectrophotometer at a wavelength of 412 nm. In the process of determining the lysozyme activity, 3mL of *Micrococcus lysodeikticus* in 0.05 M sodium phosphate buffer was combined with mucus samples of 100µL and the reading was taken at 540nm in 0.5 and 4.5 minutes (Zheng et al. 2009). In order to determine the protein concentration, the method of Lowry et al. (1951) was used. Total immunoglobulin (Ig) amounts were determined with the application of a method previously reported in the literature (Hoseinifar et al. 2016). According to this method, each sample was blended with a polyethylene glycol solution (12%) followed by the precipitation of immunoglobulin molecules. In the next step of the procedure, total protein levels were measured again. Differences in protein contents were taken as reflections of the total Ig contents of the skin mucus. The kinetic potentiometric method was applied in the evaluation of the liver enzymes of the fish, including alkaline phosphatase (ALP), aspartate transaminase (AST), and alkaline transaminase (ALT). All measurements were made using commercial diagnostic kits (Pars Azmun Company, Iran). Readings were performed with a spectrophotometer (DR/4000, HACH, USA) at 405nm (Mirghaed et al. 2017; Ahmadniaye Motlagh et al. 2020a).

Analyses of intestinal bacterial: In the final step of the experiment, all fish were anaesthetized with the administration of clove powder (5mg L^{-1}) followed by disinfecting with 70% ethanol and then the intestines were removed in their entirety (Ahmadnia-Motlagh et al. 2017). Total viable counts (TVCs) of intestinal heterotrophic bacteria and lactic acid bacteria (LAB) were obtained based on results for 1-cm samples of the posterior intestine. Samples were homogenized in 9.0 mL of normal sterile saline solution (0.90% w/v NaCl) and dilutions were prepared at 10^8. After that, 0.1mL was spread across duplicate plates of plate count agar for the TVCs of heterotrophic bacteria, MacConkey agar medium for the TVCs of enteric gram-negative bacteria, and MRS agar for the TVCs of LAB. These plates were allowed to incubate for 72h at room temperature and then counts of the bacterial colonies were performed for each sample according to colony-forming units (CFU/g; colony count×dilution^{-1}= CFU/g intestine) (Kim & Austin 2006).

Statistical analysis: Data in whole percentages was converted by the arcsine method. The Levene test was applied for determination of the homogeneity of variance while the Kolmogorov-Smirnov test was utilized to determine data normality (Zar 1999). Data were analyzed with one-way analysis of variance (ANOVA) and then the Duncan multiple range test was utilized for comparisons of the obtained mean values between groups. In this process, all statistical analyses were conducted with the help of IBM SPSS Statistics 19 (IBM Corp., USA) and findings are

Table 2. Mean (±SD) values obtained for weight (g), specific growth rate (SGR) (% BW day^{-1}), survival, hepatosomatic index (HSI), and gastrosomatic index (GaSI) of *L. fuelleborni* juveniles were supplemented with different concentrations of *A. hirtifolium* essence for 60 days (n=3).

	Concentrations of dietary *A. hirtifolium* essence			
	0	0.1	0.15	0.2
Initial weight (g)	5.67±0.14	5.48±0.25	5.65±0.28	5.45±0.18
Final weight (g)	8.15±0.85	7.88±0.77	8.01±1.14	7.76±0.66
SGR (%)	1.03±0.25	1.03±0.34	0.98±0.41	1.00±0.18
Survival (%)	77.78±9.62^{b}	66.67±16.67^{a}	66.67±28.87^{a}	83.33±28.87^{c}
HSI (%)	1.52±0.65^{ab}	2.27±0.62^{b}	1.52±0.04^{ab}	1.14±0.06^{a}
GaSI (%)	2.67±0.27	3.57±0.42	2.95±1.59	2.60±0.28

Values with different superscript letters are significantly different (P<0.05).

Table 3. Mean (±SD) values obtained for ACH50 (U mL^{-1}), lysozyme activity (µg mL^{-1}), total Ig (mg mL^{-1}), and dissolved protein (µg mL^{-1}) in skin mucus of *L. fuelleborni* juveniles were supplemented with different concentrations of *A. hirtifolium* essence for 60 days (n=3).

Skin mucus nonspecific immune parameters	0	0.10	0.15	0.20
ACH50 (U mL^{-1})	1.84±0.17^{a}	2.23±0.08^{b}	2.26±0.21^{c}	2.29±0.10^{b}
Lysozyme (U mg^{-1} protein)	2.86±0.36^{a}	3.22±0.43^{b}	3.97±0.33^{a}	3.55±0.34^{b}
Total Ig (mg mL^{-1})	2.52±0.09^{a}	2.91±0.10^{b}	3.73±0.10^{c}	3.33±0.11^{d}
Dissolved protein (µg mL^{-1})	0.19±0.02^{a}	0.23±0.09^{b}	0.32±0.02^{c}	0.25±0.03^{b}

Values with different superscript letters are significantly different (P<0.05).

presented as means±standard deviations (SDs). The obtained differences were accepted as significant at a threshold of P<0.05.

Ethical Statement: The experiment was run according to the law of animal ethics (IR.UM.REC.1400.018) in Ferdowsi University of Mashhad.

RESULTS

The results for growth parameters including final weight, SGR, HSI, GaSI, and survival rate of *L. fuelleborni* juveniles fed different concentrations of *A. hirtifolium* essence are presented in Table 2. HSI was found to increase with statistical significance (P<0.05) in the group that received *A. hirtifolium* essence at 0.10 mL kg^{-1} However, the inclusion of *A. hirtifolium* essence in the diet of the fish did not produce any significant effects on the final weight, SGR or GaSI of the fish (P>0.05). And the highest survival rate was found in the 0.20mL kg^{-1} treatment compared to other experimental groups.

At the end of the experiment, nonspecific parameters of immunity including alternative complement pathway haemolytic activity (ACH50), lysozyme activity, total Ig, and dissolved protein values of skin mucus were seen to be significantly heightened in all experimental groups in comparison to the control fish (P<0.05) (Table 3).

Table 4 presents the changes in the findings for AST, ALT, and ALP as liver enzymes among the experimental groups. Upon the conclusion of the experimental protocol, changes were not recorded in the levels of these enzymes (P>0.05).

Figures 1 and 2 present the TVCs of the experimental groups and data on the LAB and gram-negative bacteria in the intestines of *L. fuelleborni* after supplementation with *A. hirtifolium* essence. Findings for the intestinal microbiota are given as CFUs per gram of intestinal samples (CFU/g). These evaluations indicated that a significant increase occurred in the TVCs with the administration of 0.15mL kg^{-1} *A. hirtifolium* essence (P<0.05). However, gram-negative bacteria as evaluated by CFU/g were decreased in fish that received *A. hirtifolium* essence with statistical significance (P<0.05) and the minimum values were observed among the fish that had fed 0.20mL kg^{-1} *A. hirtifolium* essence. A similar pattern was observed for LAB, although, in this case, the minimum values were observed among the fish

Table 4. Mean (±SD) values obtained for liver enzyme activities of *L. fuelleborni* juveniles were supplemented with different concentrations of *A. hirtifolium* essence for 60 days (n=3).

	Different concentrations of dietary *A. hirtifolium* essence			
	0	0.10	0.15	0.20
AST (U L^{-1})	320.47±72.04	371.86±86.45	344.81±70.94	294.26±69.22
ALP (U L^{-1})	160.15±86.89	165.12±97.00	200.35±82.61	234.92±170.97
ALT (U L^{-1})	14.15±401	15.39±7.11	14.81±8.63	13.04±5.82

Values with different superscript letters are significantly different (P<0.05).

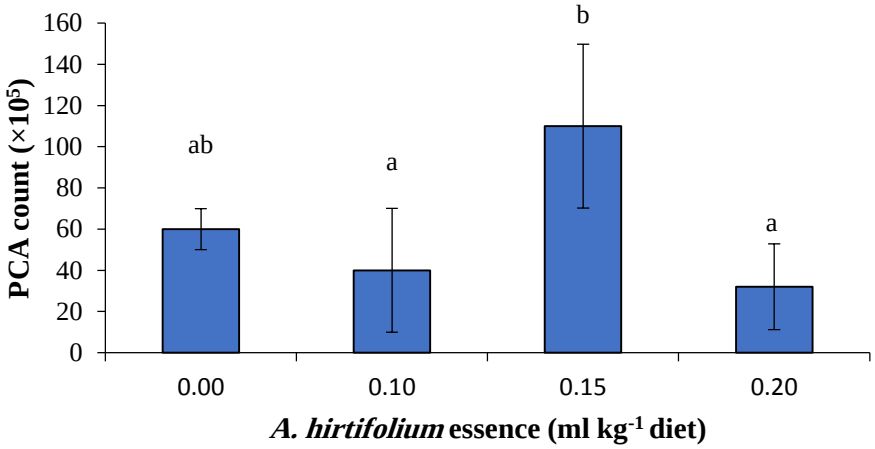

Fig.1. Total counts of *Labeotropheus fuelleborni* juveniles fed diets supplemented with *A. hirtifolium* essence at varying inclusion levels for 60 days. Statistical differences between treatment groups are indicated by different letters.

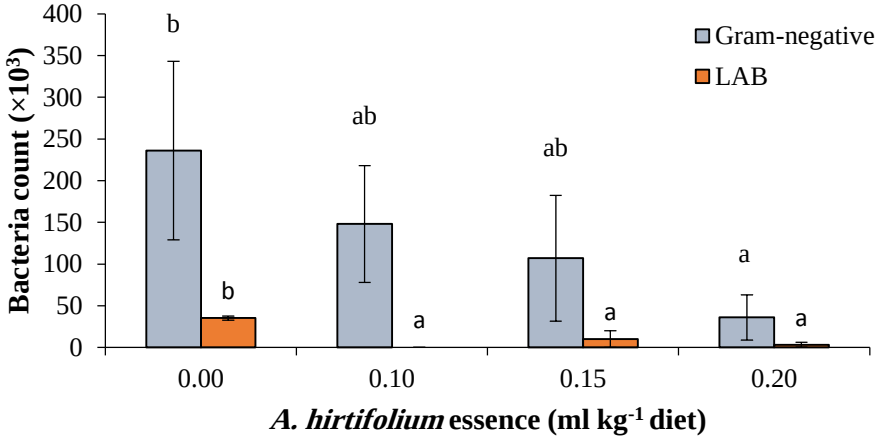

Fig.2. Bacteria counts of *Labeotropheus fuelleborni* juveniles fed diets supplemented with *A. hirtifolium* essence at varying inclusion levels for 60 days. Statistical differences between treatment groups are indicated by different letters.

that had fed treatment at 0.10mL kg^{-1} *A. hirtifolium* essence (P<0.05).

DISCUSSION

The present study has provided new data on the relationship between the dietary treatment of *L. fuelleborni* juveniles with *A. hirtifolium* essence and growth performance, biometric indexes, immune response, and intestinal microbiota. In this study, it was observed that the addition of *A. hirtifolium* essence to fish feed at doses of 0.10, 0.15, and 0.20mL kg^{-1} did not have significant effects on the growth performance of *L. fuelleborni*. Previous studies of the addition of *A. hirtifolium* to fish feed yielded different results regarding the effects of the treatment on growth performance. For example, when 1.5% *A. hirtifolium* extract was added to the diet of *C. carpio* (26.15±0.23g), an increase in growth performance as

reflected by the variables of FW, WG, SGR, and SR was observed (Rashidian et al. 2022b). In another study, 0.5%, 1%, and 2% *A. hirtifolium* extract was added to the feed of rainbow trout (*Oncorhynchus mykiss*) juveniles (25.33±0.15g) and increased growth performance (FW, WG, SGR, and SR) was achieved (Ghafarifarsani et al. 2022). Moreover, when 1% and 3% Persian shallot ethanolic extract was added to the basal diet, the growth performance (FW, WG, and SGR) of zebrafish (*Danio rerio*) increased and feed consumption decreased (Ghafarifarsani et al. 2021). It is thought that these differences between the studies in the literature are due to differences in the digestibility of the nutrients in the feed, the ratio of *A. hirtifolium* essence in the feed, the composition of the feed, the fish feeding conditions, and feed technologies.

HSI is an indicator of energy reserve status in animals and a helpful biomarker in efforts to detect dangerous effects of a variety of environmental factors (Lee et al. 2014). In the present study, we found that HSI values decreased in association with increasing doses of Persian shallot added to the feed. There is no previous study on the effects of using Persian shallot in fish or crustaceans on the levels of the HSI. However, similar to our study, a decrease in the HSI due to increasing doses of different forms of other plants from the same taxonomic family was obtained in some earlier studies (Hussein et al. 2016; Zaefarian et al. 2017; Zare et al. 2021 Gabriel et al. 2019). Researchers reported that the hydroalcoholic extract of shallots reduces liver cell damage and prevents fatty liver formation through hypoglycaemic and hypolipidaemic activities (Kazemi et al. 2010). Since lower levels of the HSI are associated with the ratio of liver fat, it can be said that the use of *A. hirtifolium* essence reduces liver fat in *L. fuelleborni* juveniles.

The GaSI is widely used to estimate the feeding intensity of fish. Its values change according to the season, feed content, maturity stage of the fish, and spawning period (Parihar et al. 2016). In our study, on the other hand, we found that the level of the GaSI did not change with the addition of *A. hirtifolium* essence. There are few studies addressing the effects of medicinal plants on the GaSI in fish. Different levels (0.2%, 0.4%, 0.8%, and 1.6%) of *Aloe vera* extract were added to *C. carpio* feed and it was determined that the GaSI increased (Khanal et al. 2021). In another study, it was reported that the GaSI increased when *Calotropis persica* seed powder was added to trout (*O. mykiss*) feeds at rates of 10, 20, 30, 40, and 50g kg^{-1} (Ahmadniaye Motlagh et al. 2019). The different results obtained in these studies may be due to differences in the plant and fish species used.

Phytobiotics are powerful immunostimulating factors and can increase skin mucus immunity (Srichaiyo et al. 2020; Mehrinakhi et al. 2021). ACH50, lysozyme activity, total Ig, and total protein exert important effects on the nonspecific immunity of fish and also act as protective factors against opportunistic pathogens (Magnadóttir 2006). In our study, the mucus lysozyme and ACH50 values of *L. fuelleborni* juveniles receiving feed supplemented with *A. hirtifolium* essence improved. Similar to our study, when the ethanolic extract of *Allium hirtifolium* was added to the feed of zebrafish (*Danio rerio*) at different rates, increases in total protein, lysozyme, and total Ig values were not ed in the skin mucus of the experimental animals (Ghafarifarsani et al. 2021). It was reported in earlier studies that Persian shallot extract added to feed at different rates increased ACH50, lysozyme activity, total Ig, and total protein levels in the skin mucus of common carp (*C. carpio*) and rainbow trout (*O. mykiss*) (Ghafarifarsani et al. 2021, 2022; Rashidian et al. 2022a,b). In a different study conducted in rainbow trout, Persian shallot powder added to the feed at rates of 0.5%, 1%, and 2% increased the total protein, lysozyme activity, and total Ig activities in the skin mucus after 56 days of feeding (Shekarabi et al. 2022). Thus, researchers have found that the inclusion of *A. hirtifolium* in fish diets improves mucosal immune responses due to the immunomodulatory properties of this plant.

ALT, AST, and ALP are aminotransferase enzymes produced within the liver and kidneys and increases in these particular enzymes signify the

presence of damage to the tissues (Huang et al. 2006). The present study demonstrated that the addition of *A. hirtifolium* essence to fish feed did not change the levels of these liver enzymes. Researchers previously reported that AST, ALP, and ALT enzyme activities decreased in trout (*O. mykiss*) and carp (*C. carpio*) fed diets that were supplemented with *A. hirtifolium* extract (Ghafarifarsani et al. 2022; Mahboub et al. 2022; Rashidian et al. 2022a). Antioxidant components such as those of Persian shallot, which contains high levels of polyphenols and flavonoids, are likely to stabilize cell membrane structures and guard the tissues against toxic damages created by free radicals, which will accordingly result in a reduction of the ALP, ALT, and AST levels (Omidifar et al. 2020). In this study, the finding that ALP, ALT, and AST enzyme activities did not change in the livers of *Labeotropheus fuelleborni* juveniles is a good indication that this administration of Persian shallot did not damage vital organs such as the liver and kidneys.

Herbal medicines can modulate the gut bacteria of fish by many mechanisms. Certain plants contain bioactive compounds such as essential oils, flavonoids, saponins, and alkaloids that have antimicrobial and immunomodulatory properties. These compounds can effectively control the population of pathogenic bacteria while supporting the growth of beneficial bacteria in the gut, resulting in an overall improvement in gut health and fish growth. Herbal medicines may also affect the gut microbiota indirectly by improving the secretion of digestive enzymes, increasing the levels of gastric acid, enhancing bile secretion, or promoting the absorption of nutrients. This can lead to better assimilation of dietary nutrients and, therefore, improved growth performance of fish (Ahmadniaye Motlagh et al. 2020b).

Plants of the genus *Allium*, which includes species of garlic, onion, and shallots, contain a variety of bioactive compounds with antibacterial and antioxidant properties (Ghafarifarsani et al. 2021, 2022; Safari & Paolucci 2017) that may be useful in modifying the gut bacteria of fish (Büyükdeveci et al. 2018; Zhou et al. 2022) and shrimp (Amoah et al. 2021; Lokesh et al. 2020). Alemayehu et al. (2018) found that the administration of garlic significantly improved the components of the gut microbiota, with increases in the relative abundance of beneficial species of bacteria such as those of *Lactobacillus* and *Bifidobacterium* alongside reductions in the abundance of pathogenic bacteria such as species of *Aeromonas* and *Pseudomonas*. Garlic supplementation also enhanced the immune response of tilapia by increasing the expression of genes involved in immune defence (Foysal et al. 2019). Overall, these studies suggest that the genus *Allium*, and particularly garlic, may be useful in modifying gut bacteria in fish and shrimp. However, further studies will be necessary for a full understanding of the mechanisms that drive these effects and the optimization of the use of these supplements in aquaculture practices. Allicin and garlic have been shown to have antimicrobial activity against enteric gram-negative bacteria. They act by targeting the cell membranes of these bacteria, which leads to disrupted membrane function and ultimately cell death (Ankri & Mirelman 1999; Fufa 2019). Several studies have reported that allicin and garlic extracts have antibacterial activity against various enteric gram-negative bacteria (Banerjee & Sarkar 2003), including *Escherichia coli* (Fujisawa et al. 2008), *Salmonella typhimurium* (Marques et al. 2008), and *Shigella flexneri* (Amer & Abd El-Rahman 2022). Additionally, allicin has been shown to inhibit the production of certain virulence factors in some enteric gram-negative bacteria, such as lipopolysaccharides and biofilm formation (Zhang et al. 2022).

LAB are a group of beneficial bacteria that produce lactic acid as a by-product of carbohydrate fermentation (Cui & Qu 2021). They are commonly found in the guts of fish and other animals, and they have been shown to play critical roles in nutrient absorption, immune modulation, and disease prevention (Ringø et al. 2020). There is limited research on how garlic specifically affects LAB in fish

gut microbiota. However, some studies have suggested that garlic and its bioactive compounds could potentially promote the growth of LAB and other beneficial bacteria in the fish gut microbiota through multiple mechanisms. For example, allicin and other sulphur-containing compounds in garlic have been shown to be responsible for increases in the production of short-chain fatty acids in the gut, which could create more favourable environments for LAB growth (Qiu et al. 2014; Ruhee et al. 2020; Afzaal et al. 2021; Mafra et al. 2021). Garlic could also stimulate the production of enzymes and mucus that protect the gut lining and support the growth of beneficial bacteria (Adibmoradi et al. 2006; Omotoso et al. 2012; Motta et al. 2015).

Limited scientific evidence is available regarding the effect of Persian shallot essence on gut microbiota. Overall, *Allium* species seem to have herbiotic properties by promoting the growth of beneficial gut bacteria in fish and other animals (Sunu et al. 2021), which could contribute to their overall health and disease resistance. However, the effects of garlic on different fish species and their gut microbiota could vary and further research is needed to fully understand the potential benefits and side effects.

CONCLUSIONS

Persian shallot (*A. hirtifolium*) is rarely applied in aquaculture and this study is the first to investigate the outcomes of administration of the essence of this plant for growth performance, biometric indexes, immune response, and intestinal microbiota in *L. fuelleborni* fry. Feeding fish diets supplemented with *A. hirtifolium* essence at up to 0.20mL kg^{-1} was found to be effective in reducing fatty liver and exerted remarkable immunomodulatory effects by improving mucosal immune responses. Furthermore, the results obtained from the microbiological analysis showed that *A. hirtifolium* essence supplementation significantly reduced the amount of LAB and gram-negative bacteria in the intestines of *L. fuelleborni* juveniles. Metagenomic studies are now required to elucidate the exact effects of dietary administration of *A. hirtifolium* essence on the gut microbiome in fish.

FUNDING

This work was supported by Ferdowsi University of Mashhad.

ACKNOWLEDGMENTS

The authors would like to thank the laboratory staff at the department of fisheries for their assistance.

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Iran. J. Ichthyol. (2023) 10(2): 79-92
P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

doi: 10.22034/iji.v10i2.962
dor: 20.1001.1.23831561.2023.10.2.7.1

مقاله کامل

اثر مثبت افزودن اسانس موسیر ایرانی (*Allium hirtifolium*) به جیره غذایی، بر باکتری‌های دستگاه گوارش و وضعیت سلامتی بچه‌ماهیان سیچلاید مارمالاد (*Labeotropheus fuelleborni*)

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چکیده: پژوهش حاضر با هدف بررسی اثرات افزودن اسانس موسیر ایرانی (*Allium hirtifolium*) در جیره غذایی بر رشد، فلور میکروبی روده، شاخص‌های رشد و پاسخ ایمنی در بچه ماهیان سیچلاید *Labeotropheus fuelleborni* انجام شد. بدین‌منظور، چهار جیره آزمایشی حاوی صفر، ۰/۱، ۰/۱۵ و ۰/۲۰ میلی لیتر در کیلوگرم اسانس تهیه شد و اثرات هر یک از آن جیره‌ها در قالب طرح های کاملاً تصادفی در طی ۶۰ روز مورد بررسی قرار گرفت. نتایج نشان داد افزودن اسانس *A. hirtifolium* به جیره غذایی ماهی تأثیری بر پارامترهای رشد نداشت، همچنین تفاوت معنی‌داری در شاخص امعاء و احشاء با تیمارهای مختلف مشاهده نشد ($P>0/05$). شاخص کبدی، در ماهیان تغذیه شده با جیره حاوی ۰/۲۰ میلی لیتر در کیلوگرم اسانس، به میزان معنی‌داری بالاترین بود ($P<0/05$). درصد بقا نیز در همین تیمار نسبت به گروه شاهد افزایش یافت ($P<0/05$). اسانس *A. hirtifolium* بر کل باکتری‌های هوازی تأثیری نداشت($P>0/05$)، اما تعداد باکتری‌های گرم منفی روده و باکتری‌های اسید لاکتیک در تیمارهای آزمایشی به‌صورت معنی‌داری کاهش یافت ($P>0/05$). مقادیر به‌دست‌آمده برای سطوح فعالیت کمپلمان، سطوح فعالیت لیزوزیم، ایمونوگلوبولین کل و پروتئین محلول در مخاط پوست ماهیانی که اسانس دریافت کردند، در مقایسه با گروه شاهد بالاتر بود ($P>0/05$). برای فعالیت آنزیم‌های کبدی شامل آلکالین فسفاتاز، آسپارتات آمینوترانسفراز یا آلانین آمینوترانسفراز هیچ تغییری ثبت نشد ($P>0/05$) نتایج حاصل از این آزمایش نشان می‌دهد که کاربرد موسیر ایرانی در آبزی‌پروری می‌تواند مفید باشد. حداکثر مقدار افزودن اسانس موسیر ایرانی به جیره بچه ماهیان *L. fuelleborni* را می‌توان بین ۰/۱۵ تا ۰/۲۰ میلی‌لیتر در کیلوگرم توصیه کرد.

کلمات کلیدی: *Allium hirtifolium*، آبزی‌پروری، موسیر ایرانی، *Labeotropheus fuelleborni*، باکتری های دستگاه گوارش.