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Research Article

Effects of Different Feeding Regimes on Growth Performance, Survival Rate, Carcass Composition, Fatty Acids Profile, and Digestive Enzyme Activities of Great Sturgeon (*Huso huso* Linnaeus, 1758) Larvae

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Six different feeding regimes (FR) were tested from 7 to 38 days post hatching (dph). Five thousand four hundred larvae (7 dph, 0.048 g) were randomly stocked in 18 fiberglass tanks contained 100 L fresh water (300 larvae per each tank). In larvae fed with FR-B, includes *Artemia* nauplii (AN) + *Artemia* biomass (AB) + microdiet (MD), the body weight (BW) at the end of trail (38 dph) similar the FR-D (AN + CHL + AB + MD), and E (AN + CHL + AB), was at high level. Survival rate was higher in larvae fed FR-A includes AN + chironomid larvae (CHL) + MD, but had a lower BW ($p < 0.05$). Moderate BW and survival rate were detected in larvae fed FR-C (AN + DP + MD). Larvae fed only MD (FR-F), had the lowest survival and the highest BW ($p < 0.05$). Different FR had a significant effect on crude protein (CP), crude fat (CF), ash, and dry weight ($p < 0.05$). The larvae fed FR-C and D had the highest CP ($p < 0.05$). The highest CF and ash in larvae were detected in FR-F and C, respectively ($p < 0.05$). Larvae from FR-A and B had higher levels of $n-3$ long chain polyunsaturated fatty acids in comparison with others FR ($p < 0.05$). The use of AB and CHL can increased the weight and survival rate of larvae, respectively. At the end of trail, the activity of pepsin, trypsin, lipase and α-amylase enzyme levels were lower in FR-B and F than others FR. Totally, by using a combination of AN + CHL + AB + MD had better results than others and ordered to *H. huso* larvae feeding. Using MD alone, can cause lesser development of the digestive system and little attractiveness, therefore it can cause starvation, and the empty digestive tract of larvae.

1. Introduction

Continued efforts have been made to determine the best foods for sturgeon larvae. In rearing some fish larvae in hatcheries, changing the type of diet can be challenging. The time of change, often refers to the cofeeding or weaning period and may be between two type of live feed or from live feeds (LF) to formulated diet [1]. One of the biggest problems is the low survival rate of sturgeon larvae. In this regard, many live and artificial feed have been used. Despite all efforts, a single feeding strategy for sturgeon larvae has not yet been developed. Although LF have been successfully used for the larvae of a number of fish (i.e., Siberian sturgeon, *Acipenser baerii*) there is no universal weaning strategy for fish larvae and this is a species-specific matter and depends on the development of the digestive system [2]. The use of LF has its own problems and in most cases, they are expensive and difficult to prepare. In addition, beside the high costs, LF may be increases the risk of disease transmission [3]. Despite the problems mentioned for LF, not using them to feed the larvae, leads to a severe reduction in larval survival. However, the use of cheaper and more efficient LF has always been one of the goals. Long-term use of LF is costly and may be problematic, especially when it lacks sufficient amounts of

nutrition for larval growth and development [4]. The effects of feeding strategy on the growth of larvae is important, but it is less critical than survival [5].

Larval feeding success depends on the species. Reduce the use of LF has always been a problem. Most efforts are made in the field of microdiet to improve the flavor, size, and quality of nutrients [1]. The ability of fish larvae to grow and survive, eventually leads to stability and an annual population of fish [6]. Larvae and egg production are important items in the aquatic production chain. In the meantime, the role of nutrition and feeding is more obvious and in general, live foods are more suitable for the initial feeding of larvae. Fish feeding is the costliest in aquaculture.

The type of feeding regimes (FR) for rearing fish larvae is a fundamental problem and determining feeding protocols based on the state of the larvae's digestive system is a vital issue [7]. Changing the type of feed is a critical issue and the best feeding strategy is to pay attention to several parameters (growth, survival, size, and quality) that directly affect the fingerlings in the during the growth period [8]. Rearing fish larvae mainly depends on the availability of suitable feed with optimum consumption and digestion; this can lead to high growth and good health of larvae [9]. LF improve survival and growth in various ways. Their enzymes, secretion of metabolite, and their movement have a major impact on their nutritional response and can improve the digestion of MD [10]. The use of MD immediately after the start of exogenous feeding can result in the low ability of larvae in digestion, unbalanced composition, undesirable physical and palatable properties, or the lack of mobility of food particles, often causes a decrease in growth and survival. Therefore, for most fish larvae, it is suggested to use a combination of LF an MD (cofeeding) to increase the success of larvae feeding. Of course, the morphogenesis and physiology of larval digestive tract might be affected by the cofeeding method [2]. In addition, in confirmation of this issue, it has been reported that the early use of MD can cause detrimental effect on fish growth performance and survival [7]. When the sturgeon larvae start exogenous feeding, their digestive system and enzymes are ready and they are anatomically complete [8, 11]. A short period of nutritional poverty after yolk sac absorption can lead to abnormal behavior, morphological development, deterioration of the condition of the digestive system and muscle tissue, food consumption coefficient, growth, and nutritional activity [12]. Feed consumption by fish depends on factors such as food and consumer size, density, physical attractiveness, and access to food and also depends on how the food is accessed [9]. The effect of rearing strategy on larval growth is also an important parameter although it may not be as critical as survival [5]. White worms (Enchytraeus albidus Henle, 1837) have long been used to feed sturgeon larvae. However, its maintenance problems eliminated its use. Daphnia is also still used to feed sturgeon larvae in some hatcheries due to its ease of supply. Nevertheless, it also be noted that LF are susceptible to protozoan, viral or bacterial contamination [4]. Tubifex has also been used to feed Russian sturgeon (Acipenser gueldenstaedtii) larvae [13].

TABLE 1: Microdiet composition (% of dry matter) used for different feeding strategies of great sturgeon larvae (Huso huso).

Proximate	Composition (%)
Crude protein	48.78
Crude fat	17.31
Ash	16.06
Fiber	2.04
Moisture	5.85
Ingredients	Fish meal, wheat gluten, soybean lecithin, fish oil, canola oil, agar (as binder), soybean meal, mineral, and vitamins

Data were determined through diet analysis.

Important aspects of hatchery management are feeding strategy, survival, and quality of larvae [8]. Successful rearing of larvae is due to factors such as the use of feed with proper digestibility and absorption, and the provision of nutrients to ensure optimal growth and survival. In addition, attention should be paid to the frequency of feeding, the type and size of feed [14]. In connection with the limiting factors of the use of microdiets is the presence of binders and proteins in them that make them difficult to digest and absorb. Conversely, amino acids are freely available in live feed and facilitate larval digestion [15]. Larval stage is one of the most critical stages of fish [3]. Combined feeding of fish larvae with live feed and microdiets can reduce the problems related to the consumption and digestion of microdiets [2, 16]. There is no universal strategy for feeding fish larvae [2]. Production of high-quality fingerlings is one of the most important factors in achieving success in fish farming [17]. Live feed can improve larval survival and growth. The enzymes in Artemia nauplii (AN) and its movement in the digestive system may improve the digestion of formulated feed [10]. However, Artemia may be deficient in some fatty acids [1]. Digestive enzymes are important in term of nutrient digestion. The activity level of digestive enzymes is a useful indicator of food utilization, digestibility, and growth performance of fish [18]. The purpose of the study was to determine the impact of feeding with LF and microdiet on growth performance, survival rate, carcass composition, fatty acids profile, and digestive enzyme activities in other to introduce the best FR in great sturgeon larvae.

2. Materials and Methods

2.1. Experimental Condition and Feeding Regimes. One thousand eight hundred larvae (7 dph, 0.048 g) were randomly stocked in 18 fiberglass tanks (103 cm lenght × 100 wide × 50 cm height) contained 100 L UV treated fresh well water (300 larvae per each tank). Larvae were reared in a flow through system (3 L min^{-1} in each tank) and each tank was equipped with an air-stone to supply oxygen and keep food suspended. The MD used include, fishmeal, wheat gluten, soybean lecithin, fish oil, canola oil, agar (as binder), soybean meal, mineral, and vitamins (Table 1). Water temperature, dissolved oxygen, and pH were (mean ± SD) 18.19 ± 0.46°C, 8.32 ± 0.34 mg L^{-1} and 7.31 ± 0.06, respectively during the research.

Six different FR (dietary treatments) by three replicate in each and by the changing the type of food as following, used to determine the best feeding strategies. The larvae were fed 12 times a day (every 2 hr from 08 : 00 to 08 : 00). Different FR were tested from 7 to 38 dph as following:

(1) Larvae were fed with live AN (7–25 dph) + frozen chironomid larvae (CHL) (14–25 dph) + microdiet (14–38 dph).

(2) Larvae were fed with live AN (7–25 dph) + frozen *Artemia* biomass (AB) (14–25 dph) + microdiet (14–38 dph).

(3) Larvae were fed with live AN (7–25 dph) + live *Daphnia* (14–38 dph) + microdiet (14–38 dph).

(4) Larvae were fed with live AN (7–25 dph) + frozen chironomid larva and frozen AB (14–25 dph) + microdiet (14–38 dph).

(5) Larvae were fed with live AN (7–25 dph) + frozen chironomid larva and frozen AB (14–25 dph).

(6) 100% microdiet (7–38 dph).

Larvae in FR 1–3, were fed with AN, CHL, AB, and *Daphnia*, each one 30%–10% of body weight (BW) of larvae (wet weight) per day from the feeding starts until it ends, respectively. In FR 4 and 5, larvae were fed with AN, CHL, and AB, each one 30%–10%, 15%–5%, and 15%–5% of BW of larvae (wet weight) per day, from the feeding starts until it ends, respectively. In addition, larvae were fed with microdiet from the beginning to the end of larval rearing period, at the rate of 20%–5% of BW of larvae per day, respectively.

2.2. *Larvae Supply Source.* The study was conducted in the aquaculture department of the International Sturgeon Research Institute, Guilan–Rasht (Iran). Eggs were obtained through artificial breeding of a female (44.5 kg) of great sturgeon (*H. huso*). For fertilization of eggs, sperm of three males (22.3–32.7 kg) of great sturgeon were used. Hormone injections were performed at two stages for female (LHRH-A2, $5 \mu g \, kg^{-1}$) and one stage for male (LHRH-A2, $4 \mu g \, kg^{-1}$). Female was injected at interval of 9–12 hr (water temperature 12–14°C) and in the rate of 10% and 90%, respectively. Males were injected (water temperature 13–16°C) with the second female injection. To fertilize, the eggs and sperm were stirred for 5 min. To remove the adhesion of the egg, 80 g of Kaolin (soft white clay) were dissolved in 4 L of water and poured on 1 kg of eggs. The eggs were stirred in water containing kaolin for 1 hr. During 1 hr, water-containing Kaolin was drained several times and water containing new Kaolin clay was adding. After 1 hr, the eggs were washed with fresh water and then transferred to McDonald's incubators. After 7 dph, 5,400 larvae were randomly selected and stocked in research tanks.

2.3. *Sampling and Growth Determination.* At all stages of sampling, larvae were euthanized using an overdose of MS-222 ($500 \, mg \, L^{-1}$) and rinsed with deionized water to collect the samples [19]. Sampling of larvae were done to determine the mean BW, mean length and survival rate of larvae by changing the FR on 7, 18, 25, and 37 dph. For this purpose, 10 larvae were randomly selected at each stage and the average larval weight was determined using a digital scale (accurately 0.001 g) and total length (TL) of randomly selected larvae was individually measured using a biometric ruler with an accuracy of 1 mm [2]. Larvae after sampling rinsed with distilled water and frozen at −70°C. The specific growth rate (SGR) by changing the type of FR (7, 18, 25, and 38 dph) was determined by using the following formula: SGR (% day^{-1}) = $[(\ln W_2 - \ln W_1)/ \, t] \times 100$; where t is experimental period = 31 days. Condition factor (CF) (g/cm^3) = $100 \times (W_2 \, (g)/L_2^3 \, (cm))$. Where W_1 and W_2 are initial and final larval weights, respectively; L2: final body length [20]. Final survival was determined by counting the fish surviving at the end of the research and the number of fish sampled during the research was considered.

2.4. *Fatty Acid Profile Analysis.* At the end of research (38 dph) the 10 whole body larvae per each tank were sampled for evaluating fatty acid profile. To determine the fatty acid profile of the whole larvae, decanter was used to extract fat from the sample. The solvent (chloroform) was then added to the fat and the glass containers containing the solvent were placed in hot water bath. As a result, the solvent evaporated and eventually the fat remained [21]. Firestone [22] method was used to esterify fat. A gas chromatographic device equipped with a capillary column and a flame ion detector was used to investigate and identify the fatty acids in the sample. By comparing the inhibition time of chromatograms, the unknown sample with the chromatograms obtained from the standard solution of methyl fatty acids, fatty acids in fish muscle were identified as a percentage.

2.5. *Carcass Composition Analysis.* Thirty-eight days post hatching, the 20 whole body larvae per each tank were sampled for evaluating moisture, dry matter, crude protein (CP), ash, and crude fat (CF) was performed using the Association of Official Analytical Chemists [23]. To measure the moisture of the sample, the minced sample was placed in the oven and after cooling, the weight of the sample was measured again and the amount of moisture was determined. To determine the percentage of dry matter, the samples were first weighed before drying and then dried for 60 min at 60°C. After transfer to the desiccator, the dry matter percentage was calculated by the weight difference. The protein content of the samples was determined by Kjeldal method with automatic Kjeldal device (Kjeltec Analyzer Unit 2300). To determine the ash, the dried powder sample was burned for 4 hr at a temperature of 500–550°C. A Soxhlet set was used to determine the fat content of the samples.

2.6. *Digestive Enzyme Analysis.* For larvae with higher than 32 dph, the head and tail of 10 larvae per each tank, were separated (through cutting) and the rest was used to measure the enzyme activity. For younger larvae, 20 whole body larvae were used to measure enzyme activity [24]. Larvae were sampled before the first daily feeding in the morning [25]. Samples were immediately frozen in liquid nitrogen and kept at −80°C until analyses. Samples were homogenized (1 : 10 w/v) of 0.15 M NaCl solution. The larval homogenates were centrifuged (model 5415 D Eppendorf Japan) at $15,000 \times g$ for 15 min at 4°C. Supernatant containing crude enzyme extract

was isolated and divided into 1 mL microtubes to measure each enzyme activity, and stored at −70°C. Determining the protein concentration of the samples was done using the Bradford [26] method. Torrissen et al. [27] method was used to measure trypsin enzyme activity (benzoyl-L-arginine-p-nitroanilide as substrate). α-amylase enzyme activity (starch as substrate) was determined on Bernfeld [28] method. The activity of lipase and pepsin enzymes (p-nitrophenyl myristate and hemoglobin as substrate, respectively) was measured using Iijima et al. [29] and Anson [30] methods, respectively. All enzymes activities were measured by spectrophotometer spectroscopy (model 6505 Jenway England) and declared as specific (U mg protein^{-1}). It is the micro moles of product formed by an enzyme in a given amount of time (minutes) under given conditions per milligram of total proteins.

2.7. Statistical Analysis. The study was carried out in a completely randomized. The assessment of normality and homogeneity of the data were done by the Kolmogorov–Smirnov's and Levene's tests, respectively. Effects of different days' post hatching, and different FR (dietary treatments) were considered as two independent factors, and their mutual effects on digestive enzyme activity, carcass fatty acid, and growth performance were analyzed using two-way ANOVA. Significant effects of independent factors were determined through one-way ANOVA and Duncan is multiple range test. Data were presented as mean ± SD. The SPSS ver. 20.0 was used to analyze the data. All tests used a significance level of $p < 0.05$.

3. Results

3.1. Larval Growth Performance and Survival Rate. The data related to the effect of different FR on larval survival is presented in Table 2 and Figure 1. Changing the FR significantly affected larval survival at 14, 25, 32, and 38 dph ($p < 0.05$). At 14 dph, no significant difference was detected between FR ($p > 0.05$). From 14 to 25 dph, significant decrease in the survival rate of the larvae was detected but survival rate from 25 to 38 dph decreased with a smaller proportion (Table 2 and Figure 1). At 25 and 32 dph, the lowest survival rate was recorded in FR-F (13.01 ± 1.34, 10.8% ± 0.53%, respectively). At the end of trail (38 dph), the highest survival was observed in FR-A (27.33% ± 2.67%) with a statistically significant difference with other FR ($p < 0.05$). The lowest survival rate was detected in FR-F (100% MD, 3.71 ± 0.60) followed by FR-E and B (14.66 ± 1.33 and 17.50 ± 2.50, respectively, $p < 0.05$). FR-C and D had intermediate values (22.66 ± 2.40, 21.02 ± 1.54, respectively; $p > 0.05$).

Different FR had an effect on the BW of larvae (Table 2). At 25 dph, the weight of larvae, in FR, A, and E was significantly lower than others FR ($p < 0.05$). At 32 and 38 dph the lowest BW was observed in FR-A (0.52 ± 0.03 and 0.741 ± 0.041 g, respectively; $p < 0.05$). The highest BW observed in FR, D, C–D, and F at 25, 32, and 38 dph, respectively ($p < 0.05$).

Changing the FR especially at 32 and 38 dph, caused significant changes in the TL of larvae in some FR (Table 2). At 14 dph, no significant difference was measured between different FR ($p > 0.05$). From 25 to 32 dph, the highest TL

was detected in FR-D (4.25 ± 0.41 and 5.94 ± 0.04 cm, respectively). At the end of trail (38 dph), the lowest and highest TL, was detected in FR-A and F (6.09 ± 0.05 and 7.34 ± 0.12 cm, respectively; $p < 0.05$).

The data related to specific growth rate (SGR) in different dph are presented in Table 3. Changing the FR significantly affected larval SGR at 25, 32, and 38 dph ($p < 0.05$). No significant differences were between different FR at 14 dph ($p > 0.05$). From 25 to 32 dph, the highest SGR was detected in FR-D (11.945% ± 0.207%) and FR, C, D, and E (10.333 ± 0.025, 10.271 ± 0.158 and 10.249% ± 0.150%), respectively. At the end of trail, the highest SGR was in FR-F (10.421% ± 0.135%) with significant difference with other FR ($p < 0.05$). The lowest SGR was detected in FR-A followed by FR-C (8.805 ± 0.281 and 9.20% ± 0.191%, respectively; $p < 0.05$). Others FR had intermediate values.

Changing the feeding strategies affected larval CF at 25, 32, and 38 dph (Table 3; $p < 0.05$). The effects of FR on the CF were greater at 32 and 38 dph. The lowest CF from 25 to 32 dph was detected in FR-D (0.557 ± 0.142, 0.300 ± 0.001, respectively). At the end of trail (38 dph), the lowest CF, was detected in FR-F followed by FR-B and C (0.311 ± 0.002, 0.325 ± 0.014 and 0.328 ± 0.017, respectively; $p > 0.05$).

3.2. Larval Carcass Analysis (CA). The data related to carcass analysis (CA) in different dph are presented in Table 4. Changing the feeding strategies affected larval CA ($p < 0.05$). The highest CP, was detected in FR-C and D (66.17% ± 0.03% and 66.07% ± 0.03%, respectively), and the lowest CP, was detected in FR-A and F (63.64 ± 0.02 and 63.84% ± 0.02%, respectively) which had significant difference with the rest FR ($p < 0.05$). The FR-B and E, had almost intermediate values ($p < 0.05$). The highest CF was detected in FR-F (14.17 ± 0.025), and the lowest CF, was recorded in FR-E and C (9.21 ± 0.015 and 9.25% ± 0.03%, respectively), with significant difference with others FR ($p < 0.05$). The rest FR (D, B and A), had intermediate values ($p < 0.05$). The higher ash, was observed in FR-C (12.21% ± 0.015%), which had significant difference with the rest FR ($p < 0.05$). The lowest ash, was detected almost similarly to FR-A and B (10.34% ± 0.02% and 10.33% ± 0.02%, respectively; $p > 0.05$). The rest FR (D, E, and F) had almost intermediate values ($p < 0.05$). The highest dry weight (DW), was observed in FR-D (95.65% ± 0.15%), and the lowest DW, was detected in FR-B (90.93% ± 0.03%) followed by FR-A (91.32% ± 0.02%), which had significant difference with the rest FR ($p < 0.05$). The FR-C, E and F, had almost intermediate values ($p < 0.05$).

3.3. Larval Fatty Acid Composition. Changes in fatty acid composition (percentage/100 g larval sample, mean ± SD, $n = 3$) of great sturgeon larvae with different FR in the end of trail (38 dph) are presented in Table 5. Changing the FR, affected larval fatty acid composition ($p < 0.05$). Feeding the larvae with different FR, caused statistically significant differences in terms of most saturated fatty acids (C10:0, C12:0, C15:0, and C18:0). Some FR did not cause significant differences in other unsaturated fatty acids (C16:0, C17:0, and C24:0), of larvae ($p > 0.05$). Totally, the average value of saturated fatty acids in the FR-D were more than

TABLE 2: Changes survival, body weight and total length of great sturgeon larvae with different feeding regimes at different days post hatching (dph).

Feeding regimes	Survival (%)				BW (g)				TL (cm)			
	14 dph	25 dph	32 dph	38 dph	14 dph	25 dph	32 dph	38 dph	14 dph	25 dph	32 dph	38 dph
A (AN + CHL + MD)	86.83 ± 3.94^a	38.22 ± 2.33^d	34.66 ± 2.53^d	27.33 ± 2.67^e	0.0760 ± 0.001^a	0.296 ± 0.014^a	0.520 ± 0.030^a	0.741 ± 0.041^a	2.42 ± 0.015^a	3.39 ± 0.05^a	5.48 ± 0.14^a	6.09 ± 0.05^a
B (AN + AB + MD)	89.99 ± 2.33^a	25 ± 20^b	23.83 ± 2.83^b	$17.5 \pm 2.15b^c$	0.0743 ± 0.003^a	0.354 ± 0.014^b	0.590 ± 0.030^b	1.066 ± 0.034^c	2.41 ± 0.015^a	3.69 ± 0.01^a	5.66 ± 0.04^{ab}	6.98 ± 0.18^d
C (AN + DP + MD)	87.33 ± 3.0^a	33.22 ± 3.90^c	30.99 ± 2.03^{cd}	22.66 ± 2.40^d	0.0765 ± 0.001^a	0.335 ± 0.012^b	0.640 ± 0.020^c	0.838 ± 0.023^b	2.42 ± 0.005^a	3.54 ± 0.04^a	5.70 ± 0.02^b	6.48 ± 0.18^b
D (AN + CHL + AB)	87.33 ± 1.9^a	31.66 ± 1.66^c	28.49 ± 3.16^c	21.02 ± 1.54^{cd}	0.0753 ± 0.002^a	0.415 ± 0.014^c	0.630 ± 0.010^c	1.045 ± 0.044^c	2.46 ± 0.005^a	4.25 ± 0.41^b	5.94 ± 0.04^c	6.77 ± 0.11^{cd}
E (AN + CHL + AB)	88.66 ± 2.73^a	25.83 ± 2.5^b	22.49 ± 1.16^b	14.66 ± 1.33^b	0.0736 ± 0.003^a	0.281 ± 0.007^a	0.635 ± 0.005^c	1.044 ± 0.027^c	2.42 ± 0.010^a	3.45 ± 0.19^a	5.80 ± 0.10^c	6.63 ± 0.17^{bc}
F (MD)	86.67 ± 2.45^a	13.10 ± 1.34^a	10.80 ± 0.53^a	3.71 ± 0.61^a	0.0743 ± 0.001^a	0.333 ± 0.008^b	0.590 ± 0.010^b	1.230 ± 0.022^d	2.43 ± 0.010^a	3.51 ± 0.03^a	5.59 ± 0.21^{ab}	7.34 ± 0.12^e

Abbreviations: AN, *Artemia* nauplii; CHL, chironomid larvae; MD, Microdiet; BW, body weight; TL, total length; dph, days post hatching. Data are mean $\pm$ SD. [a,b,c,d,e]Different superscript letters within a column indicate significant differences ($p < 0.05$).
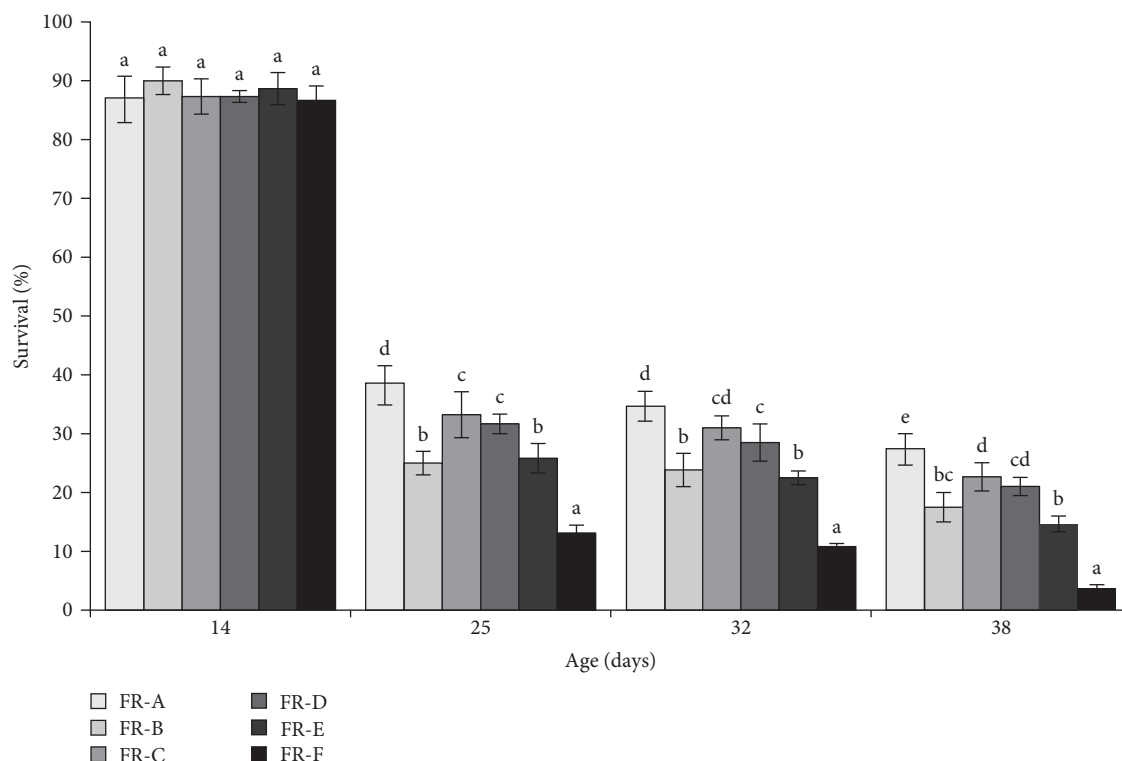
FIGURE 1: The survival rate (%) of *H. huso* larvae under different feeding regimes at different days after hatching. Data are mean $\pm$ SD. [a,b,c,d,e] Different superscript letters within a column indicate significant differences ($p < 0.05$).

other FR ($p < 0.05$). In terms of monounsaturated fatty acids (C14:1, C15:1, C17:1, C20:1, C18:1 ($n-9$) C, and C18:1 ($n-11$), no significant difference was observed in some of larvae FR ($p > 0.05$). Average of total monounsaturated fatty acids, in larvae FR-E was higher than the rest larvae FR ($p < 0.05$). In larvae FR-C, followed by FR-E and F had the highest monounsaturated fatty acids ($p < 0.05$). In terms of polyunsaturated fatty acids (PUFA), significant differences were observed in most of the FR ($p < 0.05$). The average of unsaturated fatty acids, in larvae FR-A followed by FR-B was higher than the rest FR ($p < 0.05$).

3.4. Larval Digestive Enzyme Activity. The data related to some digestive enzymes in different dph are presented in Figures 2–5. The average pepsin enzyme activity in different FR (Figure 2), increased more than 9.4 times, from 31 to 38 dph, while the average increase in pepsin activity from 14 to 31 dph was 3.4 times. At the end of trail, the highest pepsin activity was detected in FR-A (2730.364 $\pm$ 287.1) followed by FR, E, and D (2028.706 $\pm$ 72.47, and 2171.043 $\pm$ 86.35, respectively). The lowest pepsin activity was observed in FR-B (566.911 $\pm$ 34.94) followed by FR-F (1041.27 $\pm$ 34.91).

The average trypsin enzyme activity in different FR (Figure 3), increased 5.08 times (from 14 to 38 dph), 3.73 times (14–31 dph), and 1.35 times (31–38 dph). At the end of trail, the highest trypsin activity was observed in FR-D (28.471 $\pm$ 3.809), followed by FR, C, A, and E (27.833 $\pm$ 7.524, 24.426 $\pm$ 6.344, and 21.615 $\pm$ 0.878, respectively). The lowest trypsin activity was detected in FR-B (7.748 $\pm$ 1.981) followed by FR-F (14.859 $\pm$ 2.06).

The average activity of lipase enzyme in different FR (Figure 4), increased 2.63 times, from 14 to 38 dph. The average lipase enzyme activity decreased from 14 to 25 dph and 14–31 dph, 4.11 and 6.63 times, respectively. At the end of trail (38 dph), the highest lipase activity was observed in FR-A (607.263 $\pm$ 15.00) and the lower lipase activity was detected in FR-B (106.515 $\pm$ 11.676) followed by FR, C and F (222.102 $\pm$ 27.038 and 235.735 $\pm$ 18.06).

The average α-amylase enzyme activity in all FR (Figure 5), increased 6.22 (14–25 dph) and 6.02 times (14–38 dph). At the end of trail (38 dph), the lowest α-amylase enzyme activity was observed in FR-B (46.039 $\pm$ 9.697) followed by FR-F (123.823 $\pm$ 8.420). At 38 dph, the highest activity of α-amylase was observed in FR-E (195.57 $\pm$ 11.670) with significantly difference with others FR ($p < 0.05$). But in this period (38 dph) FR, A, B, C, and D had intermediate values.

4. Discusion

In the present study, according to the different feeding strategies, changes were determined in different days after hatching of larvae (e.g., 14, 25, 31, and 38 dph). One of the important things in rearing fish larvae is the survival rate of larvae [2]. In the present study, the average decrease in the survival rate of all FR, from 25 to 31 dph, was no significant (about 2.50%). Meanwhile, the average decrease in survival rate of all FR, from 14 to 25 dph was much higher (44.50%). This case is in line with the report of Gisbert et al. [8], that the majority of larval deaths occur in 9 and 18 dph. Furthermore, in the present study, the average decrease in survival rate of total FR,

TABLE 3: Changes specific growth rate (SGR) and condition factor (CF) of great sturgeon larvae with different feeding regimes at different days post hatching (dph).

Feeding regimes	SGR				CF			
	14 dph	25 dph	32 dph	38 dph	14 dph	25 dph	32 dph	38 dph
A (AN + CHL + MD)	6.469 ± 0.0266^a	10.065 ± 0.436^a	9.499 ± 0.110^a	8.805 ± 0.281^a	0.534 ± 0.017^a	0.761 ± 0.069^b	0.315 ± 0.005^{ab}	0.326 ± 0.026^{ab}
B (AN + AB + MD)	6.442 ± 0.705^a	11.177 ± 0.396^b	10.089 ± 0.081^c	10.046 ± 0.019^c	0.527 ± 0.030^a	0.704 ± 0.022^b	0.325 ± 0.009^{ab}	0.325 ± 0.014^a
C (AN + DP + MD)	6.562 ± 0.726^a	10.760 ± 0.380^b	10.333 ± 0.023^d	9.204 ± 0.191^b	0.536 ± 0.007^a	0.755 ± 0.002^b	0.345 ± 0.014^b	0.328 ± 0.017^a
D AN + CHL + AB)	6.339 ± 0.475^a	11.945 ± 0.207^c	$10.271 \pm 0.158c^d$	9.915 ± 0.195^c	0.502 ± 0.015^a	0.557 ± 0142^a	0.300 ± 0.001^a	0.335 ± 0.002^{ab}
E (AN + CHL + AB)	5.821 ± 1.096^a	9.711 ± 0.323^a	$10.249 \pm 0.150c^d$	9.869 ± 0.116^c	0.519 ± 0.022^a	0.691 ± 0.096^b	0.325 ± 0.014^{ab}	0.335 ± 0.018^b
F (MD)	6.057 ± 0.655^a	10.686 ± 0.239^b	9.982 ± 0.165^b	10.421 ± 0.135^d	0.518 ± 0.017^a	0.769 ± 0.001^b	0.329 ± 0.032^b	0.311 ± 0.020^a

Abbreviations: SGR, specific growth rate; CF, condition factor. [a,b,c,d]Different superscript letters within a column indicate significant differences ($p < 0.05$).

TABLE 4: Changes in carcass analysis of great sturgeon larvae with different feeding regimes at the end of trail (38 dph).

Feeding regimes	Crude protein (%)	Crude fat (%)	Ash (%)	Dry matter (%)
A (AN + CHL + MD)	63.64 ± 0.02[a]	10.935 ± 0.015[b]	10.340 ± 0.020[a]	91.320 ± 0.020[b]
B (AN + AB + MD)	64.21 ± 0.03[b]	12.065 ± 0.025[c]	10.330 ± 0.020[a]	90.830 ± 0.030[a]
C (AN + DP + MD)	66.17 ± 0.03[d]	9.250 ± 0.030[a]	12.215 ± 0.015[c]	94.340 ± 0.030[c]
D (AN + CHL + AB)	66.07 ± 0.03[d]	13.240 ± 0.020[d]	10.930 ± 0.030[a]	95.650 ± 0.150[d]
E (AN + CHL + AB)	65.46 ± 0.02[c]	9.215 ± 0.015[a]	11.775 ± 0.015[b]	94.310 ± 0.190[c]
F (MD)	63.84 ± 0.02[a]	14.175 ± 0.025[e]	10.060 ± 0.020[a]	94.735 ± 0.035[c]

Abbreviations: AN, *Artemia* nauplii; CHL, chironomid larvae; MD, Microdiet. Data are mean ± SD. [a,b,c,d]Different superscript letters within a column indicate significant differences ($p < 0.05$).

TABLE 5: Changes in fatty acid composition of great sturgeon larvae with different feeding regimes at the end of trail (38 days post hatching).

Fatty acids	Feeding regimes					
	A	B	C	D	E	F
C10:0	0.333 ± 0.015[b]	0.185 ± 0.004[a]	0.950 ± 0.02[f]	0.480 ± 0.01[c]	0.631 ± 0.010[d]	0.785 ± 0.005[e]
C12:0	0.228 ± 0.004[c]	0.115 ± 0.091[a]	0.227 ± 0.138[d]	0.492 ± 0.162[e]	0.315 ± 0.221[b]	0.463 ± 0.245[e]
C15:0	0.490 ± 0.010[a]	0.630 ± 0.010[b]	0.945 ± 0.045[c]	0.955 ± 0.005[c]	0.645 ± 0.015[b]	0.615 ± 0.005[b]
C16:0	18.90 ± 0.010[b]	16.750 ± 0.600[a]	19.255 ± 0.055[b]	19.775 ± 0.075[c]	18.965 ± 0.095[b]	17.160 ± 0.060[a]
C17:0	0.720 ± 0.010[b]	0.700 ± 0.010[b]	0.700 ± 0.010[b]	0.735 ± 0.005[b]	0.625 ± 0.005[a]	0.650 ± 0.050[a]
C18:0	6.525 ± 0.025[d]	6.400 ± 0.100[c]	6.365 ± 0.065[c]	5.810 ± 0.080[b]	5.785 ± 0.035[b]	4.710 ± 0.060[a]
C20:0	0.383 ± 0.015[d]	0.360 ± 0.010[c]	0.225 ± 0.015[a]	0.245 ± 0.005[a]	0.315 ± 0.005[b]	0.455 ± 0.015[e]
C24:0	0.250 ± 0.010[d]	0.145 ± 0.005[c]	0.000[a]	0.000[a]	0.000[a]	0.0250 ± 0.010[b]
C14:0	1.693 ± 0.015[a]	1.675 ± 0.015[a]	1.850 ± 0.050[b]	4.535 ± 0.015[e]	3.900 ± 0.070[d]	2.025 ± 0.015[c]
C14:1	0.200 ± 0.010[b]	0.360 ± 0.010[d]	0.360 ± 0.010[d]	0.360 ± 0.010[d]	0.150 ± 0.010[a]	0.310 ± 0.01[c]
C15:1	0.175 ± 0.005[d]	0.160 ± 0.01[c]	0.060 ± 0.010[b]	0.560 ± 0.010[e]	0.000 ± 0.000[a]	0.050 ± 0.010[b]
C17:1	0.630 ± 0.010[d]	0.853 ± 0.015[e]	0.415 ± 0.005[b]	0.475 ± 0.015[c]	0.380 ± 0.010[a]	0.650 ± 0.030[b]
C20:1	0.165 ± 0.005[a]	0.145 ± 0.005[a]	0.125 ± 0.005[a]	0.000 ± 0.000[a]	0.120 ± 0.010[a]	0.540 ± 0.440[b]
C20:2	1.070 ± 0.030[a]	0.812 ± 0.578[a]	1.200 ± 0.010[a]	0.750 ± 0.010[a]	0.721 ± 0.535[a]	1.350 ± 0.050[a]
C18:1 (n−9) C	35.603 ± 0.015[a]	33.807 ± 5.873[a]	38.200 ± 0.300[ab]	34.600 ± 0.500[a]	38.025 ± 0.065[ab]	42.430 ± 0.350[b]
C18:1 (n−11) C	0.520 ± 0.010[d]	0.580 ± 0.010[e]	0.235 ± 0.015[a]	0.300 ± 0.010[b]	0.310 ± 0.010[b]	0.445 ± 0.005[c]
C18:2 (n−6) C	8.223 ± 0.025[a]	8.943 ± 0.095[b]	8.250 ± 0.150[a]	10.335 ± 0.035[e]	9.805 ± 0.045[d]	9.220 ± 0.030[c]
C18:3n6	0.870 ± 0.020[c]	0.803 ± 0.015[b]	0.515 ± 0.015[a]	0.515 ± 0.015[a]	0.890 ± 0.010[c]	1.050 ± 0.050[d]
C18:3n3	1.073 ± 0.025[c]	1.300 ± 0.010[e]	0.900 ± 0.020[a]	1.400 ± 0.020[f]	1.140 ± 0.030[d]	1.000 ± 0.010[b]
C20:3n9	0.775 ± 0.005[d]	0.575 ± 0.025[b]	0.645 ± 0.015[c]	0.445 ± 0.015[a]	0.485 ± 0.015[a]	0.538 ± 0.053[b]
C20:3n6	1.170 ± 0.010[e]	0.745 ± 0.015[c]	0.695 ± 0.015[b]	0.200 ± 0.010[a]	0.765 ± 0.015[c]	0.880 ± 0.030[d]
C20:3n3	2.750 ± 0.020[e]	2.330 ± 0.040[d]	1.845 ± 0.035[b]	1.995 ± 0.015[c]	1.965 ± 0.035[c]	1.080 ± 0.030[a]
C20:n3 EPA	2.155 ± 0.015[c]	2.315 ± 0.015[d]	2.195 ± 0.025[c]	2.000 ± 0.110[b]	1.950 ± 0.020[b]	1.255 ± 0.035[a]
C22:5n3 DPA	0.740 ± 0.010[c]	0.850 ± 0.050[d]	0.960 ± 0.020[e]	0.205 ± 0.005[a]	0.440 ± 0.030[b]	0.240 ± 0.010[a]
C20:4n6 ARA	0.280 ± 0.010[b]	0.300 ± 0.010[b]	0.290 ± 0.010[b]	0.305 ± 0.005[b]	0.115 ± 0.005[a]	0.350 ± 0.030[c]
C22:4n6 DTA	0.550 ± 0.010[d]	0.335 ± 0.005[c]	0.245 ± 0.025[a]	0.525 ± 0.005[d]	0.240 ± 0.020[a]	0.285 ± 0.015[b]
C22:6 ω 3	8.903 ± 0.055[f]	7.950 ± 0.150[e]	7.265 ± 0.055[d]	6.205 ± 0.025[b]	6.035 ± 0.135[a]	6.375 ± 0.065[c]

Data are mean ± SD. Fatty acid composition expressed as percentage/100 g larval sample. [a,b,c,d,e] Different superscript letters within a column indicate significant differences ($p < 0.05$).

from 31 to 38 dph was 6.97% ($p < 0.05$). In this regard, from 25 to 38 dph, the positive aspects of adding CHL (FR-A, D, and E) and *Daphnia* (FR-C) were detected. Due to the nutritional characteristics of CHL, the high-protein levels and the presence of most essential amino acids, causing it to be become a suitable feed for sturgeon's larvae [31]. In addition, *Daphnia magna* is an important live food for feeding fresh water fish larvae, and beside being a good source of protein, it contains digestive enzymes such as proteinases, peptidases,

amylases, lipases, and even cellulose [32]. In addition, in this relation, Abo-Taleb et al. [33], reported that, *Daphnia magna* contains high content of animal protein (30.8%–61%) and noticeable amount of vitamins, antioxidants, nonsaturated fatty acids, and chitosan. In the present research, at the end of trail (38 dph) feeding the larvae with CHL (FR-A) increased the survival by 18.294% compared to *Daphnia* (FR-C). The decrease in survival rate in others FR that used AB (FR-B) and CHL in combination with AB (FR, D, and E) compared to
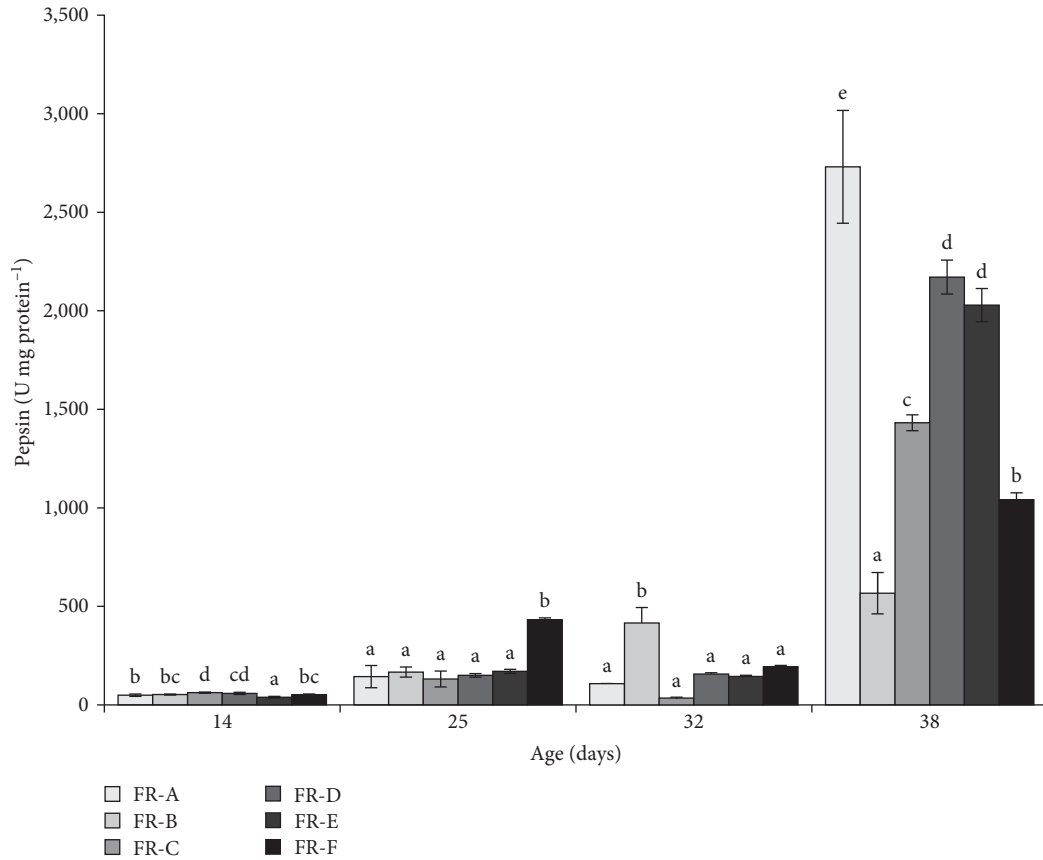
FIGURE 2: Pepsin enzyme activity of *H. huso* larvae under different feeding regimes at different days after hatching. Data are mean ± SD. [a,b,c,d,e]Different superscript letters within a column indicate significant difference ($p < 0.05$).
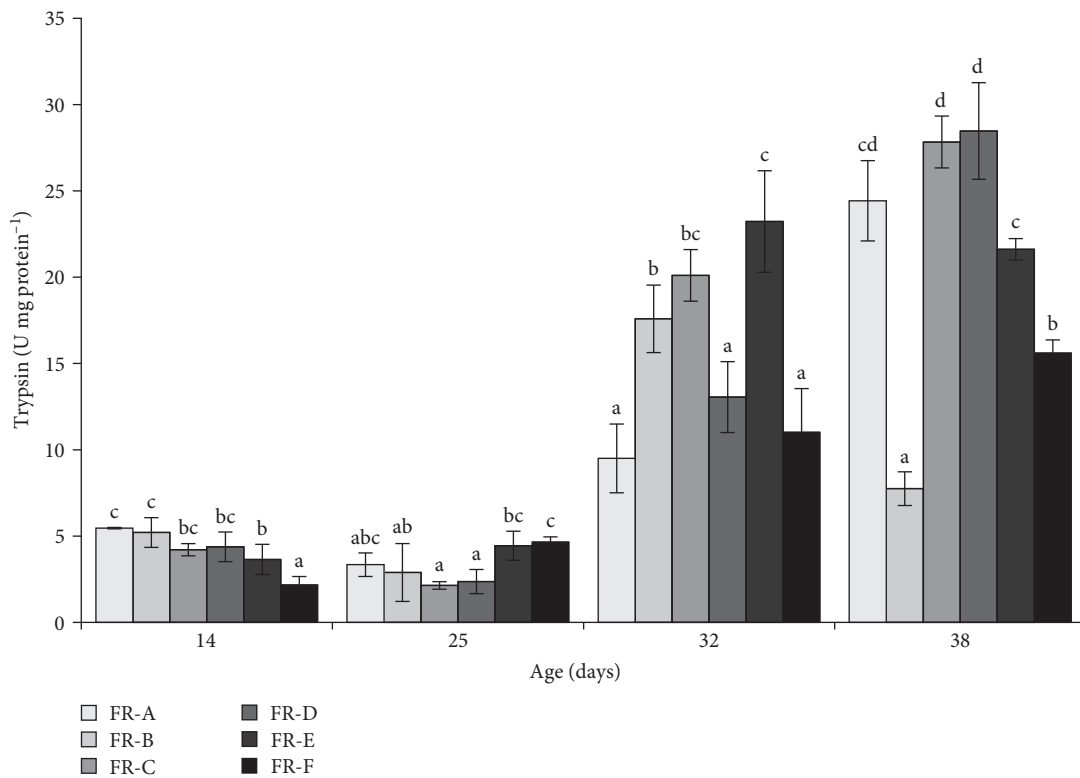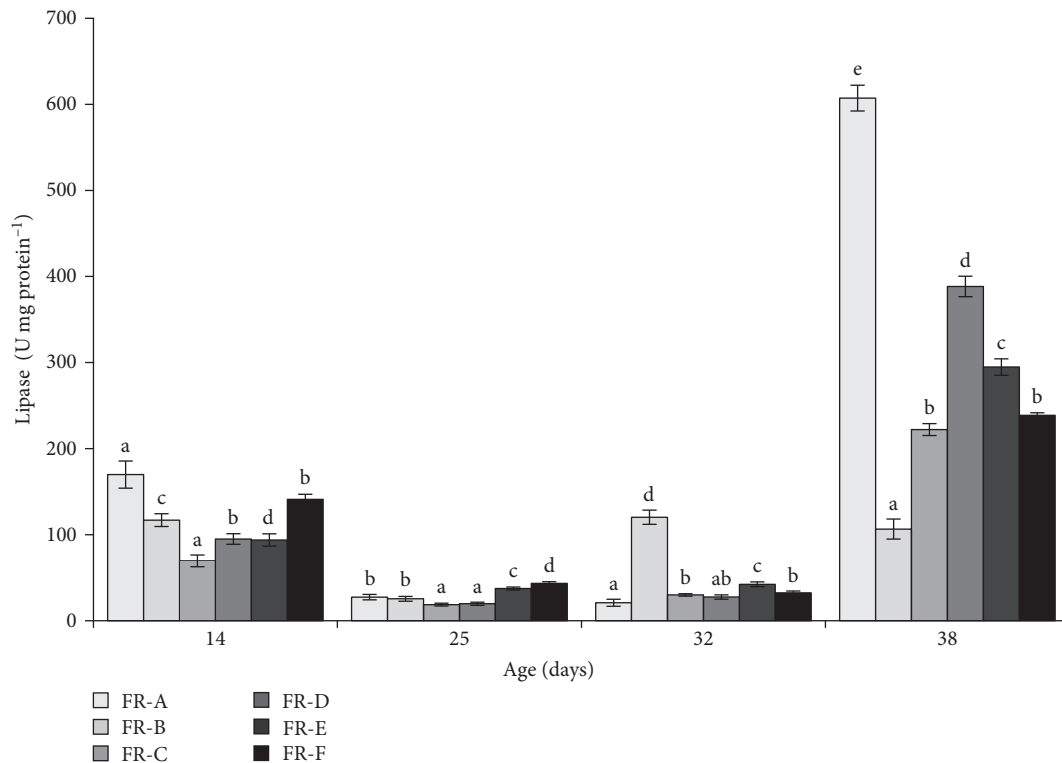

FIGURE 3: Trypsin enzyme activity of *H. huso* larvae under different feeding regimes at different days after hatching. Data are mean ± SD. [a,b,c,d]Different superscript letters within a column indicate significant difference ($p < 0.05$).

FIGURE 4: Lipase enzyme activity of *H. huso* larvae under different feeding regimes at different days after hatching. Data are mean $\pm$ SD. [a,b,c,d,e]Different superscript letters within a column indicate significant difference ($p < 0.05$).

FR-A, indicates the role of using CHL in increasing larval survival rate (Table 1). This issue was especially evident at the end of trail (38 dph). In the present research, the low survival rate of larvae fed only with microdiet (FR-F) compared to other FR is the line with the research of Agh et al. [11], which reported that the low survival rate of larvae is caused by the starvation of the larvae and the empty digestive tract due to little attractiveness of microdiet. In determining BW of larvae, the positive effects of using AB, especially in some FR (B, D, and E), where it was used on the 32 and 38 dph were noticeable (Table 2). BW changes at 14 dph, in FR, A–F were not significant ($p > 0.05$), because the larvae were fed the same only AN ($p > 0.05$). Nevertheless, the increasing trend started at 25 dph. At this time, BW in FR-D was significantly more than other FR. Others FR had middle range (except FR, A, and E). At 32 dph, the lowest BW was detected in FR-A ($p < 0.05$). The highest BW was detected in FR, C, D, and E, and middle values in FR, B, and F. At 38 dph, in FR-F (100% microdiet), the high BW, was due to very low survival of larvae (for example 86.42% lower than FR-A). At this time, FR-D, B, and E had a statistically significant difference with FR, C, A, and F ($p < 0.05$). The stating the reason for the higher growth in FR-F compared to others FR, it should be mentioned that, the higher density of larvae can lead to increased competition for food and space, and as a result, more energy consumption lead to higher metabolic ratios and hence growth is reducing [4]. In terms of nutritional value, both AN and Adults can meet the nutritional requirements of a wide type of organisms. Compared to newly hatched nauplii, the nutritional value of on-growing and adult *Artemia* is superior [34]. In addition, in expressing the importance of using AB, it has been reported that, *Artemia* is rich in amino acids and is an excellent food source for newly hatched fish [35]. Also in expressing the importance of live foods, it should be stated that, they are a sources of easy to digest proteins, while other protein sources, such as fishmeal, have low digestibility. In addition, compared to microdiet, live foods, in addition to providing essential micronutrients, may activate zymogens or digestive hormones or stimulate the secretion of endogenous enzymes [2]. In this regard, Ljubobratović et al. [10], reported that, live feed facilitates the digestion process and product of their autolysis may accelerate the release of trypsinogen from the pancreas and the activation of gastric zymogen. In this connection, the lower growth in larvae that only feed on microdiet can be caused by the lesser development of the digestive system and as a result, the inability to properly digest microdiet. Also reported that, the mortality rate of larvae fed only microdiet may 2.5 times higher than larvae solely fed live feed [36]. In addition, Bauman et al. [37], reported that, lake sturgeon does not prefer microdiet until 14 day postexogenous feeding. Also, Meyer et al. [38], reported that, natural diets can achieve higher growth than formulated diets in some cultured sturgeon species such as the Chinese sturgeon (*A. sinensis*), great sturgeon (*H. huso*), and Persian sturgeon (*A. persicus*) [38].

At 25 dph, SGR in FR, C, A, B, and F were significantly more than FR-D ($p < 0.05$). At this stage, it is possible that, the combined use of CHL + AB + microdiet caused such condition. At 32 dph, SGR in FR, C, and F was significantly
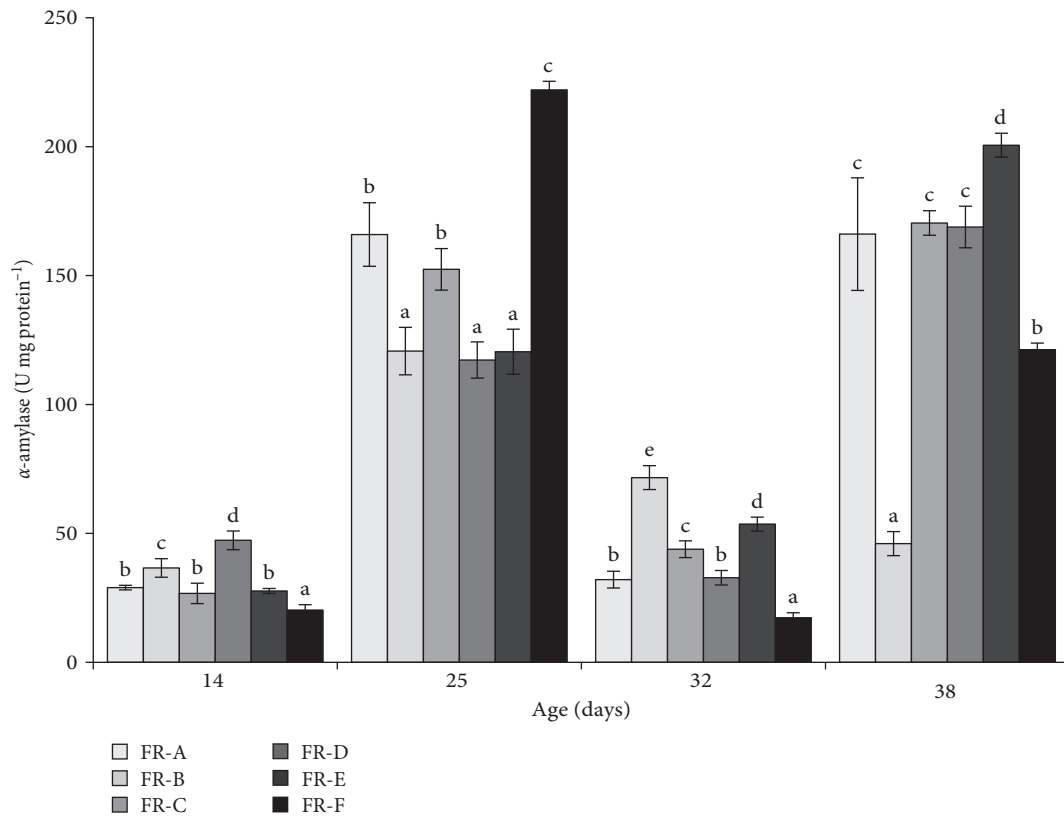
FIGURE 5: α-amylase enzyme activity of *H. huso* larvae under different feeding regimes at different days after hatching. Data are mean $\pm$ SD. [a,b,c,d,e]Different superscript letters within a column indicate significant difference ($p < 0.05$).

more than FR and D ($p < 0.05$). This issue can also indicate the role of *Daphnia* in increasing the growth rate larvae at this stage, like other live feed. At the end of the trail (38 dph), SGR in FR-F was more than others FR (A-E, $p < 0.05$). Considering the dependance of SGR on the increase in larval weight, the higher SGR in FR-F was due to the significantly lower survival rate compared to others FR ($p < 0.05$, Table 3).

Changing the FR especially at 32 and 38 dph, caused significant changes in the length of larvae in some FR. At 14 dph, no significant difference was measured between different FR ($p > 0.05$). From 25 to 32 dph, the highest TL was detected in FR-D (4.25 ± 0.41 and 5.94 ± 0.04 cm, respectively). At the end of trail (38 dph), the lowest and highest TL, was detected in FR-A and F (6.09 ± 0.05 and 7.34 ± 0.12 cm, respectively, Table 2; $p < 0.05$).

A large difference in the size of the larvae can increase the risk of cannibalism [5]. In addition, reducing the heterogeneity of larval population growth can highly reduce food wastage and consequently improve water quality. Cofeeding can make the size of the larvae more homogeneous by improving the nutritional condition of the larvae. In addition, cofeeding procedure improved the nutritional status of larvae to avoid high size variation of larvae in other taxa. Changing the type of food is a critical period in the rearing of fish larvae, and the use of live foods together with microdiet can reduce competitive behavior and cannibalism [5]. In the present study, at the end of trial, the highest CF was detected

in FR, D and E, with no significant differences with FR-A ($p > 0.05$). The lowest CF was observed in FR, B, C, and F (Table 3; $p < 0.05$). This issue indicates the positive effects of some live foods on the CF of larvae. Fish body composition is often used as an indicator of meat quality and fish health. The type of diet, feeding and growth rate, and water temperature are known as influencing factors on body composition. The higher amount of carcass protein and lesser raw fat in FR-C shows that, the fish probably store more tissue protein than the initial amount and use fat as an energy source to provide energy to the fish more than protein [39]. It is also reported in this connection that, a lower body lipid content in fish muscle mass is the result of lower feeding rate than optimum rate, while some believe that a higher feeding rate than needed, lead to storage of ample energy store (usually in the form of fat) in the viscera, muscle and liver [40]. In the present study, FR-E followed by A and C had a similar status in terms of fat utilization and protein storage (Table 4). This issue can be caused by the lower values of CP in the mentioned FR. The amount of ash in FR-C followed by FR-E was significantly more than other FR ($p < 0.05$). This issue is probably due to the lesser body muscle mass and higher proportions of bone mass of larvae in the mentioned FR [39]. A significant difference in terms of DW was observed in most FR ($p < 0.05$). DW in FR-D followed by FR, C and E was significantly more than other FR. The lowest DW was observed in the FR and B followed by FR and A. In this

connection, the change in moisture is known to be caused by the feeding ratio [39].

Changes in fatty acid composition of great sturgeon larvae with different FR at the end of trail are presented in Table 5. In the present study, changing the FR, affected larval fatty acid composition. In most of the FR, feeding the larvae with different diets, caused statistically significant differences in terms of saturated fatty acids (C10:0, C12:0, C15:0, C16:0, C17:0, C18:0, and C24:0; $p < 0.05$). In terms of monounsaturated fatty acids (C14:1, C15:1, C17:1, C20:1, C18:1 ($n-9$), and C18:1 ($n-11$) C), no significant difference was observed in some of FR ($p > 0.05$). In terms of PUFA, statistically significant differences were observed in most of the FR ($p < 0.05$). Essential fatty acids, especially DHA, play an important role in the development of nerve and visual tissues, and it is clear that the DHA play a more important role in the growth and membrane structure than the EPE [17]. Sturgeons need both $n-3$ and $n-6$ fatty acids and store these fatty acids, which are affected by dietary fatty acids, in meat and liver [41]. Long chain PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have a greater tendency to be stored in tissue, tend to be mostly retained in the body tissues. Also, pointed out that increasing the supply of this fatty acid, more than the larvae needs, leads to an increase in oxidation for energy production [42]. Most of the researches on larval lipid nutrition have focused on the requirements for fatty acids, especially highly unsaturated fatty acids (HUFA) due to their importance in larval quality and growth. They have also mentioned that, skeletal anomalies and cranial anomalies was caused by the lack of $n-3$ HUFA in AN and dietary $n-3$ HUFA levels, respectively [42]. In the research conducted in connection with longfin yellowtail (*Seriola rivoliana*), feeding the fish with high levels of $n-3$ HUFA can lead to the skeleton anomalies. Insufficient amounts of $n-3$ HUFA can lead to decreased appetite, growth, swimming activity, survival and especially skeletal abnormalities [4]. In expressing the effect of the type of oil source, it was found that, in the fish that were fed with a diet containing fish oil, $n-3$ fatty acids were higher in the whole body and liver than in the fish that were fed with vegetable oil [41].

The data related to some digestive enzymes in different dph are presented in Figures 2–5. During the initial of larval development, protein digestion is carried out in an alkaline environment in the lumen zone. Knowing the changes in digestive enzyme activity in fish larvae can indicate the process of development of digestive system, which plays an essential role in the process of feeding [17]. The activity pattern of enzymes at the time of the first feeding of fish larvae is species specific [43]. In this connection, it has been reported that the amount of dietary protein has an effect on protease activity, but protease activity is different among species. It has been found that there is no correlation between protease activity and protein level diets in some fish species (*Labeo rohita* fingerlings, *Dentex dentex*, *Cyprinus carpio*, *Anarhichas minor*, and *Cherax quadricarinatus*) [18]. Among the different enzymes, the activity of pancreatic enzymes activity (e.g., amylase, trypsin, and lipase) is usually used as an indicator of the activity and readiness of the digestive system [44]. In the present study, changing in the activity levels of pepsin (Figure 2) and trypsin (Figure 3) showed that, the type of FR changed significantly the enzyme activity level. So, in the case of pepsin, the highest activity was observed at the end of trail (38 dph). In this time, trypsin activity in FR-A followed by FR-E was significantly more than others FR ($p < 0.05$). In this connection, it has been reported that the lack of amino acids that are responsible for the synthesis and secretion of trypsin can lead to the lack of proper activity of the trypsin enzyme [18]. At 38 dph, pepsin activity in FR-A followed by FR-D and E was significantly more than other FR ($p < 0.05$). CHL have a high-protein levels and the most essential amino acids, so it is a suitable feed for sturgeon's larvae [31]. Lipase activity has been reported in the larvae of several fish species, including *Acipenser fulvescens* [45]. It has been reported that the change of lipase enzyme activity (Figure 4) follows the same pattern as trypsin enzyme activity. Excessive amounts of protein may turn into massive fat deposits, and thus inhibit the lipase activity [18]. The lipase activity in FR-A followed by FR, D, and E was significantly more than others FR (e.g., in FR-A was 61.18% higher than FR-F; $p < 0.05$). At the end of trail (38 dph), α-amylase enzyme activity (Figure 5) in FR and E was significantly higher than other FR ($p < 0.05$). Amylase activity has been reported in the larvae of several fish species, including *Acipenser fulvescens* [45, 46]. In the present study, the lowest α-amylase enzyme activity was detected in FR-B. Dietary carbohydrate levels and its molecular form in live feed, microdiet, and changing feeding strategy can affect α-amylase activity [17]. Amylase activity has been reported in the larvae of several fish species, including *Acipenser fulvescens* [45, 46].

5. Conclusions

In summary, the results of the present research showed that changing the FR could affect growth performance, especially survival rate. It was also found that feeding the *H. huso* larvae with CHL increased survival rate, the amount of $n-3$ long chain PUFA in larvae carcass and enzymes activity. Also feeding larvae with AB led to an increase in the growth rate and the amount of $n-3$ long chain PUFA in larvae carcass. Therefore, using them in combination with CHL can give better results. Larvae from FR-A and B, had higher levels of $n-3$ long chain PUFA in comparison with others FR ($p < 0.05$). In addition, in term of the activity of digestive enzymes, at the end of trail, the activity of pepsin, trypsin, lipase, and α-amylase were lower in FR-B and F than other FR (A, C, D, and E), respectively ($p < 0.05$). The use of MD alone to feed the larvae caused a sharp decrease in survival rate and enzymes activity. Considering all the factors, the combined use of AN, AB, CHL, and MD led to better results and ordered to *H. Huso* larvae feeding.

Data Availability

The data that support the findings of this study are available upon reasonable request by the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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