

Article



Growth, Muscle Nutrition Composition, and Digestive Enzyme Activities of the Juvenile and Adult *Siniperca chuatsi* Fed on Live Baits and a Formulated Diet

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Abstract: The present study aimed to evaluate the feasibility of a formulated diet to replace live baits as feed for *Siniperca chuatsi*. A 2 \times 2 factorial design with three replicates was used to investigate the effects of conventional live baits (LB) and a formulated diet (FD) on the growth, muscle nutrition composition, and digestive enzyme activities of S. chuatsi at two growth stages. The results showed that the feed conversion ratio (FCR) in the FD group was significantly lower than that in the LB group. The final body weight (FBW) and weight gain (WG) were not significantly different between the two feeding modes. However, at the juvenile stage, the specific growth rate (SGR) in the FD group was significantly lower than that in the LB group. Muscle moisture, crude protein, and crude lipid contents were not influenced by dietary treatments. There were no significant differences in ΣEAA , \sum NEAA, and \sum AA compositions of the juvenile and adult stages fed the two kinds of diets. At the juvenile stage, histidine (His) content in the FD group was significantly higher than that in the LB group; at the adult stage, cysteine (Cys) content in the FD group was significantly higher than that in the LB group; at both growth stages, Met content in the FD group was significantly lower than that in the LB group. The FD group showed higher levels of \sum SFA, \sum HUFA, \sum n-3PUFA, n-3/n-6, EPA, and DHA than the LB group did, whilst the opposite occurred in the MUFA levels. In addition, fish fed with LB had significantly higher values of pepsin and intestinal trypsin activities in the juvenile fish compared to the FD group, but the activities were not significantly different between the two feeding modes at the adult stage. The activities of stomach and intestine amylase in the FD group were significantly higher than those in the LB group. Overall, these results showed that under long-term feeding conditions, S. chuatsi fed with the artificial diet had no significant difference in muscle crude protein, crude lipid, amino acid composition, and digestive capacity, but showed superiority in the composition of fatty acids such as EPA and DHA levels compared to the live baits group. Therefore, the artificially formulated diet might be a more appropriate feeding approach for sustainable development of the S. chuatsi industry.

Keywords: formulated diet; live baits; *Siniperca chuatsi*; growth stage; proximate composition; digestive enzymes

1. Introduction

As a freshwater carnivorous species, Chinese perch (*Siniperca chuatsi*) originally feeds mostly on live prey fish when they were originally domesticated in the intensive culture model [1,2]. They were found to have a very peculiar food preference that involves capturing live fish from the first feeding stages [2]. Chinese perch has a great economic value in China, with the total production increasing to 370,000 tons in 2020 [3]. The traditional feeding mode, which mainly utilizes live bait and chilled fish as feed, is so far the predominant approach used in *S. chuatsi* farming in China. However, feeding live



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). fish to Chinese perch has problems such as declining traditional natural bait resources, water quality deterioration, carrying pathogens, and imbalanced nutritional composition, which ultimately adversely affects the green and sustainable development of the *S. chuatsi* breeding industry [4–6]. Due to these problems, intensive Chinese perch culture is becoming increasingly reliant on formula feed.

Diets have a considerable influence on the nutritional composition and taste of cultured animals [7]. In addition, the nutritional status of feed is a key element affecting the innate immune response in fish [8]. Therefore, understanding the nutritional requirements of fish is important for developing effective foods. The composition of amino acids and fatty acids makes a great contribution to meat quality [9]. Growth and digestive enzyme activities are important criteria that must be considered in growing species [10]. Previous studies have compared the feeding of trash fish and the formulated diet in fish and crustaceans. Shao et al. reported that *Eriocheir sinensis* fed with the formulated diet as a replacement for trash fish had no adverse effect on meat quality, and could increase the content of muscle highly unsaturated fatty acids [9]. Woodcock et al. showed that feeding with the formulated diet decreased growth and survival rates and had a lower caloric value, with a lower protein and glycogen level of flesh when compared with trash fish in juvenile whelks [7]. Recent studies demonstrated that feeding with the formulated diet improved the digestive capacity, content of phospholipids, protein utilization, amino acids composition, the relative abundance of firmicutes at the phylum level, and the immune function in different species when compared with trash fish feeding [11-13]. After years of selective breeding, Chinese perch can be successfully domesticated and fed with the artificial diet through some special procedures. Researchers have explored the effects of the formulated diet on the physiological metabolic response, intestinal health, and molecular basis of S. chuatsi [14–17]. However, further research is needed if live bait is to be completely replaced by formulated feed. To date, during long-term farming, the dynamic effects of live baits replacement with the formulated diet on the biochemical composition and digestive enzyme activities have rarely been evaluated in *S. chuatsi*. Consequently, it is quite important to clarify the response of Chinese perch at different growth stages when feeding with various diets, which might be helpful to produce an effective low-cost food for the species in the future.

Building a successful breeding industry requires optimizing its feed for fast growth, high survival rate, low cost, and good feed efficiency while providing better meat biochemical composition. Hence, this study compared the effect of live baits and a formulated diet on the growth, biochemical composition, and digestive enzyme activities of juvenile and adult Chinese perch. Our result might provide a valuable reference for selecting a suitable rearing pattern in industrial breeding of this breed.

2. Materials and Methods

2.1. Diets and Experimental Design

A 2 \times 2 factorial design with two fish growth stages (juvenile and adult) and two diets (live baits and formulated feed) was used in this study. The juvenile stage was sampled after 14 days of feeding, and the adult stage was sampled after 120 days of feeding. The dietary treatments included live baits (LB) and a formulated diet (FD). The live baits used in the experiment was the fry of crucian carp, which was provided by the Huangma aquaculture base of Jiangxi Fisheries Research Institute (Nanchang, China). The formulated diet was commercial feed, which was purchased from Jiangmen Yuewang Agricultural Technology Co., Ltd. (Jiangmen, China). The general nutritional compositions of LB and FD are presented in Table 1. The amino acids and fatty acid compositions of LB and FD are presented in Table 2. Healthy juvenile *S. chuatsi* were obtained from Le'an special aquatic product development Co., Ltd. (Shangrao, China), which is a provincial breeding farm of Poyang Lake *S. chuatsi*. Fish were maintained in a net cage (2 m \times 3 m \times 2 m) for 3 days and fed live baits prior to the feeding experiments. Before the feeding experiment,

according to the previous research, Chinese perch were domesticated to accept the artificial diet before the formal feeding experiment [18].

Table 1. Proximate composition of live baits and formulated diet (% dry matter).

Item	Moisture	Crude Protein	Crude Lipid	Ash
LB	$74.5\pm2.23^{\text{ b}}$	52.55 ± 1.05 $^{\rm a}$	12.55 ± 0.94	12.55 ± 0.82
FD	9.10 ± 0.42 a	$56.55\pm1.31~^{\rm b}$	13.53 ± 0.73	12.54 ± 0.63
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Different letters in the same column indicate significant differences among groups (p < 0.05).

Table 2. The amino acids (g/100 g, dry) and fatty acid (% total fatty acids) composition of live baits and formulated diet.

AA (Amino Acids)	LB	FD	Fatty Acids	LB	FD
EAA			C14:0	3.55	1.06
Arg	2.87	2.63	C16:0	21.81	15.76
His	2.09	1.54	C18:0	2.90	3.70
Val	2.11	1.88	C20:0	0.28	0.28
Phe	2.34	1.83	∑SFA	28.54	20.79
Leu	4.17	3.46	C16:1n-7	5.29	2.61
Ile	1.55	1.58	C18:1n-9	24.24	33.63
Thr	2.27	2.17	C20:1n-9	1.18	0.72
Met	0.89	0.67	∑MUFA	30.70	36.97
Lys	4.03	3.88	C18:2n-6	16.19	28.36
Trp	0.63	0.50	C18:3n-6	3.93	0.54
NEAA			C18:3n-3	1.61	2.40
Asp	5.08	4.46	C20:2n-6	0.67	0.71
Ser	2.60	2.21	C20:3n-6	0.58	0.83
Glu	7.87	7.13	C20:4n-6	0.78	1.44
Ala	3.25	3.13	C20:3n-3	0.20	0.13
Gly	2.83	3.29	C20:5n-3	2.73	0.18
Tyr	1.60	1.46	C22:6n-3	3.32	0.94
Pro	2.34	1.79	Others	8.74	6.72
Cys	0.52	0.33	∑HUFA	7.62	3.52
ΣEAA	22.95	20.13	∑PUFA	30.02	35.53
∑NEAA	26.07	23.79	∑n-3PUFA	7.87	3.65
∑AA	49.02	43.92	∑n-6PUFA	22.16	31.88

 Σ EAA: total essential amino acids; Σ NEAA: total non-essential amino acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acid; Σ PUFA, total poly-unsaturated fatty acid; Σ HUFA, total highly unsaturated fatty acid.

After the acclimation, 1200 healthy juveniles (initial weight 20 ± 1.8 g) were distributed into 6 net cages (200 fish per net cage) and randomly assigned in triplicates to the treatments. During the trial, fish in all net cages were hand-fed with their respective experimental diets twice (06:00 and 18:00) a day to apparent visual satiation for 120 days. The water depth in the net cage was 2 m. Each net cage was provided with microporous oxygenation equipment, and the water was aerated daily for 24 h to maintain dissolved oxygen levels at or near saturation. The temperature was maintained at 28 ± 2 °C.

2.2. Sampling and Data Collection

After the 14-day and 120-day feeding trials, final body weight (FBW) and weight gain (WG) were determined after a 24 h fast. The wet weight of all fish from each tank was individually measured to an accuracy of 0.01 g. The fish (anaesthetized on ice) were dissected to obtain the stomach, intestine, and muscle, which were stored at -80 °C for subsequent analyses.

2.3. Muscle Proximate Composition

Muscle proximate composition including crude protein, crude lipid, and moisture were measured according to standard methods described by AOAC [19]. Briefly, the total

nitrogen content was determined using the Dumas combustion method with a protein analyzer (FP-528, Leco, San Joes, CA, USA), and crude protein contents were then calculated as $\%N \times 6.25$. Crude lipid contents in the feeds and muscle were determined via the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Hoganas, Sweden). Moisture contents were determined by drying the samples to a constant weight at 105 °C. The contents of amino acids and fatty acid in the muscle was performed at the Jiangxi analysis and Testing Center, Nanchang, China, based on the standard methods of AOAC [19].

2.4. Digestive Enzymes

The fish stomach and intestine were homogenized in 50 mM Tris-HCl solution and centrifuged $(10,000 \times g \cdot min^{-1})$ at 4 °C for 10 min. The supernatants were collected and kept frozen at -80 °C for later determination. The lipase (A054-1-1), pepsin (A080-1-1), trypsin (A080-2-2), and amylase (C016-1-1) activities were measured by a microplate photometer (MultiskanTM Go, Thermo Fisher Scientific, Waltham, MA, USA) using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). The specific activity of lipase was expressed as the amount of enzyme needed to form 1 µmol of hydrolyzed substrate per minute (U) per protein g. Pepsin is defined as 1 protein produced by decomposing protein per mg of tissue protein at 37 °C per minute µg tyrosine, which is equivalent to one enzyme activity unit. Trypsin contained in each mg of protein changes the absorbance by 0.003 per minute, which is an enzyme activity unit. Each mg of protein in the tissue reacts with the substrate at 37 °C for 30 min. Hydrolyzed starch of 10 mg is defined as one amylase activity unit.

2.5. Statistical Analysis

All statistical analyses were performed using SPSS (version 24.0; SPSS Inc., Chicago, IL, USA). The data represent the mean \pm standard deviation (SD) of three replicates. Two-way ANOVA and follow-up Tukey tests were used to determine the statistically significant difference (p < 0.05) among different groups.

3. Results

3.1. Growth Performances

Effects of live baits and the formulated diet on the growth performances of *S. chuatsi* are summarized in Table 3. The FCR and FI in the FD group were significantly lower than those in the LB group (p < 0.05). FBW and WG were not influenced by the treatments (p > 0.05). However, at the juvenile stage, the specific growth rate (SGR) in the FD group was significantly lower than that in the LB group (p < 0.05).

Table 3. Effects of live baits and formulated diet on the growth performances of *Siniperca chuatsi* at different growth stages ¹.

Items	Juver	Juvenile Fish		Adult Fish			Two-Way ANOVA <i>p</i> -Value		
	LB	FD	LB	FD	D ¹	G ¹	$\mathbf{D} imes \mathbf{G}$		
FBW ²	$32.67\pm1.53~^{\rm a}$	$28.33\pm2.08~^{a}$	353.33 ± 45.09 ^b	$346.67 \pm 30.55 \ ^{\rm b}$	0.728	0.000	0.951		
WG ³	$63.33\pm7.64~^{a}$	$41.67\pm10.41~^{\rm a}$	$1666.67 \pm 225.46^{\text{ b}}$	$1633.33 \pm 152.75 \ ^{\rm b}$	0.728	0.000	0.951		
SGR ⁴	3.68 ± 0.39 ^b	$2.48\pm0.62~^{\rm a}$	2.39 ± 0.11 $^{\rm a}$	$2.38\pm0.07~^{a}$	0.023	0.009	0.026		
FCR ⁵	$4.34\pm0.65~^{\rm b}$	1.23 ± 0.10 $^{\rm a}$	4.22 ± 0.66 ^b	1.29 ± 0.08 $^{\rm a}$	0.000	0.919	0.748		
FI ⁶	$13.32\pm0.74~^{\rm c}$	2.57 ± 0.58 $^{\rm a}$	$6.25\pm1.07^{\text{ b}}$	1.90 ± 0.10 $^{\rm a}$	0.000	0.000	0.000		

¹ Data are expressed as mean \pm S.D. (n = 3). D: diets. G: growth stages. ² FBW: final body weight (g). ³ WG: weight gain (%) = 100 × (final body weight (g) – initial body weight (g))/initial body weight (g). ⁴ SGR: specific growth rate (%/day) = 100 × (In final body weight – In initial body weight)/feeding days. ⁵ FCR: feed conversion ratio = dry diet fed/wet weight gain. ⁶ FI: feed intake (g/100 g ABW/d) = feed consumption (g)/(ABW (g) × feeding days). ABW: average body weight (g) = (final body weigh (g) + initial body weight (g))/2. Different letters in the same line indicate significant differences among groups (p < 0.05).

3.2. Muscle Proximate Compositions

The result of muscle proximate composition is shown in Table 4. At the end of the experiment, there were no significant differences in moisture, crude protein, and crude lipid observed in both juveniles and adults fed with the live baits and the formulated diet (p > 0.05).

Table 4. Effects of live baits and formulated diet on the proximate composition in muscle of *Siniperca chuatsi* at different growth stages ¹.

Items	Juvenile Fish		Adult Fish		Two-Way ANOVA <i>p</i> -Value			
	LB	FD	LB	FD	D ¹	G ¹	$\mathbf{D} imes \mathbf{G}$	
Crude protein (%)	18.13 ± 0.25	18.23 ± 0.21	18.30 ± 0.10	17.97 ± 0.29	0.393	0.709	0.132	
Crude lipid (%)	2.57 ± 0.25	2.20 ± 0.36	2.10 ± 0.30	2.13 ± 0.32	0.380	0.172	0.298	
Moisture (%)	78.80 ± 0.79	79.33 ± 0.38	79.43 ± 0.57	79.57 ± 0.32	0.323	0.208	0.545	

¹ Data are expressed as mean \pm S.D. (n = 3). D: diets. G: growth stages.

3.3. Muscle Amino Acid Composition and Relative Retention Rate

The amino acid contents of the muscle are shown in Table 5. In general, there were no significant differences in total essential amino acids (Σ EAA), total non-essential amino acids (\sum NEAA), and total amino acids (\sum AA) compositions of the juvenile and adult S. chuatsi fed the two kinds of diets, while the \sum EAA composition at the juvenile stage was significantly higher than that at the adult stage (p < 0.05). Among the essential amino acids (EAA), the His content was significantly affected by the diets, the growth stage, and by the interaction of the two factors (p < 0.05). The His content of juvenile S. chuatsi fed the formulated diet was significantly higher than the His content of those fed the live baits (p < 0.05). The contents of Val, Ile, and Trp in the muscle of S. chuatsi were significantly affected by growth stage. The contents of Val, Ile, and Trp in the muscle of the juvenile stage were significantly higher than those in the adult stage (p < 0.05). In addition, at both growth stages, the muscle Met content of S. chuatsi fed with the formulated diet was significantly lower than that of fish fed with live baits (p < 0.05). Among the non-essential amino acids (EAA), the contents of Glu followed by Asp were highest. The contents of Ser, Tyr, and Cys in the muscle were significantly affected by the diets (p < 0.05). At the adult stage, the muscle Cys content of the FD group was significantly higher than that of the LB group (p < 0.05). The relative retention rates of dietary EAA in the muscle fed with live baits and the formulated diet at different growth stages are shown in Table 6. The relative retention rate of essential amino acids, such as His, Val, Phe, Leu, and Trp, in the muscles of S. chuatsi fed with live baits was higher than that in the group fed with the formulated diet. The highest amino acid relative retention rate was Met, followed by Lys or Ile.

Table 5. Effects of live baits and formulated diet on the amino acid composition in muscle of *Siniperca chuatsi* at different growth stages $(g/100 \text{ g}, \text{wet})^{1}$.

AA (Amino Acids)	Juvenile Fish		Adult Fish		Two-Way ANOVA <i>p</i> -Value		
	LB	FD	LB	FD	D ¹	G ¹	$\mathbf{D} imes \mathbf{G}$
			EAA ²				
Arg	0.94 ± 0.05	0.97 ± 0.02	0.98 ± 0.01	0.97 ± 0.02	0.413	0.212	0.212
His	0.43 ± 0.01 a	0.47 ± 0.01 ^b	0.43 ± 0.01 a	$0.43\pm0.02~^{\mathrm{a}}$	0.010	0.002	0.010
Val	0.73 ± 0.02 ^b	0.71 ± 0.01 ^b	0.66 ± 0.02 a	0.68 ± 0.02 a,b	0.740	0.001	0.125
Phe	0.72 ± 0.02	0.71 ± 0.01	0.70 ± 0.03	0.69 ± 0.04	0.467	0.194	0.916
Leu	1.36 ± 0.01	1.36 ± 0.01	1.36 ± 0.02	1.34 ± 0.02	0.172	0.172	0.172

	Juvenil	e Fish	Adu	ılt Fish	Two-W	Two-Way ANOVA <i>p</i> -Value			
AA (Amino Acids)	LB	FD	LB	FD	D ¹	G 1	$\mathbf{D} imes \mathbf{G}$		
Ile	0.64 ± 0.01 ^{a,b}	0.65 ± 0.03 ^b	0.59 ± 0.01 a	$0.62 \pm 0.03^{\ a,b}$	0.170	0.011	0.392		
Thr	0.82 ± 0.03	0.86 ± 0.02	0.81 ± 0.03	0.82 ± 0.04	0.178	0.098	0.401		
Met	0.45 ± 0.04 ^{a,b}	0.36 ± 0.04 a	0.51 ± 0.08 ^b	0.40 ± 0.03 ^{a,b}	0.010	0.138	0.701		
Lys	1.62 ± 0.02	1.61 ± 0.01	1.60 ± 0.04	1.59 ± 0.02	0.310	0.222	0.907		
Trp	0.20 ± 0.02	0.21 ± 0.02	0.17 ± 0.01	0.17 ± 0.02	0.640	0.015	0.875		
	NEAA ³								
Asp	1.77 ± 0.03	1.76 ± 0.02	1.79 ± 0.01	1.74 ± 0.05	0.118	0.920	0.217		
Ser	0.77 ± 0.01	0.76 ± 0.01	0.78 ± 0.02	0.75 ± 0.02	0.024	0.837	0.174		
Glu	2.69 ± 0.06	2.72 ± 0.06	2.78 ± 0.04	2.74 ± 0.09	0.930	0.186	0.309		
Ala	1.08 ± 0.01	1.11 ± 0.02	1.10 ± 0.02	1.09 ± 0.04	0.468	0.806	0.240		
Gly	0.81 ± 0.02	0.89 ± 0.09	0.80 ± 0.02	0.85 ± 0.04	0.061	0.488	0.556		
Tyr	0.57 ± 0.01	0.55 ± 0.01	0.56 ± 0.01	0.53 ± 0.02	0.016	0.227	0.408		
Pro	0.57 ± 0.04	0.64 ± 0.06	0.54 ± 0.02	0.57 ± 0.01	0.069	0.042	0.341		
Cys	0.11 ± 0.02	0.12 ± 0.01	0.08 ± 0.02	0.12 ± 0.01	0.019	0.182	0.182		
$\sum EAA^2$	7.88 ± 0.13	7.88 ± 0.04	7.78 ± 0.12	7.68 ± 0.05	0.365	0.021	0.396		
$\sum NEAA^{3}$	8.37 ± 0.13	8.56 ± 0.11	8.44 ± 0.12	8.39 ± 0.22	0.443	0.605	0.193		
$-\Sigma AA$	16.23 ± 0.21	16.43 ± 0.06	16.23 ± 0.25	16.13 ± 0.25	0.688	0.247	0.247		

Table 5. Cont.

¹ Data are expressed as mean \pm S.D. (n = 3). D: diets. G: growth stages. ² \sum EAA: total essential amino acids. ³ \sum NEAA: total non-essential amino acids. Different letters in the same line indicate significant differences among groups (p < 0.05).

Table 6. Relative retention rate of dietary PUFA and EAA in the muscle of *Siniperca chuatsi* fed with live baits and formulated diet at different growth stages.

AA (Amino Acids) —	Juvenil	Juvenile Fish Adult		lt Fish	sh Fatty Asida		Juvenile Fish		Adult Fish	
	LB	FD	LB	FD	- Fatty Actus	LB	FD	LB	FD	
Arg	1.69	1.63	1.81	1.65	C18:2n-6	0.87	1.14	0.91	1.42	
His	1.32	1.09	1.36	1.01	C18:3n-3	0.83	1.13	0.93	1.62	
Val	1.84	1.63	1.71	1.58	C20:4n-6	1.53	1.42	1.26	1.67	
Phe	1.85	1.45	1.86	1.44	C20:5n-3	0.90	0.99	1.07	0.61	
Leu	1.85	1.58	1.91	1.57	C22:6n-3	1.68	1.68	1.77	1.70	
Ile	1.91	2.03	1.81	1.96						
Thr	1.79	1.84	1.82	1.77						
Met	3.18	1.95	3.72	2.20						
Lys	1.97	1.93	2.01	1.93						
Trp	1.89	1.62	1.65	1.33						

Relative retention rate = The percentage content of fatty acids (amino acids/dry matter) in muscle tissues/corresponding percentage content of fatty acids (amino acids/dry matter) in feed.

3.4. Muscle Fatty Acid Composition and Relative Retention Rate

The fatty acids of the juvenile and adult *S. chuatsi* fed the live baits and the formulated diet are presented in Table 7. Different diets had a significant effect on the muscle fatty acid composition of *S. chuatsi* at different growth stages. The main fatty acid was C18:1n-9. The \sum SFA, \sum HUFA, \sum n-3PUFA, n-3/n-6, EPA (C20:5n-3), and DHA(C22:6n-3) levels of *S. chuatsi* in the FD group were significantly higher than those in the LB group (p < 0.05), while the percentages of \sum MUFA, \sum n-6PUFA, C18:2n-6 (LA), and C20:4n-6(ARA) in the FD group were significantly lower than those in the LB group (p < 0.05). At the different growth stages, the muscle \sum MUFA, n-3/n-6, and EPA levels of the juvenile *S. chuatsi* fed with the formulated diet were significantly higher than those of the adult group (p < 0.05). However, there were no significant differences between the juveniles and adults fed with live baits (p > 0.05). The C18:3n-3 (LNA) level of the juvenile *S. chuatsi* was significantly lower than that of the adult group, irrespective of dietary treatments (p < 0.05). The relative

retention rates of dietary PUFA in the muscle fed with live baits and the formulated diet at different growth stages are shown in Table 6. The relative retention rate of C18:2n-6 and C18:3n-3 in the muscle of *S. chuatsi* fed with the formulated diet was higher than that of the group fed with live baits, and the highest relative retention rate was C22:6n-3.

Table 7. Effects of live baits and formulated diet on the muscle fatty acid composition in muscle of *Siniperca chuatsi* at different growth stages (% total fatty acids) ¹.

Fatter A -: Ja	Juveni	le Fish	Adul	t Fish	Two-W	Two-Way ANOVA <i>p</i> -Value			
Fatty Acids	LB	FD	LB	FD	D 1	G ¹	$\mathbf{D} imes \mathbf{G}$		
C14:0	1.15 ± 0.04 a	3.05 ± 0.13 c	1.11 ± 0.11 a	2.30 ± 0.14 ^b	0.000	0.000	0.001		
C16:0	17.02 ± 0.38 ^a	$20.61\pm0.63~^{\mathrm{b}}$	17.78 ± 0.19 a	20.58 ± 0.57 ^b	0.000	0.219	0.194		
C18:0	3.46 ± 0.11 a	3.35 ± 0.30 a	3.54 ±0.04 ^{a,b}	$3.97\pm0.11~^{\rm b}$	0.143	0.007	0.125		
C20:0	0.14 ± 0.00 a	$0.16\pm0.03~^{\mathrm{a,b}}$	0.14 ± 0.00 a	0.18 ± 0.01 ^b	0.006	0.096	0.186		
∑SFA	$20.62\pm0.41~^{\rm a}$	$25.93\pm2.33^{\text{ b}}$	$21.45\pm0.23~^{\text{a}}$	$24.74\pm0.66^{\text{ b}}$	0.000	0.894	0.031		
C16:1n-7	3.62 ± 0.14 ^a	$5.92\pm0.35~^{\rm c}$	3.35 ± 0.13 ^a	4.43 ± 0.09 ^b	0.000	0.000	0.001		
C18:1n-9	33.28 ± 0.15 ^b	$26.98\pm0.95~^{a}$	32.72 ± 0.72 ^b	25.73 ± 0.73 $^{\rm a}$	0.000	0.056	0.417		
C20:1n-9	0.67 ± 0.04 ^a	$0.87\pm0.08~^{\rm b}$	0.67 ± 0.05 $^{\rm a}$	$0.80\pm0.03~^{\mathrm{a,b}}$	0.001	0.286	0.345		
∑MUFA	$37.57 \pm 0.31 \ ^{\rm c}$	$33.77\pm1.36^{\text{ b}}$	$36.74\pm0.86~^{\rm c}$	$30.96\pm0.68~^{\rm a}$	0.000	0.007	0.089		
C18:2n-6	$24.65\pm0.34~^{\rm c}$	18.46 ± 0.59 $^{\rm a}$	$25.72\pm0.53~^{\rm c}$	$22.96\pm0.87^{\text{ b}}$	0.000	0.000	0.001		
C18:3n-6	0.75 ± 0.04 ^a	$0.96\pm0.07~^{b}$	$0.76\pm0.05~\mathrm{a,b}$	0.61 ± 0.12 a	0.523	0.006	0.004		
C18:3n-3	1.98 ± 0.01 $^{\rm a}$	$1.82\pm0.04~^{\rm a}$	2.23 ± 0.13 ^b	$2.61\pm0.12~^{\rm c}$	0.074	0.000	0.001		
C20:2n-6	$0.83\pm0.02~^{\mathrm{c}}$	$0.56\pm0.04~^{\rm b}$	$0.76\pm0.04~^{ m c}$	0.42 ± 0.01 a	0.000	0.000	0.098		
C20:3n-6	1.11 ± 0.02 d	$0.57\pm0.01~^{\rm b}$	$0.93\pm0.07~^{\mathrm{c}}$	$0.37\pm0.04~^{\rm a}$	0.000	0.000	0.576		
C20:4n-6	$2.20\pm0.05~^{\rm c}$	1.11 ± 0.18 $^{\rm a}$	1.81 ± 0.19 ^b	$1.31\pm0.05~^{\rm a}$	0.000	0.265	0.005		
C20:3n-3	$0.13 \pm 0.00 \ ^{b}$	$0.15\pm0.00~^{\rm c}$	0.12 ± 0.00 a	$0.12\pm0.00~^{\mathrm{a}}$	0.006	0.000	0.004		
C20:5n-3	0.16 ± 0.01 $^{\rm a}$	$2.70\pm0.14~^{\rm c}$	0.19 ± 0.01 $^{\rm a}$	1.67 ± 0.11 ^b	0.000	0.000	0.000		
C22:6n-3	1.58 ± 0.05 $^{\rm a}$	$5.59\pm0.45^{\text{ b}}$	1.67 ± 0.12 $^{\rm a}$	5.65 ± 0.11 ^b	0.000	0.594	0.890		
Others	7.37 ± 0.31	6.39 ± 1.03	6.50 ± 0.57	6.30 ± 0.23	0.134	0.215	0.309		
∑HUFA	5.17 ± 0.10 $^{\rm a}$	10.12 ± 0.71 ^b	$4.72\pm0.35~^{a}$	9.11 ± 0.13 ^b	0.000	0.015	0.271		
∑PUFA	$33.38 \pm 0.43~^{ m a,b}$	$31.91 \pm 1.32~^{\text{a}}$	34.20 ± 0.51 ^{b,c}	$35.72\pm0.82~^{\rm c}$	0.952	0.001	0.016		
∑n-3PUFA	$3.84\pm0.05~^{\rm a}$	10.25 ± 0.57 ^b	$4.22\pm\!0.04~^{a}$	$10.05\pm0.24~^{\mathrm{b}}$	0.000	0.653	0.143		
∑n-6PUFA	$28.70\pm0.42~^{\rm c}$	$21.10\pm0.77~^{a}$	$29.22\pm0.51~^{\rm c}$	$25.25\pm0.71~^{\mathrm{b}}$	0.000	0.000	0.001		
n-3/n-6	0.13 ± 0.00 a	$0.49\pm0.02~^{ m c}$	0.14 ± 0.00 a	0.40 ± 0.01 ^b	0.000	0.000	0.000		

¹ Data are expressed as mean \pm S.D. (n = 3). D: diets. G: growth stages. Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acid; Σ PUFA, total poly-unsaturated fatty acid; Σ HUFA, total highly unsaturated fatty acid. Different letters in the same line indicate significant differences among groups (p < 0.05).

3.5. Digestive Enzymes Activities

Digestive enzymes activities of the juvenile and adult *S. chuatsi* fed with live baits and the formulated diet are presented in Table 8. The activities of pepsin and intestinal trypsin determined in this study were significantly affected by diets, growth stages, and their interactions (p < 0.05). At the juvenile stage, the activities of pepsin and intestinal trypsin in the FD group were significantly lower than those in the LB group, while the activity of stomach amylase was significantly higher than that of the LB group (p < 0.05). However, there was no significant difference in intestine amylase activity (p > 0.05). At the adult stage, the activity of stomach and intestinal amylase in the FD group was significantly higher than that of the LB group (p < 0.05), whilst there were no significant differences in the activities of pepsin and intestinal trypsin (p > 0.05). Both the dietary treatments and growth stages had no significant effect on lipase activity (p > 0.05).

Items	Juveni	ile Fish	Adul	Adult Fish			Two-Way ANOVA <i>p</i> -Value		
itenis	LB		LB	FD	D ¹	G ¹	$\mathbf{D}\times\mathbf{G}$		
Stomach Pepsin (U/mgprot)	159.70 ± 10.73 ^b	113.17 ± 11.01 ^a	164.03 ± 22.56 ^b	169.03 ± 10.11 ^b	0.039	0.007	0.015		
Intestine Trypsin (U/mgprot)	3311.22 ± 365.93 ^b	2034.76 ± 363.90 ^a	$3344.55 \pm 596.50 \ ^{\text{b}}$	3411.44 ± 452.11 ^b	0.050	0.028	0.034		
Stomach Lipase (U/gprot)	1.93 ± 0.49	1.99 ± 0.26	1.96 ± 0.49	2.16 ± 0.42	0.612	0.696	0.794		
Intestine Lipase (U/gprot)	2.94 ± 0.29	3.11 ± 0.22	3.01 ± 0.32	3.14 ± 0.23	0.352	0.730	0.891		
Stomach amylase (U/mgprot)	$0.39\pm0.08~^a$	$0.74\pm0.10^{\text{ b}}$	$0.45\pm0.13~^{a}$	$0.82\pm0.12^{\text{ b}}$	0.000	0.305	0.856		
Intestine amylase (U/mgprot)	3.95 ± 0.55 ^{ab}	$3.56\pm0.68~^{a}$	$4.21\pm0.43~^{\text{ab}}$	$5.35\pm0.51^{\text{ b}}$	0.271	0.012	0.043		

Table 8. Effects of live baits and formulated diet on the digestive enzyme activities of *Siniperca chuatsi* at different growth stages ¹.

¹ Data are expressed as mean \pm S.D. (*n* = 3). D: diets. G: growth stages. Different letters in the same line indicate significant differences among groups (*p* < 0.05).

4. Discussion

The development of formulated feed to successfully substitute live baits in the culture of S. *chuatsi* is essential for sustainable production of this species, and can also contribute toward safeguarding the environment. The primary objective of this study was to evaluate the influence of formulated diets, relative to live baits, on growth, proximate composition, amino acid and fatty acid profile, and digestive enzyme activities. This study showed that *S. chuatsi* can successfully be grown using a formulated diet.

After 14 days of feeding, the SGR in the FD group was significantly lower than that of the LB group. However, after 120 days of feeding, fish accepted the formulated diet readily and *S. chuatsi* fed the formulated diet achieved a high growth, with no significant difference compared to fish fed with the live baits. This shows that the formulated diet was suitable for long-term feeding until the adult stage in the present study. Similar results were observed in the juvenile hybrid grouper [13], Asian seabass, and tiger grouper [20]. Ye et al. [13] reported that feed bioeconomics, digestive capacity, and immunity of the hybrid grouper were superior in the formulated diet group compared to the trash fish group. Nevertheless, the result of the adult stage in this study is contrary to that of Li et al. [17], who reported that growth of the hybrid mandarin fish hybrid fed a formulated diet was significantly lower compared with the live baits. The difference may be related to different evaluation species, culture environment, and feed composition.

The composition of nutrients such as protein and fat in fish muscle can be used as important indicators for flesh quality [21]. In the present study, after the 120-day growth trials, the muscle proximate composition of the muscle of Chinese perch fed with the formulated diet had no significant differences in protein and lipid contents compared to those fed the live baits, suggesting that fish fed with the formulated diet also had good flesh quality, similar to the results from Shao et al. [9].

The body need for protein is actually the need for amino acids [22]. In this study, there was no significant difference in muscle protein content between the two feeding modes. Therefore, further evaluation of amino acid types, composition, and nutritional value is an

important way to compare protein quality. In the present study, there were no significant differences in ΣEAA , $\Sigma NEAA$, and ΣAA compositions of juvenile and adult *S. chuatsi* fed the two kinds of diets, implying that there would be no significant difference in the amino acid supply from S. chuatsi from the two feeding modes to consumers. Studies in mice suggest that His can increase histamine production in vivo, which can reduce animal feed intake through nervous system regulation, and then affect animal growth [23]. Methionine deficiency decreases protein accretion and synthesis, and then affects normal growth [24]. After 14 days of feeding, juvenile *S. chuatsi* fed the formulated diet showed higher His and lower Met compositions compared to fish fed the live baits, which is in line with the lower SGR observed in fish fed the formulated diet. After 120 days of feeding, adult S. chuatsi fed the formulated diet showed a lower Met and higher Cys. However, there was no significant difference in His content compared to fish fed the live baits, and the SGR was also not affected, implying that the content of Cys may affect the demand of fish for Met. Studies on Ictalurus punctalus, Sciaenops ocellatus, and Oreochromis niloticus showed that Cys can replace the amount of Met in the feed [24–26], which was somewhat corroborated in the current study. The amino acid profile of the muscle has been identified as an index of the essential amino acid obtained from feed [27]. The ΣEAA composition at the juvenile stage of *S. chuatsi* was significantly higher than that at the adult stage in this study; therefore, it can be considered that the total amount of essential amino acids of juvenile S. chuatsi is higher than that of adult fish.

The fatty acid composition of fish can be used to judge the nutritional value of its fat, because fish is an important source of n-3 series unsaturated fatty acids in human food, which is of great significance to improve human nutrition and health [28]. The muscle fatty acids composition can reflect the nutritional level of the body, as well as the feeding status and health of the fish [29]. In the present study, S. chuatsi fed the formulated diet produced muscle with higher levels of Σ HUFA, Σ n-3PUFA, n-3/n-6 ratios, EPA, and DHA than those fed the live baits, which suggests that these formulated diets could produce better muscle fatty acid nutrition than live fish through balanced fatty acid matching. These findings demonstrated that the EPA and DHA levels increased in the muscle of S. chuatsi, and is possibly related to a higher level of circulating fatty acids, which could be translated into the body due to nutrients richness in the diet, and is in agreement with previous studies in *Salmo salar* [30–32]. *S. chuatsi* fed with live baits had markedly high amounts of MUFAs in their muscle compared to fish fed with the formulated diet. The amount of MUFAs can be attributed mainly to the FA 18:1n-9 as reported in a previous study [33]. The n-3/n-6 ratio is useful in comparing nutritional value as n-6 PUFA in excess could cause inflammation [34]. Therefore, a higher n-3/n-6 PUFAs ratio is deemed a better composition [35]. As recommended by the FAO, a dietary n-3/n-6 PUFAs ratio of at least 0.1–0.2 is optimal [36]. Our results show that the S. chuatsi fed with the formulated diet had a significantly higher ratio of n-3/n-6 than fish fed the live baits, suggesting that S. chuatsi fed with the formulated diet would be of more benefit to consumers.

The activities of fish digestive enzymes are related to feed absorption, physiological state, intestinal environment, and other factors [37]. Zhu et al. [38] reported that the addition of exogenous enzyme preparation can enhance the activities of digestive enzymes, improve the apparent digestibility of dry matter and crude protein in feed, reduce the content of protein in feces, and significantly improve the growth performance. In the present study, after the 14-day feeding trial, the juvenile *S. chuatsi* fed with the formulated diet showed lower activities of pepsin and intestinal trypsin than those fed with live baits, which also correlates with the SGR. This is in agreement with an earlier report by Caruso et al. [39], which showed that after feeding *pagellus acarne* for 20 days with live bait and the formulated diet, the protease activity of the live bait group was higher than that of the feed group and was related to the growth performance. However, after the 120-day feeding trial, there were no significant differences in the activities of pepsin and intestinal trypsin between FD and LB groups for the adult *S. chuatsi*, which could have contributed to the similarity in growth, as seen in the SGR. These results suggest that with

the extension of feeding time, nutritionally balanced formulated diets with relatively high protein content could be more suitable for *S. chuatsi*, thus showing good protease activity and growth rate [37], which is consistent with recent studies [13]. Amylase activity is related to carbohydrate utilization [40]. The activity of stomach and intestinal amylase in the FD group was significantly higher than that in the LB group, which indicated that the starch content of the formulated diet was higher than that of the live baits, as more of the enzyme was needed in the former for digestion and absorption. Similar results were also observed in *Chelonia mydas* [41].

5. Conclusions

Our results showed that under long-term feeding conditions, *S. chuatsi* fed the artificial diet had no significant difference in growth performance, muscle crude protein, crude lipid, amino acid composition, and digestive capacity, but showed superiority in fatty acid composition such as EPA and DHA levels compared to the live baits group. Therefore, the artificial formulated diet would be a more appropriate feeding approach for sustainable development of the *S. chuatsi* industry.

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Data Availability Statement: The data that support the findings of this study are available upon request from the authors.

Conflicts of Interest: The authors declare no conflict of interest.

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