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Comparative study on body index, nutrient composition, and digestive enzyme activity of *Misgurnus anguillicaudatus*, *Paramisgurnus dabryanus, and Paramisgurnus dabryanus* ssp

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Keywords: body index, digestive enzyme, *Misgurnus anguillicaudatus*, nutrient composition activity

Abstract

The current research work was undertaken to compare and analyze the body index, nutrient composition, and digestive enzyme activity of Pond loach (*Misgurnus anguillicaudatus*), large-scale loach (*Paramisgurnus dabryanus*), and Taiwan loach (*Paramisgurnus dabryanus ssp*). Viscerosomatic ratio (VR), condition factor, (CF), W/L and H/L were highest in Taiwan loach ($P < 0.05$). Muscle protein content was highest, whereas lipid content was lowest in pond loach ($P < 0.05$). The content of total amino acids (TAA), total essential amino acids (EAA), and delicious amino acids (DAA) in the muscle of pond loach was highest ($P < 0.05$). The content of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and linoleic acid was highest in pond loach, Taiwan loach and large-scale loach, respectively ($P < 0.05$). The trypsin activities and amylase activities of the pond loach were significantly higher than those of the large-scale loach and Taiwan loach in the intestine and liver ($P < 0.05$). These results indicate that the three kinds of loaches are of high nutritional value and have breeding prospects, among which pond loach has higher nutritional value.

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Introduction

Loach is widely distributed in various lakes and rivers in China. With delicious taste and high nutritional value, loach is reputed as ginseng in water and is an important freshwater economic fish in China (Fu et al., 2015). According to the China Fisheries Statistics Yearbook 2020 (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2020), the production of Chinese loach in 2019 was 356,900 tons. The protein composition of loach is higher than that of most conventional cultured fish, and its amino acid content is comprehensive, which is regarded as a good tonic for health. Loach, as a traditional export commodity in China, has a large market demand gap and is especially popular in Japan and South Korea.

Pond loach (*Misgurnus anguillicaudatus*) and large-scale loach (*Paramisgurnus dabryanus*) are the main cultured loaches in China. Compared with pond loach, large-scale loach has the advantages of larger body size and faster growth. In recent years, in addition to pond loach and large-scale loach, Taiwan loach (*Paramisgurnus dabryanus* ssp) has been introduced to some areas in the mainland of China. The taxonomic status of Taiwan loach is still unclear, and it is presumed to be a subspecies of *Paramisgurnus dabryanus* (Liang et al., 2018). Similarly, Taiwan loach has the characteristics of fast growth, short culturing cycle, high disease resistance, and mature fry breeding technology. At present, the three kinds of loaches are easily confused in the process of culturing and circulation, and the comparison of nutritional value among them is still not clear. Growth performance (Huang et al., 2015) and genetic variation (Fu et al., 2015) have been investigated in these three loaches. The comparison between pond loach and large-scale loach mainly focuses on meat quality (Xu and Hu, 2020), propagation performances (Fu et al., 2015), fecundity (Chu et al., 2012), and so on. You et al. (2017) found that large-scale loach and Taiwan loach are rich in muscle nutrient composition, which is fish with culturing prospects. The evidence showed that although the large-scale loach and Taiwan loach were similar, the discriminant function could be established by using the morphological indexes to distinguish them scientifically and quickly (Huang et al., 2016). Nevertheless, studies on the body index and nutrient composition including body composition, fatty acid content, and amino acid content of these three loaches are few. In addition, with the improvement of people's living standards, people have higher requirements for the nutrition and quality of aquatic products. Therefore, it is very necessary to study the nutritional value of these three loaches.

The intestine plays a key role in the digestion and absorption of nutrients. In addition, it is important to understand the digestive function of the loach to improve its cultural efficiency. Therefore, this study aimed to compare the body index, nutrient composition, and digestive enzyme of *Misgurnus anguillicaudatus*, *Paramisgurnus dabryanus*, and *Paramisgurnus dabryanus* ssp. Our findings will provide the theoretical basis for the comparative study of the nutritional value of these three kinds of loach and also guide the scientific selection of culturing loach varieties in production.

Materials and methods

Experimental fish and sample collection. The experimental fish were obtained from Linyi aquaculture farm (Linyi, China). 30 healthy and homogenous-sized loaches were randomly selected from pond loach (20.95±1.20), large-scale loach (17.02±0.53), and Taiwan loach (30.85±1.50), respectively, to determine the body index. Nine fish were taken from each group and anesthetized with MS-222 (100 mg/L; Sigma Chemical Company, St. Louis, MO, USA), and dorsal muscles were then immediately collected and stored at - 80°C, in which three fish were used for the dorsal muscle composition analysis, three for the dorsal muscle amino acid composition analysis and the last three for the dorsal muscle fatty acid composition analysis. After dorsal muscle samples were collected, the intestine and liver were excised and stored at - 80°C.

Chemical analysis

The analysis of the composition of the dorsal muscle was performed following the methods of AOAC (2003): Moisture was determined by oven drying at 105 °C until constant weight. According to the method described in Huang et al. (2021), Crude protein (N × 6.25) was determined by the Kjeldahl method using the semiautomatic Kjeldahl system (1030-Auto-analyzer, Tecator, Hoganos, Sweden) after acid digestion; crude lipid by ether extraction; ash by incineration at 550 °C for 4 h. Using the method in line with Fang et al. (2021), we measured the free amino acid contents by employing an automatic amino acid analyser. Fatty acids in the dorsal muscle were analyzed according to the protocols developed by Xu et al. (2022). Briefly, the fatty acid composition in the dorsal muscle was determined using a gas chromatograph (GC, Shimadzu GC-2010, Shimadzu, Japan). The intestine and liver samples were ground with buffer solution in the kit, and the activities of protease, amylase, and lipase in the intestine and liver were determined according to the requirements of the kit (Nanjing Jiancheng Bioengineering Research Institute Co. Ltd.). The protease activity was assayed following the Forint phenol-reagent method and the activities of lipase and amylase were assayed by the Colorimetric method.

Statistical analyses

All data were presented as mean ± SEM. The data were analyzed for significance by one-way ANOVA and then followed by Tukey's multiple range test using SPSS Software version 19.0 for Windows (International Business Machines Corporation, Armonk, New York, USA). P value less than 0.05 was considered statistically significant.

Result

Body index

As shown in **Table 1**, body weight / body length (W/L) was highest in the Taiwan loach, followed by the pond loach ($P < 0.05$). body height / body length (H/L) and condition factor (CF) of Taiwan loach were significantly higher than that of pond loach and large-scale loach ($P < 0.05$). Taiwan loach had the highest viscerosomatic ratio (VR) ($P < 0.05$), while those pond loach and large-scale loach had no significant differences ($P > 0.05$). Hepatosomatic index (HSI) and Intestosomatic index (ISI) were not significantly different between pond loach, large-scale loach, and Taiwan loach ($P > 0.05$).

Table 1 Overview of body index in three loaches.

Items	**MA**	**PD**	**PDS**
W/L	1.49 ± 0.06^{b}	1.30 ± 0.03^{c}	2.05 ± 0.08^{a}
H/L	0.14 ± 0.00^{b}	0.14 ± 0.00^{b}	0.15 ± 0.00^{a}
HSI[1]	1.16±0.06	0.99±0.06	1.04±0.08
VR[2]	4.19 ± 0.14^{ab}	3.78 ± 0.11^{b}	4.70 ± 0.24^{a}
ISI[3]	0.95±0.06	0.98±0.04	0.84±0.06
CF[4]	0.78 ± 0.02^{b}	0.77 ± 0.01^{b}	0.92 ± 0.02^{a}

Note, MA, *Misgurnus anguillicaudatus*; PD, *Paramisgurnus dabryanus*; PDS, *Paramisgurnus dabryanus ssp*. W, body weight; L, body length; H, body height. Values are presented as mean ± SEM (n = 3). Values with different superscripts in the same row are significant ($P < 0.05$).

[1]Hepatosomatic index, (HSI) = 100 × (liver weight, g) / (body weight, g);

[2]Viscerosomatic ratio, (VR) = 100 × (viscera weight, g) / (body weight, g);

[3]Intestosomatic index, (ISI) = 100 × (Intestine weight, g) / (body weight, g);

[4]Condition factor, (CF) = 100 × W/L^3, where W is weight (g), and L is length (cm);

Muscle composition

The results for the dorsal muscle composition are presented in **Table 2**. Pond loach showed significantly higher crude protein compared to that large-scale loach and Taiwan loach ($P < 0.05$). On the contrary, the crude lipid was highest in large-scale loach and Taiwan loach ($P <$

0.05), with no significant differences between large-scale loach and Taiwan loach ($P > 0.05$). There were no significant differences in dorsal muscle moisture and ash between pond loach, large-scale loach, and Taiwan loach ($P > 0.05$).

Table 2 Overview of muscle composition (%) in three loaches.

Items	**MA**	**PD**	**PDS**
Moisture	76.89±0.62	75.38±1.04	73.81±0.87
Crude protein	18.15±0.41[a]	17.45±0.38[b]	17.10±0.45[b]
Crude lipid	3.62±0.19[b]	4.03±0.14[a]	4.21±0.13[a]
Ash	1.35±0.10	1.24±0.07	1.17±0.09

Note, MA, *Misgurnus anguillicaudatus*; PD, *Paramisgurnus dabryanus*; PDS, *Paramisgurnus dabryanus ssp*. W, body weight; L, body length; H, body height. Values are presented as mean ± SEM (n = 3). Values with different superscripts in the same row are significant ($P < 0.05$).

Free amino acid compositions in muscle

As shown in **Table 3**, the content of total amino acids (TAA), total essential amino acids (EAA), and delicious amino acids (DAA) in the muscle of pond loach were highest ($P < 0.05$). Meanwhile, we found that the Asp, Glu, Gly, Ala, and Phe content of pond loach was significantly higher than that of the other groups. The Tyr and Pro content of pond loach and large-scale loach was significantly higher than that of Taiwan loach ($P < 0.05$). The content of Thr, Val, Leu, and Lys in the muscle of pond loach showed the highest level and was significantly higher than that of large-scale loach and Taiwan loach ($P < 0.05$). The content of Met for the pond loach was the highest, followed by the large-scale loach, and the Taiwan loach has the lowest Met content ($P < 0.05$). We also found that the differences in the contents of Ser, Cys, Ile, His, and Arg were not significant among all groups ($P > 0{\cdot}05$).

Table 3 Free amino acid compositions (% tissue) in the muscle of three loaches.

Amino acids	**MA**	**PD**	**PDS**
EAA[1]			
Arg	5.27±0.03	5.02±0.06	4.63±0.04
His	2.03±0.04	1.97±0.07	2.07±0.03
Ile	3.35±0.07	2.97±0.09	2.85±0.03
Leu	7.04±0.04[a]	6.34±0.07[b]	6.00±0.05[b]
Lys	8.50±0.07[a]	7.48±0.11[b]	7.06±0.03[b]
Met	1.10±0.01[a]	0.67±0.03[b]	0.37±0.02[c]
Phe	3.57±0.05[a]	3.12±0.06[b]	3.08±0.04[b]
Thr	4.21±0.05[a]	3.94±0.02[b]	3.69±0.01[b]
Val	3.56±0.05[a]	3.21±0.08[b]	3.12±0.02[b]
NEAA[2]			
Glu	16.36±0.13[a]	15.24±0.08[b]	14.16±0.04[c]
Pro	2.79±0.05[a]	2.80±0.02[a]	2.52±0.03[b]
Gly	4.52±0.03[a]	4.16±0.06[b]	3.89±0.02[b]
Ala	5.72±0.09[a]	5.19±0.06[b]	4.89±0.04[b]
Cys	0.81±0.02	0.82±0.03	0.82±0.01
Asp	9.63±0.04[a]	8.54±0.05[b]	8.09±0.03[c]
Tyr	2.55±0.03[a]	2.43±0.06[a]	1.74±0.04[b]
Ser	3.98±0.02	3.75±0.05	3.44±0.02
TAA[3]	84.98±0.82[a]	77.64±1.03[b]	72.43±0.79[c]
EAA	36.97±0.98[a]	33.21±0.07[b]	30.92±0.08[b]
DAA[4]	42.35±0.21[a]	38.68±0.33[b]	35.85±0.04[c]

Note, MA, *Misgurnus anguillicaudatus*; PD, *Paramisgurnus dabryanus*; PDS, *Paramisgurnus dabryanus ssp*. Values are presented as mean ± SEM (n = 3). Values with different superscripts in the same row are significant ($P < 0.05$).
[1]EAA, total essential amino acids;
[2]NEAA, non-essential amino acid;
[3]TAA, total amino acids;
[4]DAA, delicious amino acids (Asp, Glu, Gly, Ala, Tyr, Phe).

Fatty acid composition in muscle

There was no significant difference in SFA among all groups (**Table 4**). The level of PUFA in pond loach was highest among all groups while Taiwan loach had the highest MUFA ($P < 0.05$). The large-scale loach showed the highest proportion of linoleic acid (18:2n-6) in the muscle ($P < 0.05$). The muscle proportions of arachidonic acid (C20:4n-6) and total n-6 PUFA contents were highest in pond loach and large-scale loach ($P < 0.05$), with no significant differences between pond loach and large-scale loach ($P > 0.05$). The percentage of linolenic acid (18:3n-3) and EPA (20:5n-3) were highest in the muscle of the pond loach ($P < 0.05$). Muscle DHA (22:6n-3) content was highest in the Taiwan loach, followed by the pond loach ($P < 0.05$). Both EPA + DHA and total n-3 PUFA contents in the muscle of pond loach were higher than those in other groups, followed by the Taiwan loach ($P < 0.05$).

Table 4 Fatty acid composition (% of total fatty acid) in the muscle of three loaches.

Fatty acids	**MA**	**PD**	**PDS**
C14:0	1.39±0.03^{b}	1.04±0.04^{c}	1.68±0.07^{a}
C15:0	0.65±0.02^{a}	0.56±0.01^{a}	0.31±0.01^{b}
C16:0	14.71±0.47^{b}	14.78±0.33^{b}	17.63±0.32^{a}
C17:0	1.14±0.07^{a}	0.86±0.04^{b}	0.28±0.01^{c}
C18:0	5.90±0.24^{a}	4.28±0.16^{b}	3.10±0.15^{c}
C20:0	0.28±0.01	0.29±0.02	0.22±0.04
C22:0	0.49±0.01^{a}	0.48±0.03^{a}	0.14±0.01^{b}
∑SFA	24.56±0.83	22.29±0.63	23.36±1.14
C16:1	8.38±0.44^{c}	13.11±0.47^{a}	8.91±0.36^{c}
C18:1	6.60±0.23^{c}	10.67±0.06^{b}	21.50±0.17^{a}
C20:1	0.33±0.02^{b}	0.34±0.01^{b}	1.24±0.04^{a}
C22:1	0.18±0.01^{b}	0.16±0.01^{b}	0.41±0.01^{a}
∑MUFA	15.49±0.68^{c}	24.28±0.50^{b}	32.06±0.17^{a}
C18:2n-6	5.81±0.35^{b}	6.84±0.27^{a}	3.21±0.10^{c}
C20:2n-6	0.85±0.02	0.59±0.04	0.68±0.03
C20:3n-6	1.33±0.01^{a}	1.12±0.02^{b}	0.72±0.02^{c}
C20:4n-6	5.26±0.29^{a}	5.68±0.32^{a}	0.90±0.06^{b}
C22:5n-6	3.15±0.12^{a}	2.01±0.06^{b}	1.10±0.10^{c}
∑n-6	16.40±0.16^{a}	16.24±0.13^{a}	6.61±0.05^{b}
C18:3n-3	4.04±0.06^{a}	3.34±0.21^{b}	2.75±0.04^{b}
C20:5n-3	5.42±0.14^{a}	2.67±0.25^{b}	1.74±0.12^{c}
C22:6n-3	3.82±0.13^{b}	2.07±0.15^{c}	5.49±0.12^{a}
∑n-3	13.28±0.24^{a}	8.08±0.19^{c}	9.98±0.13^{b}
EPA+DHA	9.24±0.12^{a}	4.74±0.07^{c}	7.23±0.04^{b}
∑PUFA	29.68±0.25^{a}	24.32±0.37^{b}	16.59±0.13^{c}

Notes. Values are presented as mean ± SEM (n = 3). Values with different superscript letters in the same row are significant ($P < 0.05$). MA, *Misgurnus anguillicaudatus*; PD, *Paramisgurnus dabryanus*; PDS, *Paramisgurnus dabryanus* ssp.; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Linolenic acid, 18:3n–3; Linoleic acid, 18:2n–6; Arachidonic acid, 20:4n–6; EPA: eicosapentaenoic acid (20:5n–3); DHA: docosahexaenoic acid (22:6n–3).

Digestive enzyme activity in the intestine and liver

The results of digestive enzyme activity measurements are presented in **Figure 1**. The trypsin activities and amylase activities of the pond loach were significantly higher than those of the large-scale loach and Taiwan loach in the intestine and liver ($P < 0.05$). The activity of lipase was higher in the intestine of the large-scale loach and Taiwan loach than that of the pond loach ($P < 0.05$). In addition, the highest level of lipase appeared in the liver of the Taiwan loach, while the pond loach had the lowest level ($P < 0.05$).

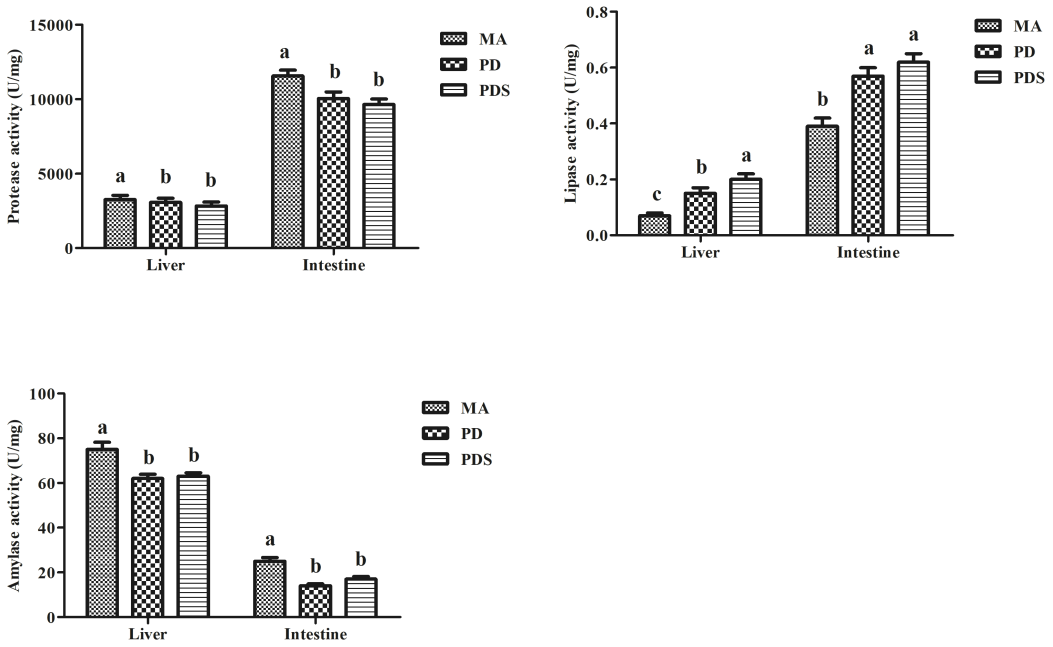

Figure 1 Digestive enzyme activity in the muscle and liver of three loaches. Note, MA, *Misgurnus anguillicaudatus*; PD, *Paramisgurnus dabryanus*; PDS, *Paramisgurnus dabryanus* ssp. Values are presented as mean ± SEM (n = 3). Values with different superscripts in the same row are significant ($P < 0.05$).

Discussion

In fish classification, morphological observation is the most intuitive classification method, and it is important to use the key morphological indicators to identify fish. In the present study, the morphology of the three loaches was different. Compared with the traditional discriminant method of loach based on the number of lateral line scales, Huang et al. (2016) established the discriminant function through 8 morphological indexes, which could distinguish large-scale loach and Taiwan loach 100%. In this study, large-scale loach and Taiwan loach can be distinguished successfully by the morphological function equation. In addition, studies have shown that body height/body length and body weight/body length can be used as the characteristic indexes to identify pond loach and large-scale loach. Similarly, in the present study, compared with pond loach and large-scale loach, Taiwan loach has a higher body weight/body length ratio and body height/body length ratio, while pond loach and large-scale loach have different body weight/body length ratio. High HSI and VSI are often related to poor growth and fish health due to increased levels of dietary carbohydrates (Moreira et al., 2008; Habte-Tsion et al., 2013). However, a 60-day culturing experiment showed that there was no significant difference between pond loach and large-scale loach, while the weight gain of Taiwan loach was highest, indicating that Taiwan loach grew fast than pond loach and large-scale loach (Huang et al., 2015). This may be related to their independent genetic structure (Fu et al., 2015). Our study indicated that there are morphological differences among these three loaches, and they can be identified according to the body indicators.

The protein and lipid content of fish determines its nutritional value. In the present study, our results showed that the three loaches showed high protein and low fat characteristics. Compared with large-scale loach and Taiwan loach, pond loach showed higher crude protein content in muscle, while the crude lipid content in pond loach was the lowest. On the contrary, Xu et al. (2020) reported that the crude protein content of pond loach was lower than large-

scale loach, which may be related to the different culturing environments and feeding methods. Generally, the taste and flavor of fish improve as the lipid content of fish increases (Szczesniak, 1963). In this study, the crude lipid content of large-scale loach and Taiwan loach was significantly higher than pond loach, indicating that the meat quality of large-scale loach and Taiwan loach was oilier and juicier. Therefore, combined with the results of crude protein, pond loach may be more suitable for people to reduce fat and increase muscle consumption.
The muscle amino acid and fatty acids composition is usually used as an indicator of nutritional value. Meanwhile, muscle free amino acids and fatty acids are major flavor contributors and important flavor precursors in fish, respectively (Grigorakis, 2007). In general, EAA and DAA in fillets can be used to evaluate the flavor and quality, and it is generally accepted that Glu and Asp promote umami, while Ala and Gly are essential for sweetness (Lioe et al., 2018). In this study, high EAA and DAA contents were observed in the three loaches, which were similar to Nile tilapia (*Oreochromis niloticus*) (Wu et al., 2022a), but higher than grass carp (Wu et al., 2022b). Our result indicated that the three kinds of loach have high amino acid nutritional value. In the present study, Leu, Lys, Met, Phe, Thr, and Val were significantly higher in EAA of the pond loach compared to the other loach. In addition, the content of DAA was also highest in the pond loach, including Asp, Glu, Ala, and Gly, followed by large-scale loach. Therefore, pond loach has better nutritional and flavor values of fillets to some extent.
The fatty acid composition of the muscle is another indicator of nutritional value, and lower SFA and higher unsaturated fatty acids especially long-chain polyunsaturated fatty acids (LC-PUFA) mean better nutritive value. Linolenic acid (18:3n–3) and linoleic acid (18:2n–6) are essential fatty acids of freshwater fish. LC-PUFA such as EPA and DHA have potential benefits for human health including reducing blood pressure (Rasmussen et al., 2006). Our results shown that the percentage of linolenic acid and linoleic acid were higher in pond loach groups. In addition, both n-3 PUFA, n-6 PUFA, EPA and DHA were also present at high levels in pond loach, indicating that the nutritional quality of pond loach is higher than that of large-scale loach and Taiwan loach.

For aquatic animals, the hepatopancreas and intestines are important digestive organs, and the activity of digestive enzymes can be directly reflected in the nutrient digestion capacity, nutritional status, and growth performance of aquatic animals (Haghparast et al., 2019). Among the class of digestive enzymes amylase, lipase and protease are the most important ones. However, the digestive capacity of fish tissues is different (Mardones et al., 2022). Similarly, in this study, the intestinal protease and lipase activities were higher than those in the liver, indicating that the intestinal undertook more protein and lipid digestion functions. In addition, previous studies have shown that there was a strong correlation between digestive enzyme activities and growth performance (Luo et al., 2020; Wang & Xu, 2006). In this work, we observed that the protease activity of three loaches was extremely high, suggesting that they had a strong ability to decompose proteins. This may be responsible for their high protein and total amino acid content. In the present study, the activities of protease and amylase in the liver and intestine of pond loach were significantly higher than that of large-scale loach and Taiwan loach, while lipase was the lowest. However, large-scale loach and Taiwan loach showed the same digestive ability with no difference in intestinal and liver digestive enzyme activities. Our results suggest that the digestive capacity of pond loach is different from large-scale loach and Taiwan loach, which may be due to their different intestinal microbiota microbial (Cai et al., 2022).

Conclusion

In summary, our findings showed that pond loach, large-scale loach and Taiwan loach can be classified by morphological indicators. Both three kinds of loaches have the characteristics of high protein and low lipid, and high essential amino acid and PUFA content. Thus, the three kinds of loaches are of high nutritional value and have breeding prospects. In terms of amino

acid and fatty acid composition, pond loach has higher nutritional value than large-scale loach and Taiwan loach, and the protease and amylase activities of pond loach were the highest. Therefore, through comprehensive comparison, pond loach has a higher nutritional value.

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