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# Effects of starvation and refeeding on digestive enzyme activity of *Megalobrama pellegrini*

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## ABSTRACT

The aim of this research is to explore the effects of starvation and refeeding on the digestive enzyme activity of *Megalobrama pellegrini*, to find out its regularity and enrich its starving physiology. In this experiment, *Megalobrama pellegrini* were divided into five groups, as starved for 0(control), 2, 4, 7, 14day and refed for 14day with enough food. The specific activities of protease, lipase and amylase were determined during starvation and refeeding. The results showed that effects of different level of protease, lipase and amylase were produced by starvation. Along with starved time extension, the activities of protease, lipase and amylase overall presented downward trend, and the activity presented stabilized in the late starvation. The amylase and lipase activity of intestinal significantly decreased at first and then after a slight downward trend in rising. The specific activities of protease, lipase and amylase were increased rapidly after refeeding, and reached a peak when refeed two days. As continue refeeding the activity gradually recovered to the level before starvation.



## KEYWORDS

*Megalobrama pellegrini*;
Starvation;
Refeeding;
Digestive enzymes.

## INTRODUCTION

Digestive enzyme is a kind of ferment which is effective on digestion, and is secreted by digestive gland and alimentary canal. We can find some studies about the researches of fish's digestive enzyme, but most of them focus on the following aspects: the type and the physics and chemistry characteristics[1-3] of fish's digestive enzyme, the distributed law[4,5] of the digestive enzyme in fish's organs, the environment and the physics and chemistry characteristic's effects[6-8] on digestive enzyme, etc. In the recent years, the cultivation scale developed rapidly, and as a result, more and more studies which were about the influence of fodder on fish's digestive enzyme appeared[9].

*Megalobrama pellegrini*, because of its high economic value and better growth performance, and due to the loss of habitat the population sizes of

*Megalobrama pellegrini* decreased sharply, is become one kind of important breeding species gradually. Now, many researches focus on the biological characterization, life history type, disease control and artificial breeding, however, more starvation physiology information about it is still lacking. In this study, the change process of the activity of protease, amylase and lipase of *Megalobrama Pellegrini* during starvation and re-feeding was analyzed. This work is helpful for us to provide theoretical reference to the cultivation of *Megalobrama Pellegrini*, and could direct aquiculture more correctly and more scientifically.

## MATERIALS AND METHODS

### Source and taming of tested fish

The tested *Megalobrama Pellegrinis* were provided by Chongqing Dongping Aquatic Products Co. Ltd., which is the science research exercitation base of the Department of Fisheries, College of Animal Science and Technology of Southwest University. In June, 2009, 800 *Megalobrama Pellegrinis* in oxygenated bags were brought to laboratory[10]. After 15-minute sterilizing by 3% concentrated salty water, those *Megalobrama Pellegrinis* were kept in 8 aquatic tanks which sizes were 40cm×50cm×90cm, and the water kept 40cm depth in short duration. The *Megalobrama Pellegrinis* were fed complete formula feed that produced by Chongqing Tongwei Feedstuff Co. Ltd. twice a day, at 9 a.m. and 4 p.m. respectively, and each feeding lasted for 30-minute[11]. Before next feeding, the left bait and excretion would be sucked out by siphon tube. The water should be changed depending on the circumstances.

### Experiment design

The experiment was set up one control-group and four starve-handling-groups, each group had 30 fishes, and each time experimenters settled three repetitions: after feeding for 15 days, experimenters selected 450 fishes randomly, and average them into 15 aquatic tanks. Then they divided those tanks into 5 groups, and each group included 3 parallel-groups which were starve-0-day (S0), starve-2-day (S2), starve-4-day (S4), starve-7-day (S7), and starve-14-day (S14). After starving, the re-feeding would last 14-day. During the experiment, the daily management referred to that in short duration.

### Experiment method

### Preparation of analytic sample of digestive enzyme

The experimenters took 6 tested-fishes out of every group(2 fishes per parallel-group) before starvation, after starvation and after re-feeding 2-day, 4-day, 7-day and 14-day respectively: those fishes would be dissected after being weighed, took out the hepatopancreas and intestinal track quickly, and eliminated the adiposity. The intestinal track would be cut in halves, washed by freezing normal saline and wiped up by absorbent cotton and weighed. After then, experimenters bathed organs in ice, homogenated them, and centrifugalized those organs for 30-minute under the circumstances of 4！and 12000rpm, and took the supernatant fluid to test the activity of protease, amylase and lipase of hepatopancreas and intestinal track respectively.

### Determination of the activity of digestive enzyme

The experimenters adopted the testing-boxes produced by Nanjing Jiancheng Biology Research Institute to determine the activity of protease, amylase and lipase. The tested method and the enzyme activity unit were referred to the instruction. The definition of the protease activity is: under the circumstance of pH8.0, 37°C, the trypsinase which in one milligram of protein could make the absorbance change 0.003/min called an enzyme activity unit. The definition of the amylase activity is: under the circumstance of 37°C, one milligram of tissue proteins, reacting with substrate in 1 minute in this reaction system and consuming 1 umol substrate, called an enzyme activity unit. The definition of the lipase activity is: under the circumstance of 37°C, one milligram protein, reacting with substrate in 30 minutes and hydrolysis 10 mg starch, called an enzyme activity unit.

### Data processing

The experimenters used SPASS 10.0 to count and analyze those data, and used Kolmogorov-Smimov and Levene to verify the normality and the homogeneity of the variance respectively before more analyses. On the other hand, the experimenters would use ANOVA to analyze the relevant data, and adopt Post-hoc, Tukey's

FULL PAPER

**TABLE 1 : Changes of protease activity of *Megalobrama pellegrini***

| Tissue | U/mg prot | | | | |
|---|---|---|---|---|---|
| | S0 | S2 | S4 | S7 | S14 |
| Liver | 13788.54 ± 123.21[a] | 10526.05 ± 137.63[b] | 9722.93 ± 130.75[b] | 8720.70 ± 119.99[c] | 8442.50 ± 113.87[c] |
| Intestine | 19120.28 ± 120.08[a] | 12213.97 ± 232.42[b] | 10544.00 ± 128.66[c] | 9609.13 ± 123.22[d] | 9173.31 ± 225.09[d] |

**TABLE 2 : Changes of protease activity of *Megalobrama pellegrini* after refeeding**

| Group | Tissue | Before starvation | Time of refeeding(d) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 2 | 4 | 7 | 14 |
| S2 | Liver | 13582.22 ± 133.41[a] | 10526.05 ± 137.63[b] | 14132.23 ± 110.24[a] | 13658.02 ± 143.43[a] | 13442.45 ± 101.73[a] | 13570.48 ± 150.81[a] |
| | Intestine | 19229.18 ± 127.23[a] | 12213.97 ± 232.42[b] | 21006.42 ± 273.68[a] | 20075.38 ± 245.37[a] | 19403.69 ± 202.51[a] | 19334.88 ± 236.74[a] |
| S4 | Liver | 13705.79 ± 103.56[a] | 9722.93 ± 130.75[b] | 14135.51 ± 134.62[a] | 13634.72 ± 100.39[a] | 13682.47 ± 128.56[a] | 13692.77 ± 113.35[a] |
| | Intestine | 18994.43 ± 101.69[a] | 10544.00 ± 128.66[b] | 20064.25 ± 141.34[a] | 19877.82 ± 118.71[a] | 18847.39 ± 99.53[a] | 19011.73 ± 121.65[a] |
| S7 | Liver | 13812.75 ± 150.66[a] | 8720.70 ± 119.99[b] | 14830.68 ± 131.91[c] | 14553.92 ± 108.67[c] | 13786.55 ± 142.34[a] | 13792.77 ± 122.68[a] |
| | Intestine | 19006.85 ± 131.71[a] | 9609.13 ± 123.22[b] | 21268.46 ± 133.56[c] | 20154.77 ± 113.28[c] | 18974.83 ± 130.07[a] | 19202.50 ± 103.36[a] |
| S14 | Liver | 13663.52 ± 137.07[a] | 8442.50 ± 113.87[b] | 14897.72 ± 124.61[c] | 14626.58 ± 139.07[cd] | 13908.53 ± 101.83[ad] | 13772.48 ± 124.54[a] |
| | Intestine | 18875.74 ± 144.02[a] | 9173.31 ± 225.09[b] | 22337.52 ± 305.43[c] | 21707.35 ± 214.81[cd] | 20057.83 ± 263.66[ad] | 19434.31 ± 225.33[ad] |

to compare and to verify them. The descriptive statistic data expressed by average value ± standard deviation. The significance level was hypothesized as a = 0. 05.

## RESULTS AND ANALYSIS

### Effects of starvation and refeeding on protease activity of *Megalobrama pellegrini*

### Changes of *Megalobrama pellegrini*'s protease activity during starvation

During the starvation, the protease activity of *Megalobrama Pellegrini* was in downward trend (TABLE 1). Being starved for 2 days, the protease activity declined obviously, which had marked differences from the control-group($p<0.05$); being starved for 4 days, it continued to decline, while the protease activity of hepatopancreas had no marked differences from the S2($p>0.05$), and the protease activity of intestinal track was apparently different from S2($p<0.05$); being starved for 7 days, the protease activity had an obvious decline compared with S4; after 14 days of starvation, the protease activity was tending towards stability.

### Changes of *Megalobrama pellegrini*'s protease activity during refeeding

The protease activity of *Megalobrama Pellegrini* rose again after re-feeding and reached a peak value when re-feeding had lasted for 2 days, then slowly came back to the standard before starvation. But every testing-group had their own characteristics which were not exactly the same (TABLE 2): after refeeding for 2 days, the protease activity in S2 and S4 came back to the standard before starvation; while in S7 and S14, its standard was obviously higher than before($p<0.05$), and at the 7th day of refeeding, the protease activity declined to the standard before starvation($p>0.05$).

### Changes of *Megalobrama pellegrini*'s lipase activity during refeeding

Refeeding *Megalobrama pellegrini* after starvation, the lipase activity had a similar change to the protease activity: after re-feeding for 2 days, they reached a peak value, and then went back to the standard before starvation. But they were not completely the same (TABLE 4): in S2, S4 and S7, when re-feeding contin-

**TABLE 3 : Changes of lipase activity of *Megalobrama pellegrini***

| Tissue | U/g prot | | | | |
|---|---|---|---|---|---|
| | S0 | S2 | S4 | S7 | S14 |
| Liver | $26.62 \pm 4.23^{a}$ | $24.54 \pm 3.21^{b}$ | $23.23 \pm 4.80^{b}$ | $21.10 \pm 2.30^{c}$ | $20.80 \pm 2.75^{c}$ |
| Intestine | $55.39 \pm 6.75^{a}$ | $46.97 \pm 3.87^{b}$ | $43.59 \pm 2.29^{b}$ | $50.71 \pm 1.89^{c}$ | $37.62 \pm 3.71^{d}$ |

**TABLE 4 : Changes of lipase activity of *Megalobrama pellegrini* after refeeding**

| Group | Tissue | Before starvation | Time of refeeding(d) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 2 | 4 | 7 | 14 |
| S2 | Liver | $26.57 \pm 4.73^{a}$ | $24.54 \pm 3.21^{b}$ | $27.98 \pm 3.88^{a}$ | $26.46 \pm 3.62^{a}$ | $26.13 \pm 4.01^{a}$ | $25.89 \pm 4.83^{a}$ |
| | Intestine | $57.18 \pm 5.78^{a}$ | $46.97 \pm 3.87^{b}$ | $59.44 \pm 7.03^{c}$ | $57.78 \pm 5.72^{a}$ | $56.39 \pm 6.83^{a}$ | $56.57 \pm 4.91^{a}$ |
| S4 | Liver | $27.12 \pm 5.06^{a}$ | $23.23 \pm 4.80^{b}$ | $28.23 \pm 5.17^{a}$ | $27.61 \pm 5.61^{a}$ | $27.03 \pm 4.62^{a}$ | $27.44 \pm 4.21^{a}$ |
| | Intestine | $55.89 \pm 6.32^{a}$ | $43.59 \pm 2.29^{b}$ | $58.03 \pm 7.73^{c}$ | $56.29 \pm 5.05^{a}$ | $54.32 \pm 4.71^{a}$ | $55.34 \pm 6.18^{a}$ |
| S7 | Liver | $26.88 \pm 4.01^{a}$ | $21.10 \pm 2.30^{b}$ | $27.82 \pm 5.26^{a}$ | $27.08 \pm 3.51^{a}$ | $26.62 \pm 5.25^{a}$ | $27.00 \pm 3.23^{a}$ |
| | Intestine | $55.17 \pm 4.98^{ab}$ | $50.71 \pm 1.89^{c}$ | $58.34 \pm 7.05^{d}$ | $57.09 \pm 4.36^{ad}$ | $55.08 \pm 4.75^{b}$ | $54.98 \pm 6.65^{b}$ |
| S14 | Liver | $25.97 \pm 4.66^{a}$ | $20.80 \pm 2.75^{b}$ | $28.63 \pm 3.77^{c}$ | $26.66 \pm 4.19^{a}$ | $25.62 \pm 5.23^{a}$ | $26.12 \pm 6.21^{a}$ |
| | Intestine | $55.69 \pm 6.75^{a}$ | $37.62 \pm 3.71^{b}$ | $59.87 \pm 7.15^{c}$ | $56.39 \pm 5.68^{a}$ | $55.39 \pm 6.75^{a}$ | $55.20 \pm 6.46^{a}$ |

**TABLE 5 : Changes of amylase activity of *Megalobrama pellegrini***

| Tissue | u/dl | | | | |
|---|---|---|---|---|---|
| | S0 | S2 | S4 | S7 | S14 |
| Liver | $2.75 \pm 0.09^{a}$ | $2.18 \pm 0.35^{b}$ | $1.93 \pm 0.62^{c}$ | $1.26 \pm 0.17^{d}$ | $1.15 \pm 0.14^{d}$ |
| Intestine | $1.20 \pm 0.06^{a}$ | $0.80 \pm 0.79^{b}$ | $1.12 \pm 0.08^{a}$ | $0.53 \pm 0.08^{c}$ | $0.51 \pm 0.07^{c}$ |

ued for 2 days, the liver's lipase activity went back to the standard before starvation($p>0.05$), while the intestine's were much higher than before($p<0.05$); in S14, when re-feeding had lasted for 2 days, the lipase activity in liver and intestine both were much higher than before($p<0.05$). After 4 days' refeeding, the lipase activity of every group had gone back to the standard before starvation($p>0.05$).

## Effects of starvation and re-feeding on amylase activity of *Megalobrama pellegrini*

### Changes of *Megalobrama pellegrini*'s amylase activity during starvation

During starvation, liver's amylase activity of *Megalobrama pellegrini* was in downward trend, while the amylase activity of intestine had a wavy change in different time: it declined first, picked up later and finally declined again (TABLE 5). Being starved for 2 days, the amylase activity of liver and intestine had a linear decrease ($p<0.05$); after 4 days' starvation, liver's amylase activity was still in a decrease ($p<0.05$); while intestine's had gone back to the standard of the control-group($p>0.05$); when starvation lasted for 7 days, the amylase activity in liver and intestine both had an apparent decrease($p<0.05$); and in the 14th day of starvation, both activity declined slowly($p>0.05$).

### Changes of *Megalobrama pellegrini*'s amylase activity during refeeding

When starvation had restarted, the amylase activity of *Megalobrama pellegrini* was similar to the activity of protease and lipase: it rose quickly and reached a standard higher or much higher before starvation, and then decreased slowly and stabilized to the standard before starvation (TABLE 6).

## DISCUSTION

When fish is threatened by starvation, its body's metabolism will have some changes to adapt the situation, and one of them is adjusting enzymes' activity by changes to use the substances in body reasonably and to keep alive. According to the changes of the enzymes' activity in fish body, the experimenters can analyze fish's metabolic state and nutritive state; but the abilities of adjustment are differ from the somatotype, the breed and starved degree of fishes[12].

The experiment shows that starvation has different effects on the activity of protease, lipase and amylase.

FULL PAPER

**TABLE 6 : Changes of amylase activity of *Megalobrama pellegrini* after refeeding**

| Group | Tissue | Before starvation | Time of refeeding(d) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 2 | 4 | 7 | 14 |
| S2 | Liver | $2.80 \pm 0.33^{a}$ | $2.18 \pm 0.35^{b}$ | $2.95 \pm 0.41^{a}$ | $2.85 \pm 0.31^{a}$ | $2.75 \pm 0.29^{a}$ | $2.82 \pm 0.19^{a}$ |
| | Intestine | $1.30 \pm 0.08^{a}$ | $0.80 \pm 0.79^{b}$ | $1.41 \pm 0.26^{a}$ | $1.37 \pm 0.34^{a}$ | $1.29 \pm 0.08^{a}$ | $1.26 \pm 0.06^{a}$ |
| S4 | Liver | $2.78 \pm 0.21^{a}$ | $1.93 \pm 0.62^{b}$ | $3.02 \pm 0.44^{a}$ | $2.75 \pm 0.38^{a}$ | $2.84 \pm 0.49^{a}$ | $2.71 \pm 0.19^{a}$ |
| | Intestine | $1.26 \pm 0.16^{a}$ | $1.22 \pm 0.08^{a}$ | $1.45 \pm 0.19^{a}$ | $1.33 \pm 0.20^{a}$ | $1.24 \pm 0.05^{a}$ | $1.25 \pm 0.06^{a}$ |
| S7 | Liver | $2.70 \pm 0.19^{a}$ | $1.26 \pm 0.17^{b}$ | $3.05 \pm 0.63^{a}$ | $2.85 \pm 0.28^{a}$ | $2.71 \pm 0.42^{a}$ | $2.70 \pm 0.33^{a}$ |
| | Intestine | $1.27 \pm 0.08^{a}$ | $0.53 \pm 0.08^{b}$ | $1.45 \pm 0.26^{c}$ | $1.28 \pm 0.31^{a}$ | $1.24 \pm 0.12^{a}$ | $1.26 \pm 0.27^{a}$ |
| S14 | Liver | $2.82 \pm 0.23^{a}$ | $1.15 \pm 0.14^{b}$ | $3.25 \pm 0.54^{c}$ | $3.05 \pm 0.37^{ac}$ | $2.75 \pm 0.46^{a}$ | $2.80 \pm 0.18^{a}$ |
| | Intestine | $1.29 \pm 0.15^{a}$ | $0.51 \pm 0.07^{b}$ | $1.54 \pm 0.38^{c}$ | $1.30 \pm 0.32^{a}$ | $1.26 \pm 0.08^{a}$ | $1.26 \pm 0.14^{a}$ |

As starvation time goes on, overall enzymes' activity have a declined trend, and at the beginning of starvation, the activity drop greatly and this phenomenon has been found in other fish's researches. After starvation for 1-2 days, the activity of protease and trypsinase in Clupea Harengus' intestine would decline[13]. Starvation had effect on Psetta Maxima's digestion: the non-ingested Psetta Maximas had lower activity of digestion enzyme than the ingested[14]. Ingestion would make fishes secrete chlprhydric acid which enter stomachs to improve the activity of protease; while, in most of the starved fishes, their stomachs were neutral, and the activity of protease in stomachs was low[15]. The activity of protease of starved Weever had a distinct decline[16].Sergio compared the activity of protease and lipase of juvenile Paralichthys Olivaceus in each growth-stage[17]: being incubated for 3 days and starved for 1 day, the activity of protease dropped obviously, and the activity of lipase had an apparent decline after 3 days starvation; being incubated for 25 days and starved for 2 days and 4 days, compared with control-group, the activity of protease declined greatly. Gao Lujiao discovered that starvation had clear effects on the activity of digestive enzyme of Amur Sturgeon: the activity of protease, lipase and amylase, all of them were in a downward trend[18]. Those existing researches believed that there were four reasons why starvation made the activity of enzymes change. Firstly, in starvation, the whole alimentary canal isn't stimulated by the peristalsis of food, which has effects on the secretion of enzyme[19]. Secondly, by olfaction, optesthesia and other sense organs, food would affect central nervous system of digestive gland secretion. In starvation, lacking of sense organs' stimulation would affect the feedback of information, and then change the secretion of enzyme[20]. Thirdly, starvation would cause the decline of the thickness of gastric glands and the height of intestinal epithelium cell; at the same time, it would make liver's organization structure change. All of those are substantial changes of digestive organs and lead them secret digestive enzyme less and less. Fourthly, organs lacking of energy, fishes were in a special state of anti-excitable, and on the basis of environment, organs would change the way of metabolism and the secretion of enzyme to adapt new environment. In the later period, fishes may in unrecovered-state, and those organs would suffer metabolic disorder.

In this experiment, the activity of amylase began rebound after 2-day-starvation, and reached the maximum at the 4th day (TABLE 5). The activity of lipase in intestine began to rise after the 4th day, and at the 7th day, it reached a peak (TABLE 3). This result is similar to Zheng Shuming's research to *Heterodontus*[21] and Gao Lujiao's research to Amur Sturgeon[18]. Experimenters have analyzed the reason: maybe when *Megalobrama pellegrini* feels hungry, it uses glycogen first to response and then is sebum. The short-time starved stimulation and the use of glycogen make the secretion increase, and absorb those leftovers in intestinal tract to maintain normal activity. As a result, being starved for 4 days, *Megalobrama pellegrini*'s activity of amylase in intestinal track rebounded. After a short stress response, testing fish was in a persistent state of starvation, and its whole digestive system didn't get mechanical stimulation from food, and simultaneously used sebum as the main energy, which made sebum secret more.

Fish's secretion and activity of digestive enzyme are

related to the cibation. The process of cibation is the process that digestive enzyme act on substrate in the alimentary canal. The increase of substrate will affect digestive's synthesis and secretion. In the research of juvenile *Clupea Harengus*, Pederson found that trypsase and the content of pre-enzyme have dependence on the supply of food[13]. Zambonino found the activity of pancreas and intestine respectively represent pancreatic synthetical function and secretive function. Further more, when juvenile of Sea Bass is 16-38 day-old, it cibates more, and its activity of pancreas and intestine are better[22].

In the experiment, after 2 days refeeding, *Megalobrama pellegrini*'s activity of protease, lipase and amylase quickly rebounded to the standard before starvation, and even higher than before; keep refeeding, the activity of enzyme came back to the standard before starvation gradually. This phenomenon may because at the beginning of refeeding, in the stimulation of food, olfaction, visual sense, etc. Fish's appetite improve greatly, the metabolism is flourishing, and the percent conversion of bait is high, its cibation and digestion are compensating. All of them cause its growth speed surpass those normal cibating fish, and cause enzymes' secretion increase.

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