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硕 士 学 位 论 文

日本鳗鲡对饥饿和恢复投喂的生理响应及其调节机制

Physiological responses and the regulating mechanism of Japanese eel, *Anguilla japonica* to starvation and refeeding

王晓娟

指导教师姓名: 艾春香 副教授

专业名称: 海洋生物学

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## 摘 要

采用饲养实验,以体质量( $92.07 \pm 13.29$ ) g,体长( $40.22 \pm 2.20$ ) cm 规格的日本鳎为实验对象,分别饥饿处理 0 d (S0 组,即对照组)、14 d (S14 组)、28 d (S28 组)、42 d (S42 组)、56 d (S56 组),然后恢复投喂 70 d、56 d、42 d、28 d、14 d,每组 30 尾,每组 3 平行,实验为期 70 d,饥饿组在饥饿结束取样,恢复投喂后每 2 w 取样,对照组连续取样,以探讨饥饿和恢复投喂对日本鳎生长、生化成分、消化酶活性、免疫以及肠道与肝脏组织学的影响,以期探明日本鳎在饥饿胁迫下的适应性对策及补偿生长机制,为鳎健康养殖投喂体系建立提供理论依据。本实验的主要结果和结论如下:

### 1. 饥饿和恢复投喂对日本鳎体质量、肥满度、肝指数和特殊生长率的影响

日本鳎的体质量、肥满度和肝指数在饥饿后期下降显著( $P < 0.05$ ),其中饥饿 42 d 体质量由最初的( $92.07 \pm 13.29$ ) g 降至( $74.26 \pm 10.19$ ) g,饥饿 56 d 降为( $65.76 \pm 9.07$ ) g;恢复投喂后各组日本鳎体质量均上升,与同期 S0 组差异不显著( $P > 0.05$ )。日本鳎特殊生长率随饥饿时间延长逐渐升高,分别为 S0 组: $0.3749 \pm 0.01$ , S14 组: $0.7808 \pm 0.01$ , S28 组: $1.1921 \pm 0.06$ , S42 组: $1.8234 \pm 0.04$ , S56 组: $3.2893 \pm 0.05$ ,彼此之间差异极显著( $P < 0.01$ ),而日本鳎体质量、肥满度、肝指数均与同期 S0 组差异不明显。综合日本鳎的体质量和特殊生长率指标发现, S14 组、S28 组和 S42 组表现出了完全补偿生长现象, S56 组只出现了部分补偿生长现象。

### 2. 饥饿和恢复投喂对日本鳎血糖、肌糖元、肝糖元、甘油三酯、胆固醇、生长激素含量的影响

饥饿后日本鳎血糖、肌糖元、肝糖元和血清甘油三酯含量下降,其中饥饿到 14 d,其血糖由初始 S0 组的( $240.00 \pm 23.00$ ) mg/dl 降至( $140.39 \pm 22.00$ ) mg/dl,差异显著( $P < 0.05$ ),28 d 以后其血糖含量稳定。日本鳎血清胆固醇含量在饥饿前期有所升高,饥饿 56 d 时则显著下降( $P < 0.05$ ),日本鳎血清生长激素随饥饿时间延长逐渐上升。恢复投喂后,各饥饿组日本鳎,除 S56 组肌糖元低于同期 S0 组外,其余各组血糖、肌糖元、肝糖元、甘油三酯、胆固醇和生长激素含量均与同期 S0 组差异不显著( $P > 0.05$ )。从变化顺序来看,日本鳎饥饿时先动用

肝糖元，然后脂类，最后动用肌糖元。

### 3. 饥饿和恢复投喂对日本鳗鲡消化酶活性的影响

饥饿期间日本鳗鲡肠道及肝脏中蛋白酶和淀粉酶活性呈现出先升后降的趋势，饥饿后期则略有反弹，但总体上仍低于同期 S0 组，脂肪酶活性则呈现出先降后升，高于同期 S0 组；恢复投喂后，S14 组日本鳗鲡肠道及肝脏中蛋白酶活性降低，S28 组、S42 组和 S56 组的蛋白酶活性却上升，总体上蛋白酶活性高于初始 S0 组；日本鳗鲡肠道及肝脏淀粉酶活性，S14 组和 S56 组的降低，S28 组和 S42 组的升高，总体上饥饿组低于初始 S0 组；日本鳗鲡肠道及肝脏脂肪酶活性，S14 组、S28 组和 S42 组 3 组升高，S56 组的则降低，饥饿组的日本鳗鲡肝脏脂肪酶活性与初始 S0 组的差异不大，而饥饿组的日本鳗鲡肠道脂肪酶活性则高于初始 S0 组的水平。说明恢复投喂后，日本鳗鲡肠道及肝脏蛋白酶和脂肪酶的活性升高了，而淀粉酶的活性却降低了。

### 4. 饥饿和恢复投喂对日本鳗鲡若干免疫指标的影响

饥饿后，日本鳗鲡脾指数明显下降，血清和肝脏 SOD 活性先降后升，肌肉 SOD 活性、血清和粘液溶菌活性以及血清抗菌力先升后降。恢复投喂后，各饥饿组日本鳗鲡，脾指数、血清和肝脏 SOD 活性达到或超过同期 S0 组，肌肉 SOD 活性长期饥饿组低于同期 S0 组，溶菌活性和抗菌活力长期饥饿组高于同期 S0 组。

### 5. 饥饿和恢复投喂对日本鳗鲡肠道和肝脏组织结构的影响

短期饥饿(14 d)对日本鳗鲡肠道和肝脏组织结构的影响较小，随着饥饿时间的延长，逐渐出现细胞排列紊乱，肠道直径缩小，上皮细胞高度降低，褶皱程度降低，杯状细胞增多，纹状缘断裂，肝细胞脂滴减少，界限模糊的现象。再投喂后肠道和肝脏均能恢复到正常状态，与同期 S0 组相差不明显。

**关键词：**日本鳗鲡；饥饿；生长；消化酶；免疫；组织结构

## Abstract

A feeding experiment was to determine the physiological effects of starvation and refeeding on the growth, biochemical composition, digestive enzymes and non-specific immune-response parameters and the histological structure of the intestines and liver in the Japanese eel, *Anguilla japonica* and the adaptation strategies to starvation and compensatory growth mechanisms. Japanese eel with average initial body mass of  $(92.07 \pm 13.29)$  g and body length of  $(40.22 \pm 2.20)$  cm, were divided into S0 group (the control group), S14 group, S28 group, S42 group and S56 group starved for 0, 14, 28, 42 and 56 days respectively, and refed subsequently for 70, 56, 42, 28 and 14 days. Samplings were performed right after starvation and every two weeks after refeeding. The results of this study could provide a theoretical basis for the establishment feeding technology system of Japanese eel healthy culture. The main results and conclusions of the study were as follows:

1. Effects of starvation and refeeding on the body mass, condition factor, liver index and the specific growth rates.

The body mass, condition factor and liver index decreased significantly ( $P < 0.05$ ). The body mass reduced to  $(74.26 \pm 10.19)$  g from the initial of  $(92.07 \pm 13.29)$  g after 42 days of starvation, and it reduced to  $(65.76 \pm 9.07)$  g after 56 days of starvation. The specific growth rates (S0:  $0.3749 \pm 0.01$ , S14:  $0.7808 \pm 0.01$ , S28:  $1.1921 \pm 0.06$ , S42:  $1.8234 \pm 0.04$ , S56:  $3.2893 \pm 0.05$ ) had significant difference among all the groups ( $P < 0.01$ ) after refeeding, while other parameters had no significant difference compared with those of the S0 group. The Japanese eels in S14 group, S28 group and S42 group showed completely-compensatory growth, while the eels in S56 group indicated only partially-compensatory growth after comprehensive analyzing the Japanese eel's body mass and specific growth rates.

2. Effects of starvation and refeeding on the content of glucose, muscle glycogen, liver glycogen, serum triglyceride, total cholesterol and growth hormone in serum of Japanese eel.

The serum glucose level of Japanese eel significantly decreased from

(240.00±23.00) mg/dl to (140.39±22.00) mg/dl after 14 days of starvation ( $P<0.05$ ), but maintained stable from 28 days of starvation. The total serum cholesterol content of Japanese eel increased at first and decreased significantly after 56 days of starvation ( $P<0.05$ ), while the serum growth hormone level of Japanese eel increased continuously during starvation. The measured indices except for the muscle glycogen in S56 group had no significant difference with those of S0 group ( $P>0.05$ ) after refeeding. It indicated that the liver glycogen of Japanese eel was utilized at first, the next utilization was lipid, and the last utilization was muscle glycogen during the starvation.

### 3. Effects of starvation and refeeding on digestive enzymes of intestine and liver in Japanese eel

The protease and amylase activities in intestine and liver of Japanese eel increased at first and decreased later after starvation. Protease and amylase activities slightly improved, lower than the initial level as a whole at the end of starvation. On the contrary, lipase activity decreased at the beginning and increased later. It was higher than that of the S0 group at the end of the starvation. After refeeding, protease activity of eels in S28 group, S42 group and S56 group increased besides S14 group; amylase activity of eels in S14 group and S56 group decreased, whereas that of eels in S28 group and S42 group increased; lipase activity of eels in S14 group, S28 group and S42 group increased besides S56 group, the protease activity in intestine and liver of eel after refeeding was higher than that of eels in initial S0 group in general, while the amylase activity was lower, and the lipase activity improved in intestine but didn't change much in liver. In summary, the Japanese eel protease and lipase activities in intestine and liver can enhance, but the amylase activity in intestine and liver reduced after refeeding.

### 4. Effects of starvation and refeeding on several immune parameters of Japanese eel

Spleen index distinctively dropped, and superoxide dismutase activities decreased and then increased in serum and liver of eels, while SOD activity in muscle increased firstly and then decreased after starvation. Lysozyme activities in serum and mucus of eels, as well as antibacterial activity in serum, increased firstly and then

decreased. After refeeding, spleen index, SOD activities in serum and liver caught up or exceeded those of eels in the S0 group, while SOD activity in muscle was lower than that of the S0 group after long term starvation (42 d and 56 d). On the contrary, lysozyme and antibacterial activities of eels in S42 group and S56 group were higher than those of the S0 group.

#### 5. Effects of starvation and refeeding on intestinal and hepatic histological structure in Japanese eel

There were no significant alteration both in intestinal and hepatic histological structure during the short starvation period (less than 14 days). After 14 days of starvation, the effects are different with starvation time: intestinal diameters, heights of the epithelial cells and the ruga decreased, and the microvilli was deteriorated; but the number of goblet cells increased; hepatocytes in which the lipid decreased were shrunk and looked disordered. After refeeding, both tissues recovered, and had no significant difference with the S0 group ( $P > 0.05$ ).

**Key words:** *Anguilla japonica*; starvation; growth; digestive enzymes; immunity; histological structure

## 缩略词表

缩略词	英文全称	中文全称	EC
GH	growth hormone	生长激素	----
IGF-1	insulin-like growth factor1	胰岛素样生长因子 1	----
ALT	alanine transaminase	丙氨酸转氨酶	2.6.1.2
AST	aspartate aminotransferase	天门冬氨酸转氨酶	2.6.1.1
SI	spleen index	脾指数	----
$U_L$	lysozyme	溶菌酶	3.2.1.17
$U_a$	antibacterial activity	抗菌活力	----
SOD	superoxide dismutase	超氧化物歧化酶	1.15.1.1
----	amylase	淀粉酶	3.2.1.2
----	protease	蛋白酶	3.4.4.7
----	lipase	脂肪酶	3.1.1.3
----	pepsin	胃蛋白酶	3.4.23.1
FGF2	fibroblast growth factor 2	纤维原细胞生长因子 2	----
LPL	lipoprotein lipase	脂蛋白酯酶	3.1.1.34
PNR	point-of-no-return	不可逆点	----
SGR	special growth rate	特殊生长率	----
CF	condition factor	肥满度	----
CAT	catalase	过氧化氢酶	1.11.1.6
GPX	glutathione peroxidase	谷胱甘肽过氧化物酶	1.11.1.7
GR	glutathione reductase	谷胱甘肽还原酶	1.6.4.2
G6PDH	glucose-6-phosphate dehydrogenase	葡萄糖-6-磷酸脱氢酶	1.1.1.49

## 第一章 绪论

### 1.1 饥饿对鱼类生长发育、生理生化影响及其机制

#### 1.1.1 饥饿对鱼类生长发育的影响

鱼类生长是指鱼类体质量、体长、体成分以及能量等的数量变化过程，是摄入含能物质、同化自身和异化环境的动态平衡过程(Brett, 1979)，其受到多种因素的影响，其中饥饿胁迫对鱼类生长，尤其是对其早期阶段的生长影响显著(Dou et al, 2002; Gisbert et al, 2004; Peña et al, 2005; Dou et al, 2005; Shan et al, 2008a; Shan et al, 2008b; Shan et al, 2009)。研究表明，吉富罗非鱼(GIFT, *Oreochromis niloticus*)体质量、内脏质量、内脏比、肥满度等指标随着饥饿时间的延长呈现下降趋势，说明饥饿影响吉富罗非鱼的生长(刘波等, 2009)。

在自然界中，由于摄食者和被摄食者存在时空分布格局的差异，以及季节的演替、疾病的危害、环境的突变和种间种内的竞争等原因，多数动物经常面临食物资源的匮乏，造成生长停滞，甚至出现负生长现象，这种因食物短缺或营养匮乏的鱼类在食物供给恢复正常后经常表现出一种快速生长，称为鱼类的补偿性生长(compensatory growth)或获得性生长(catch-up growth) (Miglav & Jobling, 1989; Jobling et al, 1994; Zamal & Ollevier, 1995)，也有人认为这种因食物限制抑制动物生长，而后恢复投喂时发生的动物体快速生长现象被称作补偿生长(compensatory growth)更合适(Jobling, 2010)。研究发现，许多鱼类存在补偿生长现象。杂交太阳鱼(*Lepomis cyanellus* × *L. macrochirus*)“饥饿-饱食”实验发现，循环投饵组的鱼绝对生长率是对照组鱼的2倍(Hayward et al, 1997)。红鳍笛鲷(*Lutjanus erythropterus*)饥饿后恢复投喂，其摄食率和食物转化率同时提高，表现出补偿性生长现象(王沛宾等, 2005)。大西洋庸鲷(*Hippoglossus hippoglossus* L.)短期饥饿(2 w-5 w)再投喂后摄食水平和食物转化率均提高，出现了部分补偿生长现象 (Heide et al, 2006)。金鲷(*Oncorhynchus mykiss*)幼鱼饥饿再投喂后摄食率也大大增加，出现了部分补偿生长现象(吴蒙蒙, 2008)。饥饿5 d和10 d的鲈鱼(*Lateolabrax japonicus*)幼鱼在恢复生长过程中产生了完全补偿生长效应，而饥饿15 d的仅产生了部分补偿生长效应(楼宝, 2008a)。红鲫



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