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β-Conglycinin 对不同发育时期鲤鱼消化酶活力的影响

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[摘 要] 【目的】研究 β-伴大豆球蛋白(β-Conglycinin)对鲤幼鱼、稚鱼蛋白酶和淀粉酶活力的影响。【方法】以初始体质量为(10.06±0.14) g/尾的鲤稚鱼和(110.23±0.23) g/尾的鲤幼鱼为研究对象,以鱼粉为动物蛋白源,面粉、糊精为糖源,混合油脂(m(鱼油)∶m(玉米油)=1∶1)为脂肪源,分别配制 5 种等氮(鲤幼鱼和稚鱼粗蛋白质量分数分别为 36%和 40%)、等能(鲤幼鱼和稚鱼总能分别是 15.2 和 16.9 MJ/kg) 的半精制饲料,其 β-Conglycinin 的添加量(质量分数)分别为 0(CK),2.0%,4.0%,6.0%和 8.0%,每组饲料设 3 个重复,在控温单循环养殖系统中进行为期 8 周的饲养试验,试验结束后,取鲤幼鱼、稚鱼的前、中、后肠道和肝胰脏,分别用福林-酚试剂法和淀粉酶试剂盒法,测定肠道和肝胰脏蛋白酶及淀粉酶的活力。【结果】鲤幼鱼肝胰脏蛋白酶活力各组之间差异不显著($P>0.05$);β-Conglycinin 添加量为 6.0%和 8.0%组的前肠、中肠蛋白酶活力显著低于对照组($P<0.05$);而 β-Conglycinin 添加量为8.0%组后肠蛋白酶活力显著低于对照组($P<0.05$)。在鲤稚鱼肝胰脏和后肠,β-Conglycinin 添加量为 8.0%组的蛋白酶活力显著低于对照组($P<0.05$);前肠蛋白酶活力则以 2.0%,4.0%,6.0%和 8.0%添加组显著低于对照组($P<0.05$);中肠蛋白酶活力为 4.0%,6.0%和 8.0%添加组显著低于对照组($P<0.05$)。β-Conglycinin 对鲤幼鱼和稚鱼肝胰脏、前肠、中肠及后肠淀粉酶活力均无显著影响($P>0.05$)。【结论】鲤幼鱼配合饲料中 β-Conglycinin 的添加量不应超过 6.0%;鲤稚鱼配合饲料中 β-Conglycinin 的添加量不应超过 2.0%。

[关键词] β-伴大豆球蛋白;鲤;幼鱼;稚鱼;蛋白酶;淀粉酶

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Effects of β-Conglycinin on activities of protease and amylase in juvenile and larval common carps

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Abstract:【Objective】The research investigated the effects of β-Conglycinin on activities of protease and amylase in juvenile and larval common carps.【Method】Larval and juvenile common carps with the initial weights of (10.06±0.14) g/tail and (110.23±0.23) g/tail were used as experimental objects for eight-week feeding trial at controlled temperature in single recirculating system. Five diets with identical nitrogen (total crude protein contents for juvenile and larval were 36% and 40%, respectively) and energy (total energies for juvenile and larval were 15.2 and 16.9 MJ/kg, respectively) as well as different β-Cong-

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lycinin contents (0(CK), 2.0%, 4.0%, 6.0%, and 8.0%) were provided. Fish meal was animal protein source, dextrin and flour were carbohydrate source, and mixed oil (m(corn oil) : m(fish oil) = 1 : 1) was fat source. Each group had three repetitions. The activities of protease and amylase in foregut, midgut and hepatopancreas were detected using Folin-phenol method and Amylase kit.【Result】No significant effects of β-Conglycinin on activities of protease in hepatopancreas of juvenile common carps were observed ($P>0.05$). The activities of protease in foregut and midgut of juvenile common carps in 6.0% and 8.0% groups were significantly lower than that of the control group ($P<0.05$), and that in hindgut of 8.0% group were significantly lower than that of the control group ($P<0.05$). The activities of protease in hepatopancreas and hindgut of larval common carps in 8.0% groups were significantly lower than that of the control group ($P<0.05$), that of 2.0%, 4.0%, 6.0%, and 8.0% groups were significantly lower ($P<0.05$) in foregut, and that of 4.0%, 6.0%, and 8.0% groups were significantly lower ($P<0.05$) in midgut. In addition, no significant effects of β-Conglycinin on activities of amylase in hepatopancreas and tract of both juvenile and larval common carps were observed ($P>0.05$).【Conclusion】The amount of β-Conglycinin should be less than 6.0% and 2.0% in the diet of juvenile and larval common carp, respectively.

Key words: β-Conglycinin; *Cyprinus carpio*; juvenile common carp; larval common carp; protease; amylase

随着集约化水产养殖的发展，鱼粉资源短缺，寻求鱼粉蛋白源替代品已成为国际性研究课题。大豆蛋白源是水产饲料应用最多的植物蛋白源之一。目前，国内外学者在大豆蛋白源替代鱼粉方面做了许多研究，涉及的鱼类主要有虹鳟(*Oncorhynchus mykiss*)[1]、金头鲷(*Sparus aurata* L.)[2]、大西洋鲑(*Salmo salar* L.)[3]、杂交罗非鱼(*Oreochromis niloticus*×*O. aureus*)[4]、草鱼(*Ctenopharyngodon idellus*)[5]、大黄鱼(*Pseudoscjaena crocea* R.)[6]、异育银鲫(*Carassius auratus gibelio*)[7]、齐口裂腹鱼(*Schizothorax prenanti*)[8]、埃及胡子鲇(*Clarias lazera*)[9]、鲤鱼(*Cyprinus carpio*)[10]等，研究的内容多集中在大豆蛋白源替代鱼粉蛋白后对鱼类摄食、消化、生长、健康等的影响。这些研究表明，以过量的大豆蛋白替代鱼粉蛋白，不仅会影响鱼类肠上皮细胞增生及肠道组织形态，而且会影响鱼类对饲料的消化及生长性能。其主要原因是大豆蛋白中含有多种抗营养因子，其中大豆抗原蛋白是大豆中主要的抗营养因子之一。β-伴大豆球蛋白(β-Conglycinin)比大豆球蛋白(Glycinin)具有更强的抗原性[11]，普通的热处理不能灭活β-伴大豆球蛋白的免疫活性，其能够引起鱼类消化道过敏，造成胃、肠道损伤，进而引起消化吸收障碍，甚至死亡。但由于大豆抗原蛋白具有蛋白含量高、价格低廉、来源丰富等优点，因此国内外学者就大豆抗原蛋白对动物的影响进行了广泛研究。目前，关于大豆抗原蛋白的研究主要集中在猪[12]、犊牛[13]、鼠[14]、羔羊[15]等陆生动物上，而对水产动物研究报道较少，仅见郭林英[16]研究了大豆β-伴球蛋白提取物对鲤鱼肠上皮细胞增殖及其功能的影响。鱼类消化道的消化酶是影响饲料消化吸收的主要因素，消化酶受体内外多种因素的影响。本研究分别以鲤幼鱼和稚鱼为供试动物，研究了β-Conglycinin对不同发育时期鲤鱼消化酶活力的影响，旨在为合理开发利用大豆蛋白源及大豆抗原蛋白的去除提供依据。

1 材料与方法

1.1 β-Conglycinin的分离纯化

β-Conglycinin采用简化膜中间试验方法[17]获得。

1.2 试验饲料

以鱼粉为动物蛋白源，面粉、糊精为糖源，混合油脂(m(鱼油)∶m(玉米油)=1∶1)为脂肪源，分别配制5种等氮(鲤幼鱼和稚鱼的蛋白质量分数分别为36%和40%)、等能(鲤幼鱼和稚鱼总能分别为15.2和16.9 MJ/kg)的半精制饲料，β-Conglycinin的添加量(质量分数)分别为0(对照，CK)，2.0%，4.0%，6.0%和8.0%。各原料粉碎后过孔径0.246 mm的筛，按配方准确称其质量，在吉林农业大学动物水产实验室用电动绞肉机制成粒径1.5和2.5 mm颗粒饲料。晒干后置于−20 ℃冰箱中保存备用。鲤幼鱼和稚鱼的试验饲料组成及营养成分见表1和表2。

表 1　鲤幼鱼饲料配方及营养水平(风干基础)

Table 1　Feed formulation and nutritional level for juvenile *Cyprinus carpio* (air-dry basis)

项目 Item	β-Conglycinin 添加组别 β-Conglycinin addtion group				
	CK	2.0%	4.0%	6.0%	8.0%
组分 Ingredients					
鱼粉/% Fish meal	51.0	53.0	50.3	47.4	44.7
面粉/% Flour	19.75	18.01	18.40	18.80	19.00
鱼油/% Fish oil	1.40	1.05	1.10	1.15	1.25
玉米油/% Corn oil	1.40	1.05	1.10	1.15	1.25
氯化胆碱/% Choline chlorde	0.5	0.5	0.5	0.5	0.5
微晶纤维素/% Microcrystalline cellulose	4.70	4.70	4.70	4.70	4.80
糊精/% Dextrin	19.75	18.01	18.40	18.80	19.00
预混料/% Premix	1.0	1.0	1.0	1.0	1.0
聚黏保/% Poly sticky	0.5	0.5	0.5	0.5	0.5
营养组成 Nutrient content					
粗蛋白/% Crude protein	36.00	35.99	36.01	35.99	36.01
粗脂肪/% Crude lipid	5.02	5.01	5.01	4.99	5.00
粗纤维/% Crude fiber	4.49	4.50	4.48	4.47	4.50
粗灰分/% Ash	5.81	4.50	4.56	4.54	4.52
总能/(MJ·kg^{-1}) Gross energy	15.20	15.20	15.21	15.20	15.21

表 2　鲤稚鱼饲料配方及营养水平(风干基础)

Table 2　Feed formulation and nutritional level for larval *Cyprinus carpio* (air-dry basis)

项目 Item	β-Conglycinin 添加组别 β-Conglycinin addtion group				
	CK	2.0%	4.0%	6.0%	8.0%
组分 Ingredients					
鱼粉/% Fish meal	62.00	59.3	56.5	53.6	50.86
面粉/% Flour	13.86	14.71	15.10	15.49	15.75
鱼油/% Fish oil	2.75	2.25	2.25	2.30	2.40
玉米油/% Corn oil	2.75	2.25	2.25	2.30	2.40
氯化胆碱/% Choline chlorde	0.5	0.5	0.5	0.5	0.5
微晶纤维素/% Microcrystalline cellulose	2.78	2.78	2.80	2.82	2.84
糊精/% Dextrin	13.86	14.71	15.10	15.49	15.75
预混料/% Premix	1.0	1.0	1.0	1.0	1.0
聚黏保/% Poly sticky	0.5	0.5	0.5	0.5	0.5
营养组成 Nutrient content					
粗脂肪/% Crude lipid	9.00	9.00	8.99	9.01	8.99
粗蛋白/% Crude protein	39.99	40.01	40.01	39.99	40.00
粗纤维/% Crude fiber	2.81	2.80	2.81	2.82	2.80
粗灰分/% Ash	4.37	4.75	4.48	4.38	4.70
总能/(MJ·kg^{-1})Gross energy	16.90	16.90	16.91	16.92	16.90

1.3　饲养条件及管理

养殖试验在吉林农业大学控温单循环系统中进行，试验期间连续充气，水中氨氮质量浓度低于 0.5 mg/L，溶解氧高于 5.0 mg/L，温度为 25～27 ℃，养殖试验持续 8 周。

试验鱼来源于吉林省九台市渔场，试验前饱食投喂对照组饲料，预饲 15 d，预饲试验结束后，饥饿 24 h，挑选鳍鳞完整、规格整齐、体质健壮的鲤幼鱼((110.23±0.23) g/尾) 300 尾和鲤稚鱼((10.06±0.14) g/尾) 450 尾，分别随机放养在 15 个玻璃缸中，鲤幼鱼每缸放养 20 尾，鲤稚鱼每缸放养 30 尾。放养前用质量浓度为 20 mg/L 的高锰酸钾溶液药浴 10 min，随机安排 3 个玻璃缸为一个试验组。在试验过程中，每天称取足量饲料，分 2 次投喂(09:00，16:00)，投饵方式为人工手撒，直至鱼不再到水面摄食为止，日投饵率为体质量的 3%～5%，每天记录每缸鱼的摄食饲料质量。

1.4　样品的收集与粗酶液的制备

参照吴莉芳等[18]的方法进行样品收集与粗酶液制备。(1)样品的收集。饲养试验结束前停食 24 h 后，每缸活体解剖 10 尾鱼，取出肝胰脏和其他内脏，称其质量(精确到 0.01 g)。取出肠道和肝胰脏，剔除附着物，用去离子水冲洗肠道内容物，滤纸吸干，−20 ℃冰柜保存待测。肠道从第一个回折点以

前为前肠,最后一个回折点以后为后肠,其间为中肠。(2)粗酶液的制备。称样品质量,加入10倍体积的高纯水匀浆,在4 ℃冰箱中静置过夜,5 000 r/min离心10 min,取上清液即为粗酶液,4 ℃冰箱保存、待测。粗酶液需在24 h内测定完毕。

1.5 消化酶活力的测定

蛋白酶活力采用福林-酚试剂法(Folin-phenol)测定[18];淀粉酶活力采用淀粉酶试剂盒(南京建成科技有限公司)测定[18]。

1.6 数据统计分析

采用SPSS17.5软件对鲤幼鱼、稚鱼蛋白酶及淀粉酶活力进行方差分析,若差异显著,进一步进行LSD和Duncan's多重比较,分析组间差异显著性。试验数据用"平均值±标准差"(Mean±*SD*)表示。显著性水平设定为 $P<0.05$。

2 结果与分析

2.1 β-Conglycinin对鲤幼鱼和稚鱼蛋白酶活力的影响

β-Conglycinin对鲤幼鱼、稚鱼蛋白酶活力的影响分别见表3和表4。

表3 β-Conglycinin对鲤幼鱼蛋白酶活力的影响

Table 3 Effects of β-Conglycinin on activities of protease in juvenile common carps U/mg

β-Conglycinin添加量/% Supplementation of β-Conglycinin	蛋白酶活力 Protease specific activity			
	肝胰脏 Hepatopancrea	前肠 Fore intestine	中肠 Middle intestine	后肠 Hind intestine
0(CK)	22.85±0.63 a	22.11±0.10 c	17.18±0.10 b	16.56±1.21 b
2.0	22.53±0.46 a	21.47±1.18 c	17.33±0.92 b	16.36±0.14 ab
4.0	22.12±0.87 a	21.31±1.23 c	17.02±0.44 b	16.33±0.07 ab
6.0	22.24±0.95 a	18.98±0.10 b	15.09±0.14 a	15.40±1.21 ab
8.0	21.08±1.64 a	17.40±0.07 a	15.08±0.15 a	14.73±1.17 a

注:同列数据后标不同小写字母表示差异显著($P<0.05$)。下表同。

Note:Different lowercase letters in each column indicate significant difference($P<0.05$). The same below.

表4 β-Conglycinin对鲤稚鱼蛋白酶活力的影响

Table 4 Effects of β-Conglycinin on activities of protease in larval common carps U/mg

β-Conglycinin添加量/% Supplementation of β-Conglycinin	蛋白酶活力 Protease specific activity			
	肝胰脏 Hepatopancrea	前肠 Fore intestine	中肠 Middle intestine	后肠 Hind intestine
0(CK)	23.44±0.34 a	22.53±0.07 c	19.13±0.07 c	15.71±0.17 b
2.0	23.03±0.10 ab	21.56±0.10 b	18.53±0.07 c	14.80±0.07 ab
4.0	22.60±0.36 ab	21.82±0.10 b	17.60±0.07 b	15.13±0.64 ab
6.0	22.62±0.54 ab	18.35±0.17 a	16.65±1.06 a	15.02±0.14 ab
8.0	22.19±0.63 b	18.55±0.24 a	15.89±0.17 a	14.20±1.21 a

由表3可以看出,鲤幼鱼肝胰脏蛋白酶活力各组之间差异不显著($P>0.05$)。2.0%和4.0% β-Conglycinin添加组前肠、中肠蛋白酶活力与对照组差异不显著($P>0.05$),6.0%和8.0% β-Conglycinin添加组前肠、中肠蛋白酶活力显著低于对照组($P<0.05$);8.0% β-Conglycinin添加组前肠蛋白酶活力显著低于2.0%,4.0%和6.0%添加组($P<0.05$),6.0% β-Conglycinin添加组前肠蛋白酶活力显著低于2.0%和4.0%添加组($P<0.05$);6.0%和8.0%β-Conglycinin添加组中肠蛋白酶活力显著低于2.0%及4.0%添加组($P<0.05$)。2.0%,4.0%,6.0%β-Conglycinin添加组后肠蛋白酶活力与对照组差异不显著($P>0.05$),8.0%添加组后肠蛋白酶活力显著低于对照组($P<0.05$);2.0%,4.0%,6.0%和8.0%添加组之间后肠蛋白酶活力无显著差异($P>0.05$)。

由表4可知,在鲤稚鱼肝胰脏和后肠中,2.0%,4.0%和6.0% β-Conglycinin添加组蛋白酶活力与对照组差异不显著($P>0.05$),8.0%β-Conglycinin添加组蛋白酶活力显著低于对照组($P<0.05$),2.0%,4.0%,6.0%和8.0%β-Conglycinin添加组之间蛋白酶活力无显著差异($P>0.05$);在鲤稚鱼前肠中,2.0%, 4.0%,6.0%和8.0% β-Conglycinin添加组蛋白酶活力显著低于对照组($P<0.05$),2.0%和4.0% β-Conglycinin添加组蛋白酶活力显著高于6.0%及8.0%添加组($P<0.05$);在鲤稚鱼

中肠中，2.0% β-Conglycinin 添加组蛋白酶活力与对照组差异不显著（$P>0.05$），而 4.0%，6.0%和 8.0%β-Conglycinin 添加组蛋白酶活力显著低于对照组（$P<0.05$），2.0%β-Conglycinin 添加组蛋白酶活力显著高于4.0%，6.0%和 8.0%添加组（$P<0.05$），4.0%β-Conglycinin 添加组蛋白酶活力显著高于6.0%和 8.0%添加组（$P<0.05$）。

2.2 β-Conglycinin 对鲤幼鱼和稚鱼淀粉酶活力的影响

表 5 和表 6 表明，在本试验条件下，2.0%，4.0%，6.0%和 8.0%β-Conglycinin 添加组鲤幼鱼与稚鱼肝胰脏、前肠、中肠、后肠淀粉酶活力与对照组差异均不显著（$P>0.05$）。

表 5　β-Conglycinin 对鲤幼鱼淀粉酶活力的影响

Table 5　Effects of β-Conglycinin on activities of amylase in juvenile common carps　U/mg

β-Conglycinin 添加量/% Supplementation of β-Conglycinin	淀粉酶活力 Amylase specific activity			
	肝胰脏 Hepatopancrea	前肠 Fore intestine	中肠 Middle intestine	后肠 Hind intestine
0(CK)	0.45±0.01 a	0.34±0.01 a	0.38±0.01 a	0.44±0.01 a
2.0	0.44±0.01 a	0.33±0.01 a	0.38±0.01 a	0.44±0.01 a
4.0	0.45±0.01 a	0.34±0.01 a	0.37±0.01 a	0.42±0.01 a
6.0	0.45±0.02 a	0.34±0.01 a	0.38±0.01 a	0.42±0.02 a
8.0	0.45±0.02 a	0.34±0.01 a	0.34±0.01 a	0.41±0.01 a

表 6　β-Conglycinin 对鲤稚鱼淀粉酶活力的影响

Table 6　Effects of β-Conglycininon on activities of amylase in larval common carps　U/mg

β-Conglycinin 添加量/% Supplementation of β-Conglycinin	淀粉酶活力 Amylase specific activity			
	肝胰脏 Hepatopancrea	前肠 Fore intestine	中肠 Middle intestine	后肠 Hind intestine
0(CK)	0.53±0.01 a	0.41±0.01 a	0.42±0.01 a	0.51±0.01 a
2.0	0.52±0.01 a	0.42±0.01 a	0.43±0.01 a	0.50±0.01 a
4.0	0.52±0.02 a	0.39±0.01 a	0.43±0.01 a	0.46±0.03 a
6.0	0.51±0.01 a	0.39±0.02 a	0.43±0.01 a	0.46±0.01 a
8.0	0.51±0.01 a	0.36±0.01 a	0.43±0.01 a	0.46±0.01 a

3 讨论

3.1 β-Conglycinin 对鲤幼鱼和稚鱼蛋白酶活力的影响

本研究结果表明，β-Conglycinin 对鲤幼鱼和稚鱼的前肠、中肠、后肠蛋白酶活力的影响存在一定的差异。在鲤幼鱼的配合饲料中，β-Conglycinin 添加量为 6.0%和 8.0%组前肠、中肠蛋白酶活力显著低于对照组（$P<0.05$）；在鲤稚鱼的配合饲料中，β-Conglycinin 添加量为 2.0%，4.0%，6.0% 和 8.0% 组前肠蛋白酶活力显著低于对照组（$P<0.05$），而中肠蛋白酶活力则以 4.0%，6.0% 和 8.0% β-Conglycinin 添加组显著低于对照组（$P<0.05$）。这可能是由于鲤幼鱼和稚鱼消化道结构发育程度不同，对 β-Conglycinin 的敏感性不同所致。鲤稚鱼消化系统发育尚不成熟，消化器官不发达，消化机能不完善，消化道中酶的分泌量不足，使大量未消化的营养物质进入了肠道。因此，β-Conglycinin 也可大量进入肠道，引起肠道损伤，从而导致消化酶活力降低。张帆等[6]研究了饲料中豆粕替代鱼粉对大黄鱼消化酶活性的影响，结果表明，大黄鱼肠道胰蛋白酶的活性随豆粕替代水平的升高而显著降低。Burrells 等[19]研究表明，在饲料中添加一定量的大豆蛋白，会引起虹鳟的后肠结构形态变化，并降低刷状缘的酶活性。Krogdahl 等[20]研究发现，豆粕能够引起虹鳟中肠与后肠上皮刷状缘胞外酶碱性磷酸酶、亮氨酸氨肽酶以及麦芽糖酶、乳糖酶、蔗糖酶活性下降。吴莉芳等[9]研究了去皮豆粕替代鱼粉对埃及胡子鲇消化酶活力的影响，结果表明，当去皮豆粕替代鱼粉蛋白的 45%和 60%时，埃及胡子鲇前肠和后肠的蛋白酶活力显著下降。Ksudhik 等[21]在大西洋鲑的饲料中添加一定量的大豆蛋白，引起其后肠结构发生形态变化，刷状缘的酶活性降低。关于不同添加量的 β-Conglycinin 引起不同发育时期鲤鱼肠道组织结构的变化，需进一步通过组织学方法进行研究。

3.2 β-Conglycinin 对鲤幼鱼和稚鱼淀粉酶活力的影响

鱼类的淀粉酶是碳水化合物水解酶类的一种，活性较低，同种鱼类不同消化器官淀粉酶的活力不同，另外随着鱼类年龄的增加，其淀粉酶活力也发生改变，淀粉酶活性在同一消化器官不同部位也会有

所差异。彭翔等[22]在黑鲷鱼饲料中用发酵豆粕替代0～50%的鱼粉蛋白质,研究结果表明,饲料中各组淀粉酶的活性差异不显著。吴莉芳等[23]研究了不同大豆蛋白源替代鱼粉对鲤鱼蛋白酶和淀粉酶活力的影响,结果表明,不同大豆蛋白源对鲤鱼淀粉酶活力影响不显著。钱曦等[24]研究报道,在翘嘴红鲌的饲料中,当豆粕替代鱼粉蛋白的13%和27%时,对其肝胰脏和肠道淀粉酶活力影响不显著。在本试验条件下,β-Conglycinin的添加量对鲤幼鱼和稚鱼肝胰脏、前肠、中肠、后肠淀粉酶活力影响均不显著。这主要是由于鱼类的淀粉酶对食物类型和饲料组成有明显的适应性[25]。鲤鱼属于杂食性鱼类,在天然的食谱中存在一定量的植物蛋白源,而β-Conglycinin就属于植物蛋白源。因此,鲤鱼肝胰脏和肠道淀粉酶对β-Conglycinin具有一定的适应性。

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