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# 共轭亚油酸对日本沼虾生长、抗氧化及脂质代谢的影响

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**摘要:** 为了探讨共轭亚油酸对日本沼虾生长、抗氧化及脂质代谢的影响, 实验用不同含量的共轭亚油酸(conjugated linoleic acid, CLA)替代鱼油, CLA添加水平分别为0%(CLA1)、1.5%(CLA2)、3%(CLA3)、4.5%(CLA4)和6%(CLA5), 对日本沼虾进行为期8周的摄食营养实验。实验虾的初始均重为(0.102±0.0024) g, 每组设5个平行。结果显示, 随着CLA含量的增加, 各组虾的增重差异不显著, 但存活率却有降低趋势, 其中CLA5组存活率显著低于CLA1组; 肝胰腺中丙二醛(MDA)含量、超氧化物歧化酶(SOD)和总抗氧化能力(T-AOC)活性随着CLA水平的增加呈现先降低再升高趋势, 其中CLA1组SOD活力显著高于其余各组, 当CLA含量超过4.5%(CLA4)时, 日本沼虾肝胰腺中MDA含量显著增加; 随着CLA水平的增加, 血浆中胆固醇(TC)和甘油三酯(TG)含量显著降低; 脂肪代谢相关基因B类Ⅰ型清道夫受体(SR-BI)、肉碱脂酰转移酶(CPT1) mRNA表达水平有一定的波动性, 均在CLA4组达到最高, 乙酰辅酶A羧化酶(ACC) mRNA表达水平呈先增加后降低趋势, 其中CLA4组*ACC*基因表达量显著高于其余各组($P$<0.05)。研究表明, CLA的添加对日本沼虾抗氧化能力和脂质代谢过程产生了一定的影响, CLA添加对虾的生长性能未产生显著影响, 但添加水平较高时(6%)会降低虾的存活率。

**关键词:** 日本沼虾; 共轭亚油酸; 生长; 抗氧化能力; 脂质代谢
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日本沼虾(*Macrobrachium nipponense*)又名青虾、河虾, 是我国和东南亚一些国家重要的淡水经济养殖种类之一[1]。目前, 日本沼虾的养殖在我国的长三角地区该已形成了一定的规模, 由于其肉味鲜美、营养价值高, 深受人们的青睐。然而, 近年来在实际生产中出现的个体生长缓慢、规格偏小、提前抱卵(性早熟现象)等一系列问题, 阻碍了该虾养殖业进一步发展。研究表明, 通过改善外源营养物质的平衡, 可以抑制肝胰腺脂质过度积累, 促进甲壳动物的生长[2-3]。

共轭亚油酸(conjugated linoleic acid, CLA)是一组亚油酸的位置和立体异构体多不饱和脂肪酸, 其异构体的共同结构特征是含有顺式或反式的双键[4]。目前, 受到较多关注的主要为顺9反11和反10顺12CLA[5]。作为一种不饱和脂肪酸, CLA参与了机体的一系列生理活动, 如抗氧化、提高机体免疫力、调节机体的能量和脂肪代谢[6-7]。

近年来, 有关CLA在水产动物中的研究较多。研究表明, 鱼类能将摄食的CLA积累在身体

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组织中[8]。然而，不同的鱼类摄食CLA后的生长性能与肝脏脂肪沉积却因物种不同而产生较大的差异。鲤(*Cyprinus carpio*)在摄食添加1% CLA的饲料时提高了生长性能[9]，斑点叉尾鮰(*Ictalurus punctatus*)摄食1%CLA在3周时生长性能显著提高[10]，而在其他鱼类添加CLA时生长性能未表现出显著差异[11-13]，或生长性能出现下降[14-15]。同样，对脂类代谢的研究结果也出现了一定的差异，研究表明，日粮中添加一定量的CLA能够使大西洋鲑(*Salmo salar*)[16]和大西洋鳕(*Gadus morhua*)[17]肝脏和肌肉中的甘油三酯含量降低，大黄鱼(*Larimichthys crocea*)鱼体和肌肉中脂肪含量增加[18]，但对斑点叉尾鮰[13]肝脏脂肪含量无显著影响。上述结果提示，CLA对鱼类生长及脂质代谢的影响较为复杂。因此，大量的研究都开始着手从基因表达、信号通路等方面探讨CLA对脂质代谢的影响机制[19-20]。

相比而言，有关经济虾蟹类对CLA的研究关注甚少，仅见在饲料中添加1%CLA可以提高凡纳滨对虾(*Litopenaeus vannamei*)肌肉品质的报道[21]。因此，本实验拟通过饲喂含不同含量的CLA日粮，分析日粮CLA水平对日本沼虾生长、抗氧化、脂质代谢相关的酶活性及基因表达的影响，以期为今后饲料中添加CLA以调节虾体的生长及脂质代谢提供一定的理论基础，并推动日本沼虾养殖业的可持续发展。

## 1 材料与方法

### 1.1 实验饲料

进口优质白鱼粉(粗蛋白质含量为66.69%，粗脂肪含量为9.10%)由浙江璟宝饲料股份有限公司提供。CLA由青岛澳海生物有限公司提供，CLA含量为80%，其中*cis*-9，*tran*-11和*tran*-10，*cis*-12的含量分别为37.7%和39.5%。以鱼粉和酪蛋白为蛋白源，将不同含量的CLA替代鱼油，配制等氮等脂的CLA含量分别为0%(CLA1)、1.5%(CLA2)、3.0%(CLA3)、4.5%(CLA4)和6.0%(CLA5)的半精制饲料。饲料的制作步骤如下，首先将各种原料粉碎过60目筛，按设计量逐级均匀混合，然后加入各种油脂重新混匀，再添加一定量的水混匀，最后用双螺杆挤条机压成直径为1.0 mm的颗粒饲料，于低温烘箱中进行烘干至饲料中水分含量达到约10%，密封后置于–20 °C保存备用。

### 1.2 实验动物及养殖试验

实验用虾购自于湖州日本沼虾养殖基地，暂养1周后，选择健康、体质量均匀(均重0.102 g)的日本沼虾用于试验。实验共分为5组，每组5个平行，每个平行60只日本沼虾，随机放入到体积为300 L的水族箱中，每个水族箱内放置蓝色纱网作为躲避物，以减少日本沼虾互残。每日吸污并换水(换水量约为1/2~1/3)，实验期间连续充气，保证水质条件，即水温25~29 ℃，pH 7.6~8.1，溶解氧含量大于6.5 mg/L。于每日上午、下午各投喂一次，投喂量为虾体质量的4%~5%，对日本沼虾开展为期8周的摄食营养实验。

### 1.3 样品的采集

养殖实验结束，日本沼虾饥饿一天后，称重、统计各实验组虾的成活率。用无菌解剖器取出虾的肝胰腺。将无菌注射器用抗凝剂润湿后，取出血液。最后，将肝胰腺和血液保存于–80 °C备用。

### 1.4 生长相关指标的测定

成活率(survival rae，%)=100×实验结束时存活虾个体数/实验开始时虾的个体数

增重率(weight gain，%)=100×(实验结束时虾的平均体质量 – 实验开始时虾的平均体质量)/实验开始时虾的平均体质量

特定生长率(specific growth rate，%/d)=100×(ln终末体质量 – ln初始体质量)/实验天数

### 1.5 饲料及体组织常规成分、脂肪酸组成分析

饲料中粗蛋白质含量的测定采用凯氏定氮法(FOSS，Kjeltec 2200，丹麦)，粗脂肪含量的测定采用索氏抽提法(FOSS，Soxtec™2043，丹麦)，粗灰分含量的测定采用马弗炉550 °C灼烧(14 h)法，水分含量的测定采用105 °C烘干(24 h)恒重法。

脂肪酸测定前将饲料50 °C烘干至恒重，使用氢氧化钾-甲醇法对脂肪酸进行甲酯化(AOCS, 1990)，采用HP-6890气相色谱仪进行脂肪酸分析，毛细管柱型号为Agilent 19091J-413 (30.0 mm×0.25 mm, USA)。色谱条件为柱温180 °C，进样温度220 °C，检测器温度240 °C。以面积归一法计算各脂肪酸的相对百分含量，脂肪酸含量以其

甲酯占总脂肪酸甲酯量的百分比表示。饲料组成及营养水平组成见表1，饲料脂肪酸组成见表2。

### 1.6　抗氧化酶活性的测定

用电子天平准确称取肝胰腺约0.5 g，按质量体积比1∶9加入预冷的0.86%生理盐水制成10%匀浆液，3500 r/min离心15 min，采用考马斯亮兰法测定上清液中蛋白浓度。根据各种酶活性的测定要求将上清液稀释成不同浓度，丙二醛(malondialdehyde, MDA)含量、超氧化物歧化酶(superoxide dismutase, SOD)活性以及总抗氧化能力(total antioxidant activity competence, T-AOC)的测定步骤参照南京建成生物工程研究所试剂盒说明书进行。

**表 1　实验饲料组成及营养水平**

**Tab. 1　Composition and nutrient levels of experimental diets**

| | 组别 groups | | | | |
|---|---|---|---|---|---|
| | CLA1 | CLA2 | CLA3 | CLA4 | CLA5 |
| **原料 ingredients** | | | | | |
| 酪蛋白/% casein | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| 鱼粉/% fish meal | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| 玉米淀粉/% corn starch | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| 鱼油/% fish oil | 6.00 | 4.50 | 3.00 | 1.50 | 0 |
| 共轭亚油酸/% conjugated linoleic acid | 0 | 1.50 | 3.00 | 4.50 | 6.00 |
| 大豆卵磷脂/% soybean lecithin | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| 胆固醇/% cholesterol | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| 氯化胆碱/% choline chloride | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| 矿物质预混料/% mineral premix[1] | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| 维生素预混料/% vitamin premix[2] | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| 诱食剂/% attractant[3] | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| 纤维素/% cellulose | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 |
| 羧甲基纤维素钠/% sodium methoxycellulose | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| 合计/% total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **营养水平 nutrition levels (% 风干物质 air dry matter) nutrient levels (air-dry basis)** | | | | | |
| 水分/% moisture | 9.67 | 9.67 | 10.05 | 10.21 | 9.86 |
| 粗蛋白质/% crude protein | 38.67 | 38.59 | 38.51 | 38.28 | 38.82 |
| 粗脂肪/% crude lipid | 8.45 | 8.56 | 8.52 | 8.48 | 8.62 |
| 总量 /(kJ/g)[4] gross energy | 14.09 | 14.12 | 14.10 | 14.04 | 14.18 |

注：1 每千克矿物质预混料含有(g/kg预混物)：KCl 28 g，$MgSO_4·7H_2O$ 100 g，$NaH_2PO_4$ 215 g，$KH_2PO_4$ 100 g，$Ca(H_2PO_4)_2·2H_2O$ 265 g，$CaCO_3$ 105 g，$C_6H_{10}CaO_6·5H_2O$ 165 g，$FeC_6H_5O_7·5H_2O$ 12 g，$ZnSO_4·7H_2O$ 4.76 g，$MnSO_4·H_2O$ 1.07 g，$AlCl_3·6H_2O$ 0.15 g，$CuCl_2·2H_2O$ 0.24 g，$CoC_{l2}·6H_2O$ 1.4 g，KI 0.23 g，α-纤维素 α-cellulose 2.15 g；2 每千克维生素预混料含有(/kg预混物)：VA 4 200 000 IU，VC 60 g，VE 20 g，$VD_3$ 1 200 000 IU，VK 10 g，$VB_1$ 10 g，$VB_2$ 10 g，$VB_6$ 16 g，$VB_{12}$ 20 mg，烟酸50 g，叶酸4 g，肌醇60 g，生物素 100 mg，泛酸钙35 g；3 诱食剂(mg/kg)：甘氨酸60 mg，丙氨酸60 mg，谷氨酸60 mg，甜菜碱120 mg；4 饲料总能根据蛋白质、脂肪和无氮浸出物的能量(16.7，37.6和16.7 kJ/g)来计算

Notes: 1 Mineral mixture (mg/g premix): KCL 28 mg, $MgSO_4·7H_2O$ 100 mg, $NaH_2PO_4$ 215 mg, $KH_2PO_4$ 100 mg, $Ca(H_2PO_4)_2·2H_2O$ 265 mg, $CaCO_3$ 105 mg, $C_6H_{10}CaO_6·5H_2O$ 165 mg, $FeC_6H_5O_7·5H_2O$ 12 mg, $ZnSO_4·7H_2O$ 4.76 mg, $MnSO_4·H_2O$ 1.07 mg, $AlCL_3·6H_2O$ 0.15 mg, $CuCl_2·2H_2O$ 0.24 mg, $CoC_{l2}·6H_2O$ 1.4 mg, KI 0.23 mg, α-cellulose 2.15 mg; 2 Vitamin mixture (/100g mixture): vitamin A 420 000 IU, vitamin C, 6000 mg, α-tocopherol acetate, 2000 mg, vitamin D3, 120 000 IU, vitamin K, 1000 mg, vitamin B1, 1000 mg, vitamin B2, 1000 mg, vitamin B6, 1600 mg, vitamin B12 2 mg, niacin, 5000 mg, folic acid, 400 mg, inositol, 6000 mg, biotin, 10 mg, calcium pantothenic, 3500 mg; 3 Attractant provided the following per kg of diets: glycine 60 mg, alanine 60 mg, glutamic acid 60 mg, betain 120 mg; 4 Gross energy was calculated based on protein=16.7 kJ/g; lipid=37.6 kJ/g; NFE=16.7 kJ/g

表 2 实验饲料脂肪酸组成比例(% 总脂肪酸)

Tab. 2 Fatty acid composition (% of fatty acid) of experimental diets for *M. nipponense* %

| 脂肪酸 acid | 组别 groups | | | | |
|---|---|---|---|---|---|
| | CLA1 | CLA2 | CLA3 | CLA4 | CLA5 |
| C14:0 | 5.76 | 4.25 | 3.85 | 2.44 | 1.19 |
| C16:0 | 19.72 | 16.41 | 14.76 | 10.95 | 7.12 |
| C18:0 | 4.48 | 4.27 | 4.02 | 3.64 | 3.28 |
| C21:0 | 0.14 | 6.03 | 14.32 | 21.26 | 32.12 |
| Σ SFA | 33.02 | 32.58 | 38.41 | 39.26 | 44.32 |
| C16:1 | 5.69 | 4.64 | 3.95 | 2.55 | 1.18 |
| C18:1n-9 | 16.01 | 15.06 | 14.25 | 12.81 | 11.58 |
| Σ MUFA | 31.24 | 28.39 | 21.21 | 17.93 | 13.11 |
| C18:2n-6 | 6.43 | 5.31 | 5.09 | 4.61 | 4.19 |
| *cis*-9, *trans*-11 | 0 | 2.84 | 6.70 | 11.57 | 15.06 |
| *trans*-10, *cis*-12 | 0 | 2.82 | 6.70 | 11.58 | 15.05 |
| C18:3n-6 | 0.18 | 0.14 | 0.11 | 0.64 | 0.05 |
| C20:4n-6 | 1.08 | 1.01 | 0.7 | 0.46 | 0.27 |
| C20:5n-3 | 11.21 | 10.61 | 8.24 | 5.5 | 3.16 |
| C22:6n-3 | 14.54 | 14.02 | 10.82 | 7.08 | 3.99 |
| Σ PUFA | 35.51 | 38.79 | 39.97 | 42.54 | 42.48 |
| Σ n-6 PUFA | 7.69 | 12.12 | 19.30 | 28.86 | 34.62 |
| Σ HUFA | 27.99 | 26.81 | 20.68 | 13.71 | 7.9 |
| Σ CLA | 0 | 5.66 | 13.40 | 23.15 | 31.11 |

注：表中的脂肪酸组成以2次测定的平均值表示。表中显示了主要的脂肪酸，实测脂肪酸包括： C12:0，C14:0，C15:0，C16:0，C17:0，C18:0，C20:0，C21:0，C22:0； C14:1，C16:1，C17:1，C18:1n-9，C20:1n-9，C22:1n-9，C24:1n-9； C20:2，C22:2，C18:2n-6，C18:3n-3，C18:3n-6，C20:4n-6，C20:5n-3，C22:5n-3 和C22:6n-3；Σ SFA(saturated fatty acids)指总的饱和脂肪酸含量，Σ MUFA (monounsaturated fatty acids)指总的单不饱和脂肪酸，Σ PUFA(polyunsaturated fatty acids)指总的多不饱和脂肪酸，Σ HUFA(highly unsaturated fatty acids)指碳原子数目等于或大于20的多不饱和脂肪酸，Σ CLA指总的共轭亚油酸含量

Notes: Data are mean of duplicate assay. Only the major fatty acids are shown in the table, and the detected fatty acids include: C14:0, C15:0, C16:0, C17:0, C18:0, C14:1, C16:1, C17:1, C18:1n-9, C20:1n-9, C22:1n-9, C24:1n-9; C20:2, C22:2, C18:2n-6, C18:3n-3, C18:3n-6, C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3; ΣSFA is the sum of saturated fatty acids; ΣMUFA is the sum of monounsaturated fatty acids; ΣPUFA is the sum of polyunsaturated fatty acids; ΣHUFA is the sum of highly unsaturated fatty acids with equal to and more than 20 carbons. Σ CLA is sum of CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12

### 1.7 脂质代谢相关酶活性的测定

血浆中总胆固醇(total cholesterol, TC)、甘油三酯(total triacylglycerol, TG)以及高密度脂蛋白胆固醇(high-density lipoprotein cholesterol, HDL-C)的测定参照南京建成生物工程研究所试剂盒说明书进行。TC与TG均采用酶法，主要根据一系列反应后生成的醌类化合物颜色的深浅与TC或TG的含量成正比，从而计算TC或TG的含量。HDL-C采用选择测定法，在血浆中加入沉淀剂，使血浆和沉淀剂的混合物分为上清和沉淀两部分，上清液中含有高密度脂蛋白胆固醇，然后用酶法测定上清液中胆固醇含量即为HDL-C的含量。

### 1.8 基因表达荧光定量PCR(qRT-PCR)分析

使用总RNA提取试剂盒(艾德莱，北京)提取肝胰腺总RNA，具体操作过程按试剂盒说明书进行，检测RNA的完整性及其浓度。用反转录试剂盒(TaKaRa，日本)将RNA反转录为cDNA，cDNA保存在–20 °C用于肉碱脂酰转移酶1 (carnitine palmityltransferase-1, CPT1)、乙酰辅酶A羧化酶

(acetyl coA carboxylase, ACC)和B类Ⅰ型清道夫受体(scavenger receptor class B type Ⅰ, SR-BI)qRT-PCR分析。

采用在线Primer 3设计，qRT-PCR所用引物如下：CPT1(登录号：KP690136)上游引物5′- AATTTTTGACTGGCTTCTCC-3′，下游引物5′-TCCATTCTGGAAATCATCTG-3′；ACC(登录号：KP690138)上游引物5′-CAAGGTCCACTACATGGTCT-3′，下游引物5′-ACTCTTCCCAAACTCTCTCC-3′；SR-BI(登录号：KP658863)上游引物5′-TTATCCCTGGTGTGAATGTG-3′，下游引物5′-GAACTCTTCCCATTCCAACT-3′。反应体积为20 μL，包括：10 μL的2×SYBR Green Premix Ex *Taq*(TaKaRa，日本)，上、下游引物各0.2 μL (10 μmol/L)，2 μL模板，7.6 μL dd$H_2O$。qRT-PCR反应条件：95 °C预变性30 s；94 °C 15 s，58 °C 20 s，72 °C 20 s；共40个循环；PCR反应后温度以每5 s上升5 °C的速度从60 °C上升到95 °C，绘制熔解曲线，以判断扩增产物的正确性。以日本沼虾*β*-肌动蛋白(*β-actin*，上游引物5′-GTG CCCATCTACGAGGGTTA-3′，下游引物5′-CGTCAGGGAGCTCGTAAGAC-3′)为内参，对得到的各样品循环数($C_t$)值进行均一化处理，以对照组(CLA1组)mRNA为基准，使用$-2^{\Delta\Delta C_t}$比较$C_t$值方法[22]对目的基因mRNA相对表达水平进行分析。

### 1.9　数据处理

实验结果以means±SD表示；采用SPSS 17.0软件对数据进行单因素方差分析(One-Way ANOVA)，若差异达到显著，再作Tukeys氏多重比较，$P$<0.05表示差异显著。

## 2　结果

### 2.1　CLA对日本沼虾生长存活的影响

摄食不同实验饲料后，日本沼虾的增重率和特定生长率未受到饲料中CLA含量的影响，随着CLA含量的增加，日本沼虾存活率逐渐降低，其中CLA5组存活率显著低于CLA1组($P$<0.05)(表3)。

**表 3　饲料中不同CLA含量对日本沼虾的生长存活影响**

**Tab. 3　Effects of dietary CLA on survival rate and weight gain (WG) in juvenile *M. nipponense***

| 项目 items | 组别 groups | | | | |
|---|---|---|---|---|---|
| | CLA1 | CLA2 | CLA3 | CLA4 | CLA5 |
| 初重/g initial body weight | 0.102± 0.003 | 0.101± 0.002 | 0.103± 0.002 | 0.102± 0.003 | 0.101±0.004 |
| 末重/g final body weight | 0.38±0.017 | 0.35±0.029 | 0.31±0.064 | 0.34±0.056 | 0.33±0.055 |
| 增重率/% weight gain | 275.29±16.46 | 246.91±28.18 | 203.43±63.17 | 238.24±53.18 | 219.55±56.85 |
| 特定生长率/(%/d) specific growth rate | 2.36±0.08 | 2.21±0.15 | 1.95±0.34 | 2.16±0.31 | 2.06±0.31 |
| 存活率/% survival rate | 73.50±1.32$^{b}$ | 67.63±7.80$^{ab}$ | 65.50±4.79$^{ab}$ | 64.20±3.19$^{ab}$ | 59.33±4.04$^{a}$ |

注：表中数据表示平均值±标准差($n$=5)，同行数据肩标相同字母或无字母表示差异不显著($P$>0.05)，不同小写字母表示差异显著($P$<0.05)。下表同

Notes: Values are means±SD ($n$=5). In the same line, values with no letter or the same letter superscripts mean no significant difference ($P$>0.05), while with different small letter superscripts mean significant difference ($P$<0.05). The same below

### 2.2　CLA对日本沼虾肝胰腺抗氧化能力的影响

饲料中不同浓度的CLA对日本沼虾组织中超氧化物歧化酶(SOD)活性有着显著影响($P$<0.05)，随着CLA含量的增加，SOD活性显著下降，再增加的趋势，其中CLA1组SOD活性显著高于其他组($P$<0.05)；各组总抗氧化能力(T-AOC)也存在一定差异，其中CLA2组的总抗氧化能力最低，显著低于其他组($P$<0.05)；当CLA含量超过4.5%(CLA4)时，日本沼虾肝胰腺中丙二醛(MDA)含量显著增加(表4)。

### 2.3　CLA对日本沼虾血浆中脂质代谢的影响

饲料中不同CLA含量对血浆中胆固醇(TC)、甘油三酯(TG)、以及高密度脂蛋白胆固醇(HDL-C)也产生了显著的影响，随着CLA水平的增加，血浆中TC和TG含量显著降低($P$<0.05)，同时，HDL-C逐渐升高，但各组之间无显著差异($P$>0.05)(表5)。

表 4 饲料中不同CLA含量对日本沼虾肝胰腺抗氧化酶活性的影响

Tab. 4 The MDA, SOD and T-AOC of *M. nipponense*ed fed diets with different levels of CLA

| 项目 items | 组别 groups | | | | |
|---|---|---|---|---|---|
| | CLA1 | CLA2 | CLA3 | CLA4 | CLA5 |
| 超氧化物歧化酶/(U/mg prot) superoxide dismutase | 8.40±0.54$^{c}$ | 5.40±0.99$^{ab}$ | 4.62±0.26$^{ab}$ | 3.49±1.48$^{a}$ | 5.94±1.84$^{b}$ |
| 丙二醛含量/(nmol/mg prot) malondialdehyde | 19.44±5.14$^{bc}$ | 13.33±1.32$^{ab}$ | 6.77±1.31$^{a}$ | 21.76±3.92$^{cd}$ | 29.11±1.91$^{d}$ |
| 总抗氧化能力/(U/mg prot) total antioxidant activity competence | 1.05±0.08$^{b}$ | 0.64±0.13$^{a}$ | 1.14±0.05$^{b}$ | 1.01±0.098$^{b}$ | 0.98±0.08$^{b}$ |

表 5 饲料中不同CLA含量对血浆中脂质代谢酶活性的影响

Tab. 5 The total triacylglycerol, total cholesterol and high-density lipoprotein cholesterol in plasm of *M. nipponense*ed fed diets with different levels of CLA

| 项目 items | 组别 groups | | | | |
|---|---|---|---|---|---|
| | CLA1 | CLA2 | CLA3 | CLA4 | CLA5 |
| 甘油三酯/(mmol/L) total triacylglycerol | 1.67±0.61$^{b}$ | 1.02±0.19$^{ab}$ | 0.75±0.12$^{a}$ | 0.75±0.11$^{a}$ | 1.00±0.14$^{ab}$ |
| 总胆固醇/(mmol/L) total cholesterol | 1.13±0.11$^{b}$ | 0.90±0.10$^{a}$ | 0.78±0.06$^{a}$ | 0.78±0.01$^{a}$ | 0.80±0.03$^{a}$ |
| 高密度脂蛋白胆固醇/(mmol/L) high-density lipoprotein cholesterol | 7.74±1.90 | 6.34±1.42 | 5.24±1.98 | 6.49±2.59 | 9.44±1.11 |

### 2.4 CLA对日本沼虾肝胰腺脂质代谢基因表达的影响

日本沼虾摄食不同CLA含量的饲料后，肝胰腺中*CPT*1、*ACC*和*SR-B*I基因的mRNA表达水平都发生了显著的变化(图1)。随着CLA含量的提高，*CPT*1和*SR-B*I基因的表达水平先降低，然后再上升，在CLA添加量为4.5%(CLA4)时，*CPT*1和*SR-B*I基因的表达水平达到最高，显著高于CLA2，CLA3和CLA5组($P$<0.05)；*ACC*基因的mRNA表达随着CLA水平的逐渐提高再下降，在CLA4组达到最高，显著高于其他组($P$>0.05)。

## 3 讨论

本研究结果显示，饲料中添加CLA(0~6%)对日本沼虾的增重率无显著影响。类似的研究结果也出现在一些鱼类中，如舌齿鲈(*Dicentrarchus labrax*)摄食0~1.0%的CLA、大西洋鲑摄食0~3.4%的CLA、斑点叉尾鮰摄食含0~1.0%的CLA，以及尼罗罗非鱼(*Oreochromis niloticus*)摄食0~2.5%时生长性能都未表现出显著差异[11-13, 23]。然而，杂交条纹鲈(*Morone saxatilis*×*Morone chrysops*)和黄颡鱼(*Pelteobagrus fulvidraco*)在饲料中添加1%的CLA时，生长性能出现了下降[14-15]。鲤在摄食添加1%CLA的饲料时，生长性能得到提高，但摄食5%和10%CLA时，生长受阻[9]。出现以上差异，可能与物种、CLA混合物中各同分异构体的比例、代谢速率及摄食剂量有关。

随着CLA水平的增加，日本沼虾的存活率发生下降，其中CLA5组(6%CLA)存活率显著低于CLA1组(全鱼油)，这可能与所摄食饲料中n-3PUFA和n-6PUFA比例有关。Fracalossi等[24]认为，为了使机体获得最佳的免疫能力，需要平衡该物种饲料中n-3和n-6脂肪酸的含量。因此，过多地摄食富含n-6PUFA的CLA可能会降低日本沼虾机体的免疫力。同时，从抗氧化酶的活性也发现，CLA添加量过高(>4.5%)，MDA含量显著增加。MDA是多不饱和脂肪酸的次级产物，被认为是脂质过氧化的重要标志[25]。因此，CLA4和CLA5组MDA含量的显著提高说明引起了机体脂质过氧化，从而产生了氧化应激。但是，适量地添加CLA却可以提高机体的抗氧化能力。Livisay等[26]发现随着CLA水平(0~2%)的增加，大鼠肝脏微粒体和骨骼肌匀浆的氧化稳定性增加，降低了MDA的生成。Du等[27]报道，当肉鸡日粮中添加1.25%~5%CLA时，鸡肉TBARS值随着CLA的添加显著降低，说明添加CLA提高了肉的氧化稳定性。在尼罗罗非鱼饲料中添加2.5%的CLA时，肝脏抗氧化酶SOD和GPx活性，以及MDA含量都显著降低[23]。与该研究结果相似，本实验也发现，CLA添加量从0至3%时，抗氧化酶(SOD、T-AOC)

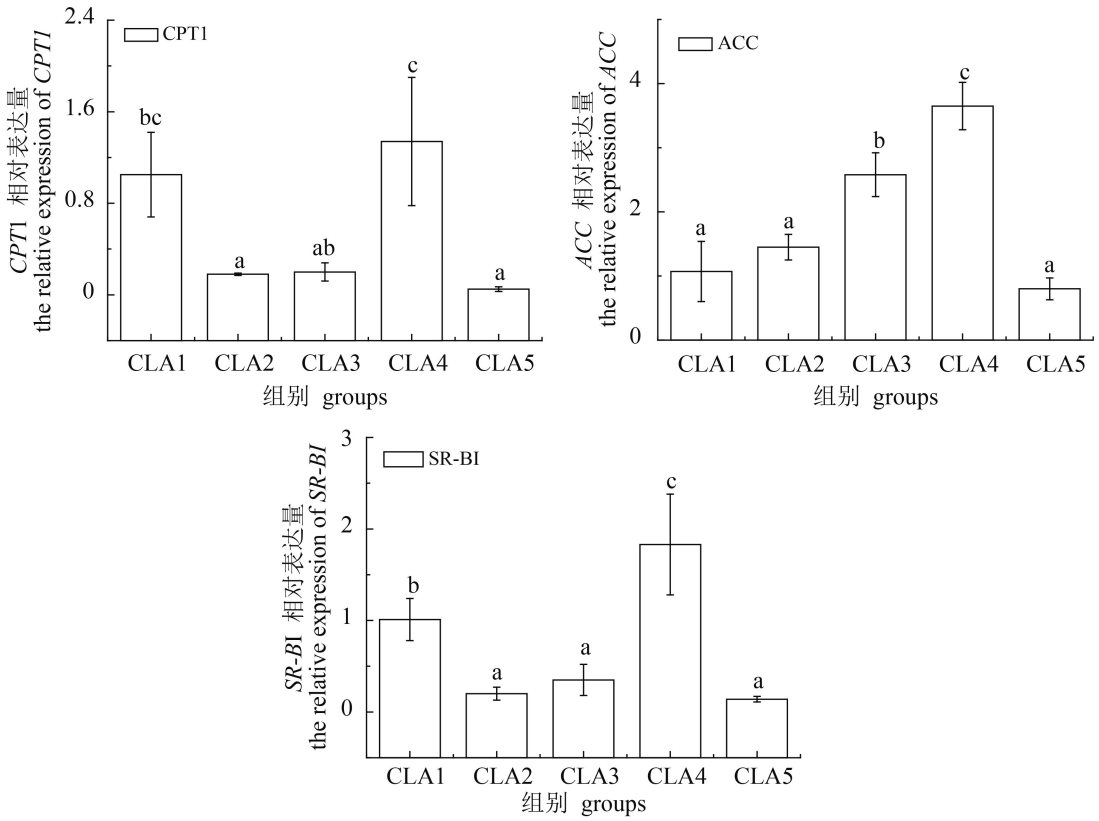


**图 1　饲料不同CLA含量对日本沼虾肝胰腺*CPT*1、*ACC*和*SR-B*I mRNA表达的影响**

不同小写字母表示差异显著($P$<0.05)

**Fig. 1　*CPT*1, *ACC* and *SR-B*I mRNA expression in the hepatopancreas of *M.nipponense* fed test diets**

Value columns with different small letters mean significant difference ($P$<0.05)

活性和MDA含量相比对照组(CLA1)都有所降低，降低的抗氧化酶活性和MDA含量表明机体有足够的能力来去除自由基的产生。

CLA具有调节机体血脂的功能，Lee等[28]研究报道，采食添加CLA的日粮12周后，新西兰白兔血清中总胆固醇和甘油三酯浓度相比对照组显著减少。Gavino等[29]报道，日粮添加CLA降低了仓鼠血浆总胆固醇和低密度胆固醇的含量。本研究中同样发现，随着CLA添加量的增加，日本沼虾血浆中甘油三酯和总胆固醇的含量显著降低。这说明CLA对低等甲壳动物的血脂也具有一定的调节作用。与Gavino等[29] 和Nicolosi等[30]研究结果相似，CLA摄食对日本沼虾血浆高密度脂蛋白胆固醇没有显著的影响。

CLA对脂质代谢影响的机理较为复杂，涉及了脂肪的合成、分解和转运复杂的生化过程。肝胰腺是甲壳动物的脂质代谢中心[31]，本实验分析了CLA对肝胰腺*ACC*、*CPT*1和*SR-B*I关键基因的表达，其中，ACC是生物素依赖的酶，能将乙酰辅酶A不可逆羧化为丙二酰，在脂肪酸生物合成中起着关键的限速作用[32-33]；CPT1是脂酰CoA进入线粒体基质转化为肉碱脂酰的重要脂质代谢酶，是脂肪酸β氧化的限速酶[34-35]；SR-BI属于CD36超家族成员，在脂质代谢平衡方面中具有重要作用[36-37]。研究表明，鱼油和CLA可以通过激活过氧化物酶体增殖受体PPARα而增加肝脏脂肪酸氧化能力[38-39]，本实验同样也发现，鱼油组和CLA4组(4.5%CLA)促进了*CPT*1基因的表达。此外，肝胰腺*SR-B*I基因表达变化趋势与*CPT*1相似，说明*SR-B*I基因的表达同样受到饲料脂肪酸组成的影响，从而调节机体脂质平衡，这与对高等动物的研究结果相似，即饲料脂肪酸组成改变了*SR-B*I基因和蛋白表达水平[40]。尽管CLA具有降低脂肪沉积的作用，但CLA却引起肝脏脂肪合成相关酶活性和基因表达上调[39, 41-42]。本实验中，在CLA添加量为0~4.5%时，日本沼虾肝胰腺脂肪合成关键基因*ACC*的表达水平逐渐显著上升，且在4.5%时达到最高。这与对小鼠的研究结

果相似，即CLA能显著增加肝脏脂肪合成相关酶基因的表达[42-43]。然而，在对草鱼的研究发现，CLA增加了脂肪合成脂肪酸合成酶(FAS)基因的表达，却降低了*ACC*基因的表达[20]。有证据表明，动物组织中*ACC*基因存在两种亚型，即*ACC*a和*ACC*b，他们具有不同的功能，前者主要维持对脂肪酸合成的调节，后者主要调节脂肪酸的氧化[44-45]。本实验中日本沼虾*ACC*基因为*ACC*a型，主要具有调节脂肪酸合成的功能。因此，CLA对*ACC*基因表达调节的差异可能与分析的是总*ACC*基因还是*ACC*亚型基因有关[20]。至于在本实验中CLA添加水平为6%时，脂肪酸代谢相关基因都显著降低，可能与较高的CLA水平引起机体脂质过氧化，从而导致代谢紊乱有关，具体的机制还有待进一步研究。

综上所述，CLA添加对日本沼虾的生长性能未产生显著影响，但添加水平较高时(6%)会降低日本沼虾的存活率；适宜的CLA添加量(0~3%)提高了机体抗氧化能力；CLA的添加降低了血浆总胆固醇和甘油三酯的浓度；CLA的添加对脂肪酸转运、氧化和合成相关基因的表达产生了一定的影响。

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# Effects of dietary conjugated linoleic acid on growth, antioxidant capacity and lipid metabolism of *Macrobrachium nipponense*

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**Abstract**: Dietary conjugated linoleic acids (CLA) have been shown to alter growth rates, improve antioxidant capacity and modulate lipid metabolism in higher animals including some species of fish, but have not been evaluated in economic crustaceans. An 8-weeks feeding trial was conducted to evaluate the effects of dietary CLA on growth, antioxidant capacity and lipid metabolism of oriental river prawn, *Macrobrachium nipponense*. Five semi-purified diets were supplemented with different levels of CLA (CLA1, 0%; CLA2, 1.5%; CLA3, 3%; CLA4, 4.5%; CLA5, 6%). Each diet was fed to juvenile prawns (mean weight, 0.102±0.0024 g) twice daily to apparent satiation in five replicates. The survival rate was influenced by dietary CLA content, and the survival rate of CLA5 significantly decreased compared with that of CLA1 ($P$<0.05). No significant difference was observed on weight gain among all groups. Activity of superoxide dismutase (SOD), total antioxidant activity competence (T-AOC) and malondialdehyde (MDA) level in hepatopancreas decreased first, and then increased as CLA level was increased. The SOD activity in prawns fed CLA1 was significantly higher than that of other treatments ($P$<0.05). The hepatopancreas MDA content was significantly increased when the CLA inclusion was above 4.5% ($P$<0.05). The total cholesterol concentration (TC) and total triacylglycerol concentration (TG) in plasma were also significantly affected by dietary CLA levels. Gradually decreased TG and TC were observed with the increase of CLA level ($P$<0.05). The mRNA expressions of carnitine palmityltransferase-1 (*CPT*1), Acetyl CoA carboxylase (*ACC*) and scavenger receptor class B type (*SR-B*I) in hepatopancreas were highly sensitive to dietary CLA. The 4.5% CLA level induced the highest *CPT*1 and *SR-B*I mRNA expression levels. Similarly, *ACC* mRNA expression was also the highest in prawns fed the CLA4 diet, which was significantly higher than that of other groups ($P$<0.05). These findings demonstrated that (1) CLA inclusion could improve the antioxidant capacity and modulate lipid metabolism; (2) Dietary CLA had little potential for improving growth responses of *M. nipponense*, and a high proportion (6%) of CLA would reduce the survival rate of prawns.

**Key words**: *Macrobrachium nipponense*; conjugated linoleic acid; growth; antioxidant capacity; lipid metabolism

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