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氧化鱼油对鲤抗应激能力的影响

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摘 要:在半纯化饲料中分别加入3%新鲜鱼油(POV 1.28 meq/kg)和POV分别为59.28、118.79和189.37 meq/kg的氧化鱼油,投喂体重100 g左右2龄鲤鱼种,15周后进行抗应激试验。结果表明:氧化鱼油可导致鲤白细胞吞噬活性增强($P<0.05$),说明鲤非特异性的细胞免疫应答水平提高,处于应激状态。病原应激和运输应激实验结果证实,长期处于应激状态的鲤,其抗应激能力减弱。

关键词:鲤;氧化鱼油;应激能力
中图分类号:S963.1 **文献标识码**:A

氧化油脂中含有多种初级和次级氧化产物,被水貂[1,2]、虹鳟[3]、斑点叉尾鮰[4]、非洲鲶[5]、银大麻哈[6]、狼鲈[7]、大西洋鲑[8]、五条鰤[9,10]和鲤[11,12]摄食后,可降低其生产性能,破坏膜结构完整性[13~15],影响其机体抗氧化酶和辅酶活性[2,16~21],降低血清中α-生育酚含量[16,22,23],因而减弱血液系统抗氧化能力,诱发血液学指标失衡,并可造成虹鳟[24~26]、狼鲈[7]、大西洋鲑[27]、鲤[12]和五条鰤[9]肝胆系统发生病变(肝脏肿大、脂肪肝、褪色、肝细胞坏死、小叶中心降解,并出现脂褐质或蜡样色素沉着),损伤肌肉组织,使鲤[11,28~30]、虹鳟[31]、狼鲈[7,32]、斑点叉尾鮰[4]和五条鰤[9]出现肌肉营养不良症。关于氧化油脂对动物抗应激能力的影响目前尚未见报道。本实验旨在探索长期摄食氧化鱼油后鲤抗应激能力的变化。

1 材料与方法

1.1 试验动物

体重100 g左右2龄鲤鱼种。依体重相近原则随机分为4个处理组,每组设4个重复,共16个试验单元,每单元投放鱼种30尾。

1.2 氧化鱼油

在鱼油中添加Fe^{2+} 30、Cu^{2+} 15、H_2O_2 600 mg/kg和0.3%的水,充分混合后,于(37±1)℃条件下搅拌氧化,在不同时间下取样得到过氧化物值(POV)分别为59.28、118.79和189.37 meq/kg的氧化鱼油,分别简写为P1、P2和P3,以F表示新鲜鱼油(对照组)。各氧化程度鱼油氧化指标测定值见表1。

1.3 试验饲料

在半纯化基础饲料中添加3%的新鲜鱼油(POV 1.28 meq/kg)和POV分别为59.28、118.79和189.37 meq/kg的氧化鱼油,形成F、P1、P2和P3处理组。试验饲料组成:酪蛋白32.00%,明胶10.00%,面粉44.00%,醋酸纤维素4.70%,鱼油3.00%,沸石粉3.00%,磷酸二氢钙2.00%,矿物盐预混料1.00%,维生素预混料0.20%,氯化胆碱0.10。营养水平:可消化能15.30(kJ/g),粗蛋白44.91%,赖氨酸2.79%,蛋氨酸1.02%,钙2.11%,磷0.74%。饲料制好后于-20℃下储存,投喂期间每周取料1次。

1.4 饲养管理

采用循环水养殖系统,养殖用聚乙烯水簇箱有效容积100 L。试验期间每日投喂2次(8:00时和15:00时各1次),日投喂量按鱼体重2%计。每周清洗养殖设施1次,水温24~28℃,溶氧6~7 mg/L,氨氮<0.2 mg/L。养殖试验期15周(1998年6

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月26日～10月13日)。

表1 氧化鱼油氧化指标

Table 1 Oxidative indices of oxidized fish oil

处理组 Treatment	POV/ (meq·kg^{-1})	TBARS/ (MDA mg·kg^{-1})	酸价/ (KOH mg·g^{-1}) AV	碘价/ (0.01g·g^{-1}) IV
F	1.28±0.01	15.52±6.85	1.18±0.02	157.48±0.44
P1	59.28±0.64	491.19±32.24	1.47±0.01	158.09±0.98
P2	118.79±0.12	1282.55±165.24	1.65±0.02	151.35±1.11
P3	189.37±0.66	2066.14±88.58	2.75±0.02	144.69±0.94

注:POV－过氧化物值 Peroxide value; F－Fresh fish oil, Pov 1.28; P1－Treated with POV 59.28; P2－Treated with POV 118.79; P3－Treated with POV 189.37. TBARS－硫代巴比妥酸反应物 Thiobarbituric acid-reacting substances; MDA－丙二醛 Malondialdehyde.

1.5 测定指标与方法

1.5.1 白细胞吞噬活性

(1)白细胞分离 将每单元平均来自于4尾鱼的抗凝血于2 000 g离心10 min,小心将血浆与红血球间白细胞吸出,于4℃保存。抗凝血物质为1%的肝素溶液,将肝素溶液均匀涂抹于5 ml试管内壁,于37℃烘箱烘干。然后,将4尾鱼的血液采集于内,制成抗凝血。

(2)白细胞与荧光乳珠孵育 将直径为1 μm的荧光乳珠(黄绿荧光的聚苯乙烯小球体)(Polysciences, Inc., Warrington, PA, USA)用血浆稀释,然后与白细胞在振荡床上常温(25℃)孵育1 h(乳珠与白细胞比例约50:1),之后于2 000 g离心10 min,沉淀用PBS悬浮后,涂片,每个血样涂5片。

(3)白细胞涂片染色 按吉姆萨(Giemsa)法[33]将制好的白细胞涂片于油镜下观察,统计白细胞吞噬百分比(P_P)和白细胞吞噬指数(I_P),其公式为:

$$\text{吞噬百分比}(P_P/\%) = \frac{\text{100个白细胞中参与吞噬的白细胞数}}{\text{100个白细胞}} \times 100$$

$$\text{吞噬指数}(I_P) = \frac{\text{100个参与吞噬的白细胞中的乳珠总数}}{\text{100个参与吞噬的白细胞}}$$

1.5.2 病原应激

用嗜水气单胞菌(*Aeromonas hydrophila*,购自华中农业大学水产学院)攻毒来测定。将菌种用肉汁蛋白胨琼脂平板培养集菌,用生理盐水稀释后注射。本测定进行2次,所用鱼每尾体重约300 g。第1次攻毒于1998年10月6日开始,从各重复随机选出5尾鱼,在每尾鱼胸鳍基部注射菌浓度为10^6 CFU/ml菌液0.2 ml,然后分别放置于经过消毒处理过的水簇箱内,3 d后未发生死亡;于10月10日每尾鱼再注射菌浓度为10^8 CFU/ml的菌液0.2 ml,之后观察7 d,记录并计算死亡率,测定期间水温保持在(21±0.5)℃。第2次测定在10月23日,从每处理组4重复的任意2重复中随机选取5尾鱼,注射菌浓度为10^8 CFU/ml的菌液0.3 ml后,分别置于经消毒处理的水簇箱内,观察5 d,记录并计算死亡率,测定期间水温保持在(29±0.5)℃。

1.5.3 运输应激

在每处理组的4重复中分别随机选取体重约300 g的鱼7尾,合并后(每组共28尾鱼)放置于盛35 L水的塑料桶中,在不充气条件下,将鱼用车从北京运至天津后折回,往返共计300 km,路途时间7.5 h(1998年10月14日11:00时至18:30时,气温16～18℃),返回实验室后立即统计临近死亡数(腹部朝上,仅鳃微动的鱼)。然后,放回充气的养殖桶,观察24 h内死亡数。

1.5.4 其它

鱼油酸价(AV)按参考文献[34],鱼油碘价(IV)、硫代巴比妥酸反应物(TBARS)和POV按参考文献[35]进行。

1.6 数据处理

用SAS软件包进行单因素方差分析,多重比较用LSD法。

2 结果

2.1 氧化鱼油对白细胞吞噬活性的影响

表2显示,氧化鱼油提高白细胞吞噬百分比(P_P)($P<0.05$),P_P增加趋势与鱼油氧化程度升高态势一致。POV为189.37 meq/kg的最高氧化程度鱼油P_P高达42.50%,高出新鲜鱼油88.89%($P<0.05$)。吞噬指数(I_P)未显示出与P_P相似的趋势,各组I_P接近($P>0.05$)。本结果反映出:鲤鱼在鱼油氧化产物刺激下,白细胞吞噬活性增强,即细胞免疫应答水平提高,表明鲤处于应激状态。

表 2　氧化鱼油对白细胞吞噬活性的影响

Table 2　Effects of oxidized fish oil on phagocytic activities of leucocytes

处理组 Treatment	吞噬百分比/% P_P	吞噬指数 I_P
F(1.28)*	22.50 ± 3.19^b	1.84 ± 0.38^a
P1(59.28)	24.50 ± 5.80^b	1.81 ± 0.16^a
P2(118.79)	38.32 ± 8.47^a	1.80 ± 0.11^a
P3(189.37)	42.50 ± 13.23^a	1.80 ± 0.16^a

* 括号内值表示过氧化物值(meq/kg)，表 3 同。Values in brackets indicate peroxide value(POV)(meq/kg), the same as table 3.

表中同列肩有不同字母者示差异显著($P<0.05$)。Values with different superscripts on the same columns show significant differences.

2.2　氧化鱼油对鲤抗病原应激能力的影响

图 1 显示，第 1 次测定中，各处理组间差异不显著($P>0.05$)，但 POV 为 59.28 和 189.37 meq/kg 的氧化鱼油处理组较对照组死亡率高出 57.14%。第 2 次测定发现，处理组与对照组出现显著差异($P<0.05$)，其中以 POV 为 118.79 meq/kg 的氧化鱼油组死亡率最高，为 70%，高出对照组 250%，但各氧化处理组间无显著差异($P>0.05$)。2 次测定中，虽然差异显著性和处理组间变化趋势不完全一致，但总体态势表明，注射嗜水气单胞菌后，氧化鱼油处理组较对照组死亡率增加。上述结果提示：摄食氧化鱼油而长期处于应激状态的鲤，其抗病原应激能力减弱。

2.3　氧化鱼油对鲤抗运输应激能力的影响

表 3 显示，对照组 2 项测定指标即返回后临近死亡率和返回后 24 h 内死亡率皆为 0，而氧化鱼油各处理组则出现不同程度死亡。虽然由于未设重复不能进行统计分析，但结果清楚表明：摄食氧化鱼油长期处于应激状态的鲤，耐受运输应激的能力减弱。

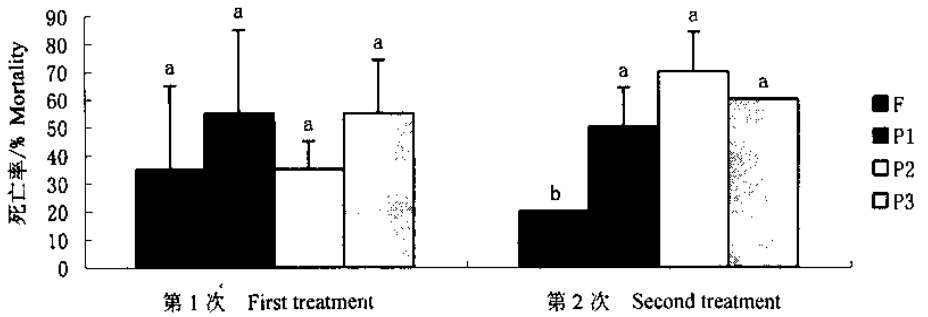

图 1　嗜水气单胞菌攻毒后鲤鱼的亡率

Fig.1 The carp mortality after injection with *Aeromonas hydrophila*

注：柱上方有不同字母者示差异显著($P<0.05$)。Columns with different letters show significant differences($P<0.05$).

表 3　鲤鱼运输应激后的死亡率

Table 3　Carp mortalities after transportation stress %

处理 Treatment	临近死亡率 Close-to-death ratio	24 h 内死亡率 Mortality within 24 h
F	0	0
P1	21.43	7.14
P2	7.14	3.57
P3	21.43	3.57

3　讨论

细胞免疫和体液免疫构成了机体疾病的防御系统。目前仅发现 IgM 一种鱼类免疫球蛋白，故细胞免疫虽然相对于体液免疫更原始，但对于鱼类或许有着更为重要的作用。在硬骨鱼类，吞噬细胞有单核细胞、嗜中性白细胞和血栓细胞 3 种，虽然血栓细胞不属白细胞，但在实际操作时，经常将其吞噬活性纳入白细胞吞噬活性来处理，本试验亦如此。白细胞吞噬活性变化是衡量机体免疫应答水平的一个有效指标。本试验出现白细胞吞噬活性增强，表明鲤对鱼油氧化产物产生非特异性的细胞免疫应答，处于应激状态。可以推断，在本次较长试验期中，长期处于应激状态将导致鲤体质下降。抗病原应激和抗运输应激结果显示：长期摄食氧化鱼油的鲤，抗病原应激能力和耐受运输应激的能力减弱，这一结论证实了推断的正确性。

在氧化鱼油对鲤抗病原应激能力影响的第 2 次测定中，死亡率普遍高于第 1 次，原因可能有两方面：一是高温条件下，受氧化鱼油毒害的鱼抗应激能力急剧减弱；另一方面，水温较高时嗜水气单胞菌繁殖速度加快，因而毒素分泌量增加，致使在比第 1 次观察时间短的情况下出现了较高死亡率。对照组在第 2 次测定中的死亡率低于第 1 次，这可能与注射

技术等方面的试验误差有关。

4 结论

氧化鱼油可导致鲤白细胞吞噬活性增强($P<0.05$),使之处于应激状态。处于应激状态的鲤,其抗病原和运输应激能力均减弱。

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Effects of oxidized fish oil on anti-stress abilities of carp, *Cyprinus carpio*

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Abstract: Juvenile carp (2 years old, average body weight about 100 g) were fed semi-purified diet supplemented with 3% of fresh fish oil (Peroxide value POV 1.28 meq/kg) and oxidized fish oil with different levels of POV (59.28, 118.79 or 189.37 meq/kg, respectively) for 15 weeks. The leucocytes of fish fed oxidized fish oil diet exhibited elevated phagocytic activities($P<0.05$). This revealed that the non-specific cell mediated immune response was increased, therefore the fish were in state of stress. Two kinds of stress tests were conducted, including bacterium-stress with *Aeromonas hydrophila* and transportation-stress with 7.5 h train way, 300 km long and air temp. 16～18℃. The mortatities in treatment groups were obviously higher than those in control fed with fresh fish oil. The results verified that the anti-stress abilities weakened when the fish are in state of stress due to dietary oxidized fish oil.

Key words: common carp; oxidized fish oil; stress tolerance