

发酵豆粕和豆粕替代部分鱼粉对银鲑幼鱼生长性能、血清生化指标、肝脏抗氧化能力和免疫相关基因表达量的影响

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FERMENTED SOYBEAN MEAL AND SOYBEAN MEAL REPLACE PARTIAL FISH MEAL ON THE GROWTH PERFORMANCE, HEMATOLOGY, LIVER ANTIOXIDANT ACTIVITIES AND IMMUNE RELATED GENES mRNA EXPRESSION OF JUVENILE COHO SALMON

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发酵豆粕和豆粕替代部分鱼粉对银鲑幼鱼生长性能、血清生化指标、肝脏抗氧化能力和免疫相关基因表达量的影响

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摘要: 为探究发酵豆粕和豆粕替代部分鱼粉对银鲑(*Oncorhynchus kisutch*)幼鱼生长性能、血清生化指标、肝脏抗氧化能力和免疫相关基因mRNA表达量的影响, 试验设置4种等氮等脂等能的饲料(粗蛋白约为42%、粗脂肪约为15%): 对照组为: 添加41%鱼粉的饲料(FM组; 鱼粉蛋白占比27%); 试验组分别为在FM组中用豆粕替代部分鱼粉(SM组; 鱼粉蛋白占比17%, 豆粕蛋白占比10%)、在FM组中用豆粕和发酵豆粕混合替代部分鱼粉(FSM5组; 鱼粉蛋白占比17%, 豆粕蛋白占比5%, 发酵豆粕蛋白占比5%)、在FM组中用发酵豆粕替代部分鱼粉(FSM10组; 鱼粉蛋白占比17%, 发酵豆粕蛋白占比10%), 饲养体重为(102.25±0.24) g的银鲑幼鱼10周。生长结果表明, FSM10组的增重率、特定生长率、日增重率、肥满度与对照组没有显著差异($P>0.05$); FSM5组特定生长率、增重率和日增重显著低于对照组($P<0.05$), 但显著高于SM组($P<0.05$); 各组间肝体比、脏体比和存活率没有显著差异($P>0.05$)。肌肉成分结果显示, 各组水分、灰分和粗蛋白没有显著差异($P>0.05$), SM组的粗脂肪显著低于对照组($P<0.05$)。血清生化试验结果表明, FSM10组血清葡萄糖、总胆固醇、白蛋白和总蛋白含量与对照组没有显著差异($P>0.05$); FSM5组血清葡萄糖、白蛋白和总蛋白含量显著低于对照组($P<0.05$), 但显著高于SM组($P<0.05$); 各组间碱性磷酸酶、谷草转氨酶和谷丙转氨酶酶活没有显著差异($P>0.05$)。肝脏抗氧化能力试验结果表明, FSM10组超氧化物歧化酶和过氧化氢酶酶活显著高于对照组($P<0.05$), 还原型谷胱甘肽含量与对照组没有显著差异($P>0.05$), 丙二醛含量显著低于对照组($P<0.05$); FSM5组过氧化氢酶酶活和还原型谷胱甘肽含量显著高于SM组($P<0.05$), 丙二醛含量显著低于SM和对照组($P<0.05$), 超氧化物歧化酶酶活与对照组和SM组没有显著差异($P>0.05$), 但显著低于FSM10组($P<0.05$)。肝脏免疫和炎症相关基因试验结果表明, FSM10组*sod-3*、*lyz*、*tlr-3*和*c3a*基因mRNA表达量显著高于对照组($P<0.05$); *tlr-7*基因mRNA表达量与对照组无显著差异($P>0.05$); 各组间*il-6*和*hsp-70*基因mRNA表达量无显著差异($P>0.05$); FSM5组*lyz*和*tlr-3*基因显著高于SM组。综上所述, 在试验条件下, 使用发酵豆粕替代10%鱼粉蛋白对银鲑生长性能和血清成分没有不良影响, 对肝脏抗氧化能力和免疫基因表达具有积极的促进作用, 因此可以在银鲑饲料中使用发酵豆粕替代10%的鱼粉蛋白。

关键词: 发酵豆粕; 生长性能; 血清生化指标; 抗氧化能力; 基因表达; 银鲑幼鱼

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银鲑(*Oncorhynchus kisutch*), 是鲑鳟中最有养殖前景的新品种, 近几年开始在中国推广养殖。目

前, 中国养殖银鲑主要是以投喂配合饲料为主, 为了缩短养殖周期以提高经济效益, 现阶段银鲑商业

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饲料中鱼粉(FM)仍处于较高水平,一般在40%—50%左右^[1],这种方式不仅成本高昂^[2],而且过量投喂的饲料沉积在水底容易造成水体环境污染,暴发疾病等^[3]。在如今水产饲料行业追求低成本、营养精准化的大背景下,寻找适宜的蛋白源替代鱼粉对于银鲑产业的发展极为重要。

目前,在鲑鳟饲料鱼粉的替代研究中,主要使用植物蛋白(如大豆蛋白、棉籽粕蛋白等)^[4, 5]、昆虫粉^[6]及一些家禽副产物^[7]作为替代源,而使用豆粕的研究相对较少。与上述材料相比,豆粕(SM)来源广泛、易于获取、质量稳定且价格低廉,被认为是替代鱼粉的优良选择。然而豆粕中的抗营养因子会阻止养殖动物对蛋白质的吸收^[8—11],严重制约了豆粕在饲料中的添加量。为了提高养殖动物的饲料消化率及对饲料中营养物质的利用率,研究必须对豆粕中抗营养因子的活性进行钝化或者消除,使豆粕成为一种优质植物蛋白源,以提高动物生产性能。

发酵豆粕(FSM)通过生产益生菌和益生元,提高动物的抗氧化能力、营养物质消化率和免疫功能。发酵豆粕的粗蛋白含量较未发酵豆粕有所提升,必需氨基酸的组成结构也更为合理^[12]。近年来,国内外已有许多研究表明,采用发酵豆粕替代鱼粉不会对水产动物生长产生负面影响^[13—16]。因此本研究使用植物乳酸菌发酵豆粕和豆粕替代饲料中不同比例鱼粉蛋白,制备4种等氮等脂等能饲料,对银鲑幼鱼进行为期10周的养殖试验,以银鲑幼鱼生长性能、血清生化指标、肝脏抗氧化能力和肝脏免疫相关基因表达量为指标,从生长性能、抗氧化酶活及基因转录水平角度讨论发酵豆粕和豆粕替代部分鱼粉蛋白对银鲑幼鱼生长、抗氧化机能及免疫机制的影响,以期对银鲑配合饲料的研发优化和银鲑养殖业的健康发展提供理论依据。

1 材料与方法

1.1 饲料制备

试验豆粕(SM)由潍坊康科润生物科技有限公司友情提供,发酵豆粕(FSM)的制备在广西民族大学海洋生态学实验室完成,营养水平及抗营养因子含量由上海璠瑞科技有限公司测定(表 1)。

以往的研究表明,饲料中植物蛋白对鱼粉蛋白的替代量以不超过10%为宜^[17—19],试验据此制备了4种等氮等脂等能的饲料(表 2),对照组为添加41%鱼粉的饲料(FM组 鱼粉蛋白占比27%);试验组分别为在FM组中用豆粕替代部分鱼粉(SM组 鱼粉

表 1 豆粕和发酵豆粕营养组成及抗营养因子含量

Tab. 1 Nutritional composition and anti-nutritional factor content of soybean meal and fermented soybean meal

指标 Index	豆粕 Soybean meal	发酵豆粕 Fermented soybean meal
营养水平 Nutrient level		
粗蛋白 Crude protein (%)	43.28±0.21	51.21±0.33
粗脂肪 Crude lipid (%)	1.84±0.05	1.93±0.09
抗营养因子 Anti-nutritional factor		
胰蛋白酶抑制因子 Trypsin inhibitors (mg/g)	66.13±1.36	15.15±0.58
大豆球蛋白 Glycinin (mg/g)	141.13±1.73	30.54±1.44
β-伴大豆球蛋白 β-Conglycinin (mg/g)	105.01±1.74	37.24±1.50
脲酶Urease (U/g)	8.01±0.18	1.90±0.15

蛋白占比17%,豆粕蛋白占比10%)、在FM组中用豆粕和发酵豆粕混合替代部分鱼粉(FSM5组 鱼粉蛋白占比17%,豆粕蛋白占比5%,发酵豆粕蛋白占比5%)、在FM组中用发酵豆粕替代部分鱼粉(FSM10组 鱼粉蛋白占比17%,发酵豆粕蛋白占比10%)。

饲料制备方法如下:所有原料按配方量称取,固体原料均匀混合后,经过超微粉碎后过60目筛;将超微粉碎后的固体原料与配方量部分鱼油、大豆油混合均匀;通过调质器调质熟化,然后用膨化机膨化挤压制成粒径为3—5 mm的颗粒,最后在鼓风干燥箱内烘干;将配方量剩余部分鱼油通过真空后喷涂工艺,将液体均匀喷涂在饲料颗粒表面即可。

1.2 饲养管理

试验于辽宁省本溪市虹鳟鱼良种场进行,试验用银鲑幼苗购买自该场,试验开始前用暂养饲料(粗蛋白42%、粗脂肪15%)暂养2周,停止投喂24h后,挑选外观正常、体质健康,初重为(102.25±0.24) g的银鲑幼鱼240尾,随机平均分成4组,每组设3个重复,每个重复20尾,放养于网箱中(1.0 m×1.0 m×1.2 m),每个网箱间隔1 m。在试验期间每天分别于8:00、12:00和16:00饱食投喂,根据摄食情况及时调整投饲量。在整个试验期间,水温10—18℃;水中溶氧量≥6.0 mg/L,养殖周期为10周。

1.3 样品采集

养殖周期结束前将鱼禁食24h,捞取各网箱鱼,称重并记录每组鱼的总重量和存活尾数,以计算生长指标。分别从各网箱中随机挑选6尾银鲑,使用MS-222进行麻醉,使用无菌注射器从尾部静脉采

表 2 饲料组成及营养水平(%干饲料)

Tab. 2 Composition and nutrient levels of diets (% dry diet)

原料名称 Ingredient	组别Group			
	FM	SM	FSM5	FSM10
鱼粉Fish meal	41.50	26.00	26.00	26.00
豆粕Soybean meal	0	22.80	10.96	0
发酵豆粕Fermenter soybean meal	0	0	9.95	19.41
鸡肉粉Chicken powder	10	10	10	10
虾粉Shrimp powder	10	10	10	10
面粉Flour	17.88	17.64	17.84	17.84
淀粉Starch	3.02	3.01	3.01	3.01
纤维素Cellulose	7.1	0	1.5	3.1
鱼油Fish oil	4.01	4.21	4.21	4.21
大豆油Soybean oil	4.01	4	4	4
磷酸二氢钙Ca(H ₂ PO ₄) ₂	1.01	1.01	1.01	1.01
矿物质预混物Vitamins premix ^a	0.52	0.52	0.52	0.52
维生素预混物Minerals premix ^b	0.52	0.52	0.52	0.52
氯化胆碱 Choline chloride	0.3	0.3	0.3	0.3
维生素C Vitamin C	0.1	0.1	0.1	0.1
营养水平Proximate composition				
粗蛋白Crude protein (%)	41.98	41.72	41.70	41.81
粗脂肪Crude lipid (%)	15.22	15.35	15.24	15.31
总能Gross energy (MJ/kg)	18.24	18.58	18.49	18.44

注: ^a每千克矿物质预混料含有(mg/kg): AlK(SO₄)₂·12H₂O, 123.7; CaCl₂, 17879.8; CuSO₄·5H₂O, 31.7; CoCl₂·6H₂O, 48.9; FeSO₄·7H₂O, 707.4; MgSO₄·7H₂O, 4316.8; MnSO₄·4H₂O, 31.1; ZnSO₄·7H₂O, 176.7; KCl, 1191.9; KI, 5.3; NaCl, 4934.5; Na₂SeO₃·H₂O, 3.4; Ca(H₂PO₄)₂·H₂O, 12457.0; KH₂PO₄, 9930.2; ^b每千克维生素预混料含有(IU or g/kg): 视网膜棕榈酸, 10000 IU; VD₃, 4000 IU; 膜接合生育醇, 75.0 IU; 甲萘醌, 22.0 g; 盐酸硫胺素, 40.0 g; 核黄素, 30.0 g; D-泛酸, 150.0 g; 盐酸吡哆醇, 20.0 g; 内消旋肌醇, 500.0 g; 生物素, 1.0 g; 叶酸, 15.0 g; 抗坏血酸, 200.0 g; 烟酸, 300.0 g; VB₁₂, 0.3 g

Note: ^acomposition (mg/kg mineral premix): AlK(SO₄)₂·12H₂O, 123.7; CaCl₂, 17879.8; CuSO₄·5H₂O, 31.7; CoCl₂·6H₂O, 48.9; FeSO₄·7H₂O, 707.4; MgSO₄·7H₂O, 4316.8; MnSO₄·4H₂O, 31.1; ZnSO₄·7H₂O, 176.7; KCl, 1191.9; KI, 5.3; NaCl, 4934.5; Na₂SeO₃·H₂O, 3.4; Ca(H₂PO₄)₂·H₂O, 12457.0; KH₂PO₄, 9930.2; ^bcomposition (IU or g/kg vitamin premix): retinal palmitate, 10000 IU; cholecalciferol, 4000 IU; α-tocopherol, 75.0 IU; menadione, 22.0 g; thiamineHCl, 40.0 g; riboflavin, 30.0 g; D-calcium pantothenate, 150.0 g; pyridoxineHCl, 20.0 g; meso-inositol, 500.0 g; D-biotin, 1.0 g; folic acid, 15.0 g; ascorbic acid, 200.0 g; niacin, 300.0 g; cyanocobalamin, 0.3 g

集全血, 4℃静置12h, 4000×g低温离心15min, 小心采集上层淡黄色的血清样品, 用于血清生化指标检测。精确分离肝脏等组织, 用生理盐水快速漂洗后用吸水纸吸干水分并记录重量, 随后置于液氮中快速冷冻, 在-80℃环境中储存, 用于计算肝体比、脏体比, 测定肝脏抗氧化能力及免疫相关基因mRNA相对表达量。用无菌的解剖刀和解剖剪取银鲑背鳍上的肌肉, 剔除鱼皮及鱼刺, 用吸水纸擦

干后置于-80℃中保存, 用于体成分测定。

1.4 样品分析

生长结果计算

$$\text{增重率(Weight gain rate, WGR, \%)} = \frac{\text{终末体质量(g)} - \text{初始体质量(g)}}{\text{初始体质量(g)}} \times 100$$

$$\text{特定生长率(Specific growth rate, SGR, \% / d)} = \frac{\ln \text{终末体质量(g)} - \ln \text{初始体质量(g)}}{\text{投喂天数(d)}} \times 100$$

$$\text{日增重(Daily growth rate, DGR, g/d)} = \frac{\text{终末体质量(g)} - \text{初始体质量(g)}}{\text{投喂天数(d)}}$$

$$\text{肥满度(Condition factor, CF, g/cm}^3\text{)} = \frac{\text{终末体质量(g)}}{\text{终末体长度(cm)}^3} \times 100$$

$$\text{肝体比(Hepatosomatic index, HSI)} = \frac{\text{肝脏质量(g)}}{\text{体质量(g)}} \times 100$$

$$\text{脏体比(Viscerosomatic index, VSI)} = \frac{\text{内脏质量(g)}}{\text{体质量(g)}} \times 100$$

$$\text{成活率(Survival rate, \%)} = \frac{\text{实验结束时鱼尾数}}{\text{实验开始时鱼尾数}} \times 100$$

体成分测定 水分含量采用105℃烘箱干燥恒重法(GB/T 6435-2014)测定, 粗蛋白质含量采用凯氏定氮法(GB/T 6432-2018)测定, 粗脂肪含量采用索氏抽提法(GB/T 6433-2006)测定, 粗灰分含量采用550℃灼烧法(GB/T 6438-2007)测定。

血清及肝脏生化指标测定 血清生化指标、肝脏抗氧化能力采用南京建成生物工程研究所生产的试剂盒进行测定, 其中葡萄糖(GLU)采用葡萄糖氧化酶法; 总胆固醇(T-CHO)采用COD-PAP法; 白蛋白(ALB)采用溴甲酚绿法; 总蛋白(TP)采用BCA法; 碱性磷酸酶(AKP)采用微量酶标法; 谷草转氨酶(GOT)和谷丙转氨酶(GPT)采用微板法。超氧化物歧化酶(SOD)采用WST-1法; 丙二醛(MDA)采用TBA法; 过氧化氢酶(CAT)采用钼酸铵法; 还原型谷胱甘肽(GSH)采用微板法。

基因mRNA表达量 在肝脏免疫相关基因表达量测定实验中, 提取肝脏总RNA、反转录和qPCR反应均采用湖南艾科瑞生物工程有限公司所生产的试剂盒进行。检测基因有超氧化物歧化酶3(Superoxide dismutase 3, *sod-3*)、溶菌酶(Lysozyme, *lyz*)、补体C3(Complement C3, *c3a*)、热休克

同源70 kD蛋白(Heat shock cognate 70 kD protein-like, *hsp-70*)、Toll样受体3 (Toll like receptor 3, *tlr-3*)、Toll样受体7 (Toll-like receptor 7, *tlr-7*)和干扰素6 (Interleukin-6, *il-6*)。基因扩增引物的设计及合成均由上海生工生物工程技术服务有限公司完成, 具体序列见表3。qPCR使用两步法完成, 具体步骤如下: 95℃、10s、1个循环; 62℃、30s并采集荧光值、40个循环; 95℃熔解曲线检测反应特异性。设置参照基因 β -actin, 根据 $2^{-\Delta\Delta C_t}$ 算法进行相对定量后, 分析肝脏中基因相对表达量。

1.5 数据处理及分析

使用SPSS 22.0软件对所有数据进行单因素方差分析(One-way ANOVA), 若差异达到显著水平($P<0.05$)则用Duncan氏法进行差异显著多重性比较, 结果以平均值 $\pm$ 标准误(mean $\pm$ SE)表示。

2 结果

2.1 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼生长性能的影响

如表4所示, FSM10组SGR、WGR和DGR与FM组没有显著差异($P>0.05$), FSM5组次之($P<0.05$),

表3 银鲑各检测基因与 β -actin参照基因引物序列

Tab. 3 Primer pairs of test genes and β -actin genes for coho salmon

基因 Gene	引物序列 Primer sequence (5'—3')	扩增长度 Amplicon size (bp)	登录号 Accession No.
β -actin	TGACCCAGATCATGT TTGAGACC CTCGTAGATGGGTAC TGTGTGGG	146	XM_0318112 26.1
<i>sod-3</i>	GGGAGCCTGCTACAT GGTAATGC CCTTCTCTCTGCTGT CGATGATGG	108	XM_0204970 14.2
<i>lyz</i>	GCTGTGTGTTCTCCT GCTTGTG TGTTCCAGCGTAGCC ATCCATTC	109	XM_0204577 70.2
<i>c3a</i>	GAGGAAAGGTGAGCC AGATG TGTGTGTGTCGTCAGC TTCG	106	XM_0317865 92.1
<i>hsp-70</i>	CCCCTGTCCCTGGGTA TTG CACCAGGCTGGTTGT CTGAGT	121	XM_0204857 29.2
<i>tlr-3</i>	GCCCTATGTCGTTCGT CCATGTG GTTGGCGATGTTGTTG TTGCTGAG	146	XM_0204971 75.2
<i>tlr-7</i>	GGTATGGAGAAGGCA GTCTGTTTGG CCATGCTAAGGTACG CCAGGTTG	106	XM_0204587 52.2
<i>il-6</i>	AGAGGACCTGTCTGC CAGTG AACGCTGGTCTTCCTC TCCC	82	XM_0205073 39.2

SM组显著低于其他各组($P<0.05$)。FSM10组与FM组、FSM5组CF没有显著差异($P>0.05$), 但显著高于SM组($P<0.05$)。各组间HSI、VSI和成活率没有显著差异($P>0.05$)。

2.2 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼肌肉组成的影响

如表5所示, 各组体成分中的水分、粗灰分和粗蛋白差异不显著($P>0.05$); 而SM组粗脂肪显著低于FSM10组和FM组($P<0.05$), 但与FSM5组没有显著差异($P>0.05$), FM、FSM5和FSM10组粗脂肪没有显著差异($P>0.05$)。

2.3 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼血清生化指标的影响

如表6所示, FSM10组血清GLU和TP与FM组

表4 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼生长、成活率及脏器指数的影响

Tab. 4 Effects of replacing fish meal with fermented soybean meal and soybean meal on the growth indices, survival rate and viscerosomatic index of juvenile coho salmon

组别Group	FM	SM	FSM5	FSM10
初始体质量IBW(g)	102.17 $\pm$ 0.44	102.33 $\pm$ 0.60	102.33 $\pm$ 0.73	102.17 $\pm$ 0.44
终末体质量FBW(g)	342.24 $\pm$ 8.66 ^a	267.46 $\pm$ 7.40 ^b	308.92 $\pm$ 7.78 ^b	357.29 $\pm$ 6.05 ^a
特定生长率SGR (%/d)	1.73 $\pm$ 0.03 ^a	1.37 $\pm$ 0.03 ^c	1.58 $\pm$ 0.04 ^b	1.79 $\pm$ 0.02 ^a
增重率WGR (%)	234.96 $\pm$ 7.96 ^a	161.31 $\pm$ 6.19 ^c	201.90 $\pm$ 7.85 ^b	249.68 $\pm$ 4.61 ^a
日增重DGR (g/d)	3.43 $\pm$ 0.12 ^a	2.36 $\pm$ 0.10 ^c	2.95 $\pm$ 0.11 ^b	3.64 $\pm$ 0.08 ^a
肥满度CF (g/cm ³)	0.93 $\pm$ 0.00 ^a	0.92 $\pm$ 0.00 ^b	0.93 $\pm$ 0.00 ^{ab}	0.93 $\pm$ 0.00 ^a
肝体比HSI	1.26 $\pm$ 0.04	1.24 $\pm$ 0.04	1.29 $\pm$ 0.02	1.36 $\pm$ 0.05
脏体比VSI	10.19 $\pm$ 0.70	11.46 $\pm$ 0.59	11.33 $\pm$ 0.72	10.25 $\pm$ 0.49
成活率SR (%)	96.67 $\pm$ 3.33	91.67 $\pm$ 1.67	93.33 $\pm$ 1.67	96.67 $\pm$ 1.67

注: 同一行相同右上角含有相同英文上标字母或无上标表示无显著差异($P<0.05$); 下同

Note: In the same row, values with different small letter superscripts are significantly different ($P<0.05$); the same applies below

表5 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼肌肉成分的影响(%干重)

Tab. 5 Effects of replacing fish meal with fermented soybean meal and soybean meal on muscle composition of juvenile coho salmon (% dry weight)

组别Group	FM	SM	FSM5	FSM10
水分Moisture	71.89 $\pm$ 0.56	72.74 $\pm$ 0.76	72.27 $\pm$ 0.66	72.30 $\pm$ 0.29
粗灰分Ash	5.00 $\pm$ 0.12	5.04 $\pm$ 0.06	4.82 $\pm$ 0.09	4.82 $\pm$ 0.03
粗蛋白 Crude protein	71.34 $\pm$ 0.40	71.35 $\pm$ 0.20	71.34 $\pm$ 0.36	70.86 $\pm$ 0.28
粗脂肪 Crude lipid	20.23 $\pm$ 0.29 ^a	19.09 $\pm$ 0.25 ^b	19.70 $\pm$ 0.50 ^{ab}	20.38 $\pm$ 0.31 ^a

没有显著差异($P>0.05$), 但显著高于FSM5和SM组($P<0.05$), FSM5组显著高于SM组($P<0.05$)。FM组血清T-CHO显著高于SM和FSM5($P<0.05$); FSM10组次之, 显著高于FSM5组($P<0.05$); FSM5组最低且与SM没有显著差异($P>0.05$)。FM组血清ALB与FSM10组差异不显著($P>0.05$), 但显著高于SM组和FSM5组($P<0.05$); FSM5组显著高于SM组($P<0.05$), 但与FSM10组差异不显著($P>0.05$)。各组间AKP、GOT和GPT差异不显著($P>0.05$)。

2.4 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼肝脏抗氧化能力的影响

如表7所示, FSM10组SOD显著高于其他各组($P<0.05$), FM组与FSM5组差异不显著($P>0.05$), 但显著高于SM组($P<0.05$)。SM组MDA显著高于其他组($P<0.05$), FM次之, 显著高于FSM5和FSM10($P<0.05$), FSM5与FSM10组差异不显著($P>0.05$)。FSM10组CAT显著高于其他各组($P<0.05$), FM组显著高于FSM5组和SM($P<0.05$)组, FSM5组显著高于SM组($P<0.05$), SM组最低。FSM10组与FM组GSH没有显著差异($P>0.05$), 但均显著高于SM组和FSM5组($P<0.05$); FSM5组显著高于SM组($P<0.05$), SM组最低。

2.5 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼肝脏免疫及炎症相关基因mRNA表达量的影响

如图1和图2所示, FSM10组*sod-3*、*lyz*、*tlr-3*和*c3a*基因相对表达量显著高于其他各组($P<0.05$); FSM10组*tlr-7*基因相对表达量显著高于SM组($P<0.05$), 与FM和FSM5差异不显著($P>0.05$); FSM5组*lyz*和*tlr-3*基因显著高于SM组($P<0.05$); FSM5组*sod-3*、*tlr-3*、*tlr-7*和*c3a*基因相对表达量与对照组无显著差异($P>0.05$); 各组间*hsp-70*和*il-6*基

因表达量差异不显著($P>0.05$)。

3 讨论

3.1 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼生长性能及肌肉成分的影响

关于在鲑鳟饲料中使用植物蛋白替代部分鱼粉的研究也有些许报道。Gu等^[20]研究发现大豆蛋白替代15%鱼粉能促进大西洋鲑(*Salmo salar*)的生长。而Hixson等^[21]研究则认为亚麻(*Camelina sati-*

表7 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼肝脏抗氧化能力的影响

Tab. 7 Effects of replacing fish meal with fermented soybean meal and soybean meal on liver antioxidant activities of juvenile coho salmon

组别Group	FM	SM	FSM5	FSM10
超氧化物歧化酶SOD (U/mg prot)	327.05±6.09 ^b	294.42±10.50 ^c	302.25±2.90 ^{bc}	357.85±12.27 ^a
丙二醛MDA (nmol/mg prot)	3.02±0.08 ^b	3.37±0.19 ^a	2.36±0.10 ^c	2.34±0.08 ^c
过氧化氢酶CAT (U/mg prot)	6.48±0.14 ^b	4.12±0.17 ^c	6.07±0.09 ^b	7.39±0.27 ^a
还原型谷胱甘肽GSH (μmol/g prot)	61.02±0.96 ^a	36.94±0.90 ^c	44.67±1.08 ^b	62.81±0.96 ^a

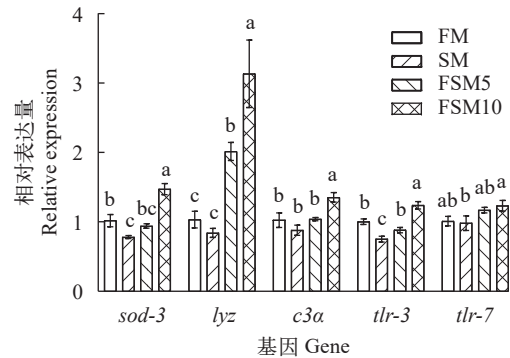


图1 银鲑幼鱼肝脏免疫相关基因mRNA表达

Fig. 1 Liver immune-related gene mRNA expression of juvenile coho salmon

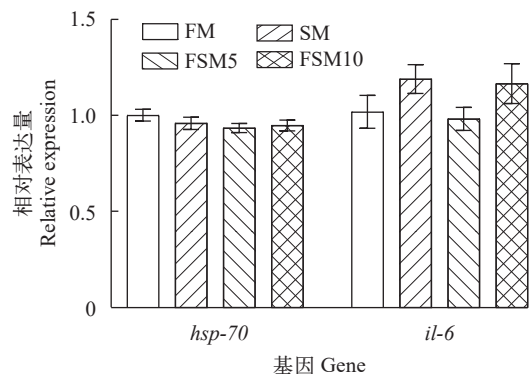


图2 银鲑幼鱼肝脏炎症相关基因mRNA表达

Fig. 2 Liver inflammatory gene mRNA expression of juvenile coho salmon

表6 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼血清生化指标的影响
Tab. 6 Effects of replacing fish meal with fermented soybean meal and soybean meal on hematology of juvenile coho salmon

组别Group	FM	SM	FSM5	FSM10
葡萄糖GLU (mmol/L)	4.32±0.26 ^a	1.88±0.31 ^c	3.17±0.05 ^b	4.02±0.18 ^a
总胆固醇T-CHO (mmol/L)	7.69±0.12 ^a	6.79±0.25 ^{bc}	6.51±0.34 ^c	7.31±0.24 ^{ab}
白蛋白ALB (g/L)	22.51±0.45 ^a	16.87±0.98 ^c	20.40±0.48 ^b	21.34±0.28 ^{ab}
总蛋白TP (μg/L)	53.88±0.92 ^a	35.21±1.61 ^c	54.00±1.06 ^b	62.80±1.74 ^a
碱性磷酸酶AKP(金氏单位/100 mL)	9.89±0.82	9.29±0.37	9.54±0.62	9.05±0.54
谷丙转氨酶GOT (U/L)	3.91±0.24	3.67±0.13	3.81±0.29	4.07±0.08
谷草转氨酶GPT (U/L)	3.77±0.21	3.53±0.24	3.83±0.22	3.83±0.16

va)粉替代鱼粉不利于大西洋鲑的生长。Rodríguez-Estrad等^[22]研究表明使用白羽扇豆(*Lupinus albus*)蛋白替代30%的鱼粉蛋白能显著促进大西洋鲑的生长,而使用发酵后的白玉扇豆替代15%的鱼粉蛋白对鱼的生长促进效果更好,但当发酵白羽扇豆替代量达到30%时显著降低鱼的生长。许多研究认为使用植物蛋白替代鱼粉对养殖水产动物的生长是有益的,然而相比于植物蛋白繁琐复杂的制备过程^[23],发酵豆粕的制备过程更加简单,成本更加低廉。因此,使用发酵豆粕替代鱼粉在水产饲料养殖中得到越来越多的关注。前人已在 大黄鱼(*Pseudosciaena crocea*)^[24]、大西洋鲑^[25]、大菱鲆(*Scophthalmus maximus* L.)^[16]、黄河鲤(*Cyprinus carpio haematopterus*)^[26]和点带石斑鱼(*Epinephelus coioides*)^[27]中观察到益生菌发酵豆粕对肉食或杂食性鱼类生长具有有益影响。本研究结果显示,FSM10组的生长性能优于SM组,而与对照组无显著差异,表明可以在银鲑幼鱼饲料中使用发酵豆粕替代部分鱼粉,且替代效果优于豆粕。可能原因如下:(1)豆粕中缺乏某些矿物质和必需氨基酸^[28, 29],伴随饲料适口性较差等原因,使鱼类采食量减少,从而抑制其生长功能^[30]。(2)豆粕中的抗营养因子会导致鱼类肠道吸收障碍,使饲料中营养物质利用率降低;本试验所制备的发酵豆粕大量降低豆粕中抗营养因子含量,减少豆粕所带来的不利影响^[31, 32]。(3)发酵豆粕相比于豆粕,粗蛋白质含量有所提高,多肽、小分子肽和氨基酸也有了一定程度的提升,营养价值得到优化^[33, 34]。(4)发酵所产生有机酸^[35]、酶类^[36]等生物活性因子不仅比鱼粉中游离的氨基酸更利于鱼体吸收,同时赋予了发酵豆粕较高的可利用性和适口性^[37],从而更好地促进动物生长,在投喂过程中也观察到各组间银鲑对饲料的摄食情况也有很大不同。

肌肉营养成分是体现水产动物营养品质的主要指标。本研究表明,使用发酵豆粕替代部分豆粕和鱼粉对银鲑肌肉水分、粗蛋白和粗灰分无显著影响,而SM组的粗脂肪显著低于对照组的原因可能是SM饲料的可消化能低,从而导致体脂沉积率低。García-Ortega等^[38]在石斑鱼(*Epinephelus lanceolatus*)的研究表明,随着大豆蛋白的替代量增加,全鱼水分含量会显著增加,脂肪和灰分会显著降低。而Liang等^[39]在七星鲈(*Lateolabrax japonicus*)的研究则认为发酵豆粕大量替代鱼粉短期(8周)会增加全鱼的水分和灰分含量,而长期(16周)投喂无显著影响。也有研究认为,植物蛋白替代少量鱼粉不会对黑海鲷(*Acanthopagrus schlegelii*)和虹鳟(*Oncorhynchus mykiss*)^[40, 41]体成分造成显著影响。这些

差异可能是由许多因素造成的,如发酵豆粕来源,养殖周期,鱼的种类和年龄、大小,饲料的组成和营养含量,实验条件和水温等。

3.2 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼血清生化指标及肝脏抗氧化能力的影响

通过测定血清生化指标能更好地分析水产养殖动物健康情况并反映饲料成分对水产养殖动物的影响^[42, 43]。血清GLU含量能直接反应机体对饲料中营养物质的吸收利用情况,本研究结果表明发酵豆粕替代10%鱼粉蛋白不会显著影响银鲑对饲料中营养物质的吸收,而未发酵豆粕会显著影响鱼体生长,这与上述生长性能所得出的结论相符。血清中约70%胆固醇来源于肝脏,在肝功能异常或损伤时,血清的胆固醇含量会显著升高,其余的30%则来源于消化道的吸收^[44]。通过对比血清中GOT、GPT及AKP含量,本研究认为四组饲料均没有对银鲑肝功能造成影响,机体没有产生应激炎症反应^[45],因此FM组血清T-CHO含量显著高于其他各组的原因可能是植物蛋白中含有的胆固醇含量较动物性蛋白低,且豆粕中存在的大豆异黄酮也具有一定清除胆固醇的作用^[46]。Kalhor等^[47]在黑鲷(*Acanthopagrus schlegelii*)和Li等^[48]在星斑川鲈(*Platichthys stellatus*)中同样得出随着植物蛋白替代量的增加,血清T-CHO含量具有降低的趋势。同时,两位学者的研究表明,随着植物蛋白替代量的增加,GOT和GPT含量显著高于对照组,该现象很可能是由于肝脏功能异常造成的^[49],这也是限制本试验在银鲑日粮中添加大量植物蛋白的原因之一。血清ALB和TP含量的提升有利于动物体更好地维持渗透压,提高代谢水平和免疫力,促进蛋白质的合成和沉积^[50, 51]。本研究结果显示,FSM10组ALB和TP含量高于对照组,但差异不显著,表明使用发酵豆粕可以在一定程度上促进银鲑幼鱼机体消化能力和吸收利用蛋白质的能力,而SM组显著低于对照组和发酵豆粕组的原因可能是普通植物蛋白源的氨基酸结构不合理导致鱼类对蛋白质的消化率和利用率较低^[52]。He等^[17]在大口黑鲈(*Micropterus salmoides*)的研究中发现豆粕替代45%鱼粉会显著降低血清中总蛋白含量,而用发酵豆粕替代45%的鱼粉对血清中总蛋白含量没有显著影响。卞宇豪等^[54]和徐茜等^[55]认为发酵豆粕替代鱼粉不会对大口黑鲈和鲫(*Carassius auratus*)的血清TP和ALB产生显著影响。李宁宇等^[56]认为使用发酵豆粕搭配少量豆粕更有利于提高日本鳗鲡(*Anguilla japoni-*

ca)黑仔鳗的蛋白质合成能力。上述研究产生差异的原因可能与不同鱼类对植物蛋白吸收能力不同有关。超氧化物歧化酶(SOD)、过氧化氢酶(CAT)和还原型谷胱甘肽(GSH)等是清除机体过多自由基和活性氧的重要抗氧化酶,能减少丙二醛(MDA)对呼吸链有关酶的损伤,同时具有细胞解毒及细胞损伤后修复等功能,较高的SOD、CAT和GSH活性水平意味着细胞具备较高的自由基清除能力^[57, 58]。本研究结果显示,FSM10组的SOD和CAT显著高于对照组,FSM5组的SOD和GSH显著高于SM组,且FSM5组SOD和CAT与对照组没有显著差异,表明发酵豆粕相比于普通豆粕和鱼粉对银鲑幼鱼清除氧自由基的能力具有显著的促进作用。研究者在凡纳滨对虾(*Litopenaeus vannamei*)^[59]、日本鳗鲡(*Anguilla japonica*)^[56]、岩鱼(*Sebastes schlegelii*)^[60]、大菱鲆^[16](*Scophthalmus maximus*)、银鲫(*Carassius auratus gibelio* var. CAS III)^[61]和虹鳟(*Oncorhynchus mykiss*)^[62]中得出了相似的结论。不少研究对发酵豆粕提升水产养殖动物抗氧化能力做了解释:一方面,豆科植物中的多酚是天然的抗氧化剂,发酵微生物不仅能释放总酚含量,还能提高维生素和胞外多糖的生物利用度,从而提高抗氧化能力^[63, 64]。另一方面,发酵豆粕中含有的免疫增强因子、抑菌素、大豆异黄酮等,可以清除自由基,抑制脂质过氧化,增强抗氧化能力^[46]。也有研究认为发酵豆粕所含有的益生菌能进入机体肠道内定植,与病原菌竞争,维持肠道微生态平衡,延缓许多慢性疾病的进展,进而提高了机体的免疫和抗病能力^[65]。总之,在多方面协同作用下,发酵豆粕使银鲑的抗氧化损伤能力有所提高。值得注意的是,FSM10组抗氧化能力的提高并没有带来生长性能的提升,结合上述研究所得出的结果^[16, 60, 62, 63],本研究认为产生该现象的原因可能是鱼体将体内的蛋白质优先用于合成功能性酶,再将蛋白质用于生长。与此对应的是,FSM10组血清ALB和TP含量的增加使抗氧化酶活力的增加,生长性能略高于对照组;而FSM5组的ALB和TP含量低于对照组,但抗氧化酶活力与对照组差异不显著,生长性能显著低于对照组;SM组的各项指标均低于对照组。然而更深入的内容有待进一步研究阐明。

3.3 发酵豆粕和豆粕替代鱼粉对银鲑幼鱼免疫及炎症相关基因mRNA表达量的影响

SOD、LYZ、C3 α 和LR等抗氧化及免疫因子可以促进抗原呈递,参与机体的抗感染和免疫调节,提高NK细胞和巨噬细胞溶酶体的活性,在机体防御病原体入侵方面发挥着重要的作用^[66]。以往的

研究表明,豆科植物中含有的黄芪多糖等植物多糖能作为免疫刺激剂,进而提高水产动物免疫基因的表达量^[67],但是这些有益成分不容易被机体消化吸收,不足以弥补抗营养因子对机体造成的影响^[68],因此豆粕替代鱼粉会使水产动物肝脏免疫基因的表达量降低^[69]。发酵豆粕不仅消除了许多抗营养因子带来的影响,其发酵过程中产生的抗菌肽^[70]和异黄酮葡萄糖苷^[71]等有益成分对银鲑相关基因表达产生积极的促进作用^[72]。本研究结果显示,FSM10组SOD、C3D和TLR基因mRNA的表达高于对照组,而FMS5组与对照组无显著差异,各组炎症基因mRNA表达量无显著差异,说明发酵豆粕能通过上调银鲑免疫相关基因的表达,达到提高银鲑免疫力的目的,银鲑没有产生炎症反应,这与上述血清生化指标和肝脏抗氧化能力所得出的结论相符。Yu等^[73]对皱纹盘鲍(*Haliotis discus hannai*)的研究表明,使用酶处理豆粕替代75%鱼粉能促进肌肉中mTOR基因mRNA表达量显著上调,而当替代量达到100%时,促炎相关基因也会显著增加。Wang等^[74]的研究同样认为用酶解豆粕替代部分豆粕可以改善南美白对虾(*Litopenaeus vannamei*)的生长和免疫状态。Yang等^[75]在七星鲈(*Lateolabrax japonicus*)饲料中添加益生菌发现抗炎基因tgf- β 1的mRNA表达量增加,促炎因子tnf- α 、il-1- β 和il-8基因mRNA的表达量降低。Zhang等^[76]发现随着豆粕添加量的增加,珍珠龙胆石斑鱼(*Pearl Gentian Groupers*)促炎基因显著上调,而抑炎因子显著下调。杨景丰等^[77]认为2%—8%发酵豆粕替代鱼粉能显著下调罗氏沼虾(*Macrobrachium rosenbergii*)hsp-70基因mRNA相对表达量而不改变免疫基因mRNA相对表达量,而当替代量达到15%时可能会导致免疫机能下降。虽然研究鱼类中免疫相关基因为评估鱼类对饲料中新成分的反应提供了一个新的角度,但基因表达水平的增加可能并没有转化为基因编码蛋白质的增加,基因编码蛋白质受多方面因素调控^[78]。事实上,mRNA表达量的丰富度与对应蛋白质丰富度之间的关系通常非常低,在哺乳动物中的mRNA丰富度可以解释约30%—40%的蛋白质丰富度差异。因此,还需要采用更全面的方法解释在饲料中添加特定膳食添加剂的结果。

4 总结

在本试验条件下,使用发酵豆粕替代10%鱼粉蛋白对银鲑生长性能和血清成分没有不良影响,对肝脏抗氧化能力和免疫基因表达具有积极的促进作用。因此可以在银鲑饲料中使用发酵豆粕替代

10%的鱼粉蛋白。

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FERMENTED SOYBEAN MEAL AND SOYBEAN MEAL REPLACE PARTIAL FISH MEAL ON THE GROWTH PERFORMANCE, HEMATOLOGY, LIVER ANTIOXIDANT ACTIVITIES AND IMMUNE RELATED GENES mRNA EXPRESSION OF JUVENILE COHO SALMON

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Abstract: In order to explore the effect of fermented soybean meal and soybean meal replacing part of fish meal on the growth performance, hematology, liver antioxidant activities and immune related gene mRNA expression of juvenile coho salmon (*Oncorhynchus kisutch*), four kinds of iso-nitrogen and iso-lipid and iso-energy feeds were set up in this experiment (crude protein is about 42% and crude lipid is about 15%). The control group was fed with 41% fish meal (FM; fish meal protein accounts for 27%); the experimental groups were replacement of partial fish meal by soybean meal in the FM diets (SM; soybean meal protein accounts for 10% and fish meal protein accounts for 17%), replacement of partial fish meal by soybean meal and fermented soybean meal in the FM diets (FSM; soybean meal protein accounts for 5%, fermented soybean meal protein accounts for 5% and fish meal protein accounts for 17%) and replacement of partial fish meal by fermented soybean meal in the FM diets (FSM; fermented soybean meal protein accounts for 10% and fish meal protein accounts for 17%). Those feeds used to feed juvenile coho salmon with an initial weight of (102.25 ± 0.24) g for 10-weeks and the results indicated that there were no significant differences in the weight gain rate (WGR), specific growth rate (SGR), daily growth rate (DGR) and condition factor (CF) between the FSM10 and FM diets ($P > 0.05$), the WGR, SGR and DGR of the FSM5 diets were significantly lower than that of the FM control groups but significantly higher than that of the SM diets ($P < 0.05$) and there were no significant differences in hepatosomatic index (HSI), viscerosomatic index (VSI) and survival rate among groups ($P > 0.05$). There was no significant difference in moisture, crude ash, crude protein among groups ($P > 0.05$), but the crude fat of the SM diets was significantly lower than that of the FM control groups ($P < 0.05$). There were no significant differences in the glucose (GLU), total cholesterol (T-CHO), albumin (ALB) and total protein (TP) between the FSM10 and FM diets ($P > 0.05$) and there were no significant differences in alkaline phosphatase (AKP), aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) among groups ($P > 0.05$), but the GLU, ALB, TP of the FSM5 diets were significantly lower than the FM control diets but higher than the SM diets. The activities of superoxide dismutase (SOD) and catalase (CAT) of the FSM10 diets were significantly higher than that of the FM control groups ($P < 0.05$) and there was no significant difference in glutathione (G-SH) between the FSM10 and FM diets ($P > 0.05$). The malondialdehyde (MDA) of the FSM10 diets were significantly lower than that of the FM control groups ($P < 0.05$). The CAT and G-SH of FSM5 of diets were higher than the SM diets but the MDA were lower than it. The SOD of FSM5 were lower than FSM10 but have not significant differences with the FM control diets. The relative mRNA expression of gene *sod-3*, *lyz*, *tlr-3* and *c3a* of the FSM10 diets were significantly higher than that of the FM control groups ($P < 0.05$), and there were no significant differences in the relative mRNA expression of gene *tlr-7* between the FSM10 and FM diets ($P > 0.05$). There were no significant differences in the relative mRNA expression of gene *il-6* and *hsp-70* among groups ($P > 0.05$). The relative mRNA expression of gene *lyz* and *tlr-3* of FSM5 diets were higher than SM diets. Overall, under the experimental conditions, using fermented soybean meal replace 10% fish meal protein had no significant differences on growth performance and hematology of juvenile coho salmon, but had positive effect on liver antioxidant capacity and immune related gene mRNA expression. Therefore, fermented soybean meal could be used instead of 10% fish meal protein in juvenile coho salmon diets.

Key words: Fermented soybean meal; Growth performance; Hematology; Antioxidant activities; Gene expression; Coho salmon (*Oncorhynchus kisutch*)