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发酵豆粕替代鱼粉对大口黑鲈幼鱼生长、脂质代谢、血清非特异性免疫及肠道菌群的影响

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摘要: 为探讨发酵豆粕替代鱼粉对大口黑鲈(*Micropterus salmoides*)幼鱼生长、脂质代谢、血清非特异性免疫及肠道菌群的影响, 试验在基础饲料中用发酵豆粕分别替代0、10%、20%、30%和40%鱼粉, 用晶体氨基酸平衡各组间蛋氨酸与赖氨酸的差异, 配制成共5种等氮等能(CP47%, GE19 MJ/kg)的试验饲料, 分别为FM、FSM10、FSM20、FSM30和FSM40。将初始体重为(22.05±0.09) g的大口黑鲈随机分成5组, 每组设3个重复, 养殖58d。生长试验结果表明: 各组之间增重率(*WGR*)、特定生长率(*SGR*)、肥满度(*CF*)、肝体比(*HSI*)和脏体比(*VSI*)均无显著差异($P>0.05$), 但替代组饲料系数(*FCR*)均显著高于FM对照组($P<0.05$), FSM40组的存活率(*SR*)和干物质表观消化率(*ADDM*)显著低于其余各组($P<0.05$)。脂质代谢方面结果显示, FSM30和FSM40组的血清甘油三酯(TG)水平显著低于FM对照组($P<0.05$), FSM40组的血清总胆固醇(T-CHO)水平显著低于FM对照组($P<0.05$)。血清非特异性免疫方面结果表明, FSM40组的溶菌酶(LZM)和超氧化物歧化酶(SOD)活性有显著增加的变化($P<0.05$)。对肠道菌群进行了Illumina Mi Seq高通量测序分析, 结果表明: 各组肠道菌群的Alpha多样性指数(Sobs、Shannon、Simpson、Ace和Chao)无显著差异($P>0.05$), 但OTU数量发生了变化。在门水平上, 优势菌群为软壁菌门(Tenericutes)、变形菌门(Proteobacteria)和梭杆菌门(Fusobacteria); 而属水平上, 优势菌群为支原菌属(*Mycoplama*)、邻单胞菌属(*Plesiomonas*)和鲸杆菌属(*Cetobacterium*)。FSM20组与FM对照组相比, 软壁菌门和支原菌属的相对丰度显著提高($P<0.05$), 变形菌门和邻单胞菌属的相对丰度显著降低($P<0.05$)。综上所述, 在试验条件下, 大口黑鲈饲料中发酵豆粕替代20%鱼粉不会对大口黑鲈幼鱼生长、脂质代谢、血清非特异性免疫及肠道菌群多样性造成负面影响, 且能显著降低肠道中邻单胞菌属在内的有害菌的相对丰度, 因此, 发酵豆粕替代鱼粉的适宜比例为20%。

关键词: 发酵豆粕; 生长; 脂质代谢; 血清非特异性免疫; 肠道菌群; 大口黑鲈

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大口黑鲈(*Micropterus salmoides*), 俗称加州鲈, 属鲈形目(Perciformes)、太阳鱼科(Cehtrachidae), 为淡水肉食性鱼类, 原产于北美洲。20世纪80年代人工繁殖成功后引入我国广东省, 经过多年养殖发展, 目前已经成为我国淡水养殖的重要品种之一。但国内养殖加州鲈主要是以投喂冰鲜鱼与配合饲料相结合的方式为主, 这种方式不仅容易污染水环境, 而且还容易暴发疾病, 故全程使用配合饲料是大口黑鲈产业可持续发展的必然趋势。然而, 全球鱼粉缺乏, 其资源已经无法满足现代水产养殖业持续增长的需要[1], 对大口黑鲈等高鱼粉饲料的影响最为显著。因此, 寻求鱼粉替代物已然成为当今水产动物营养研究的热点问题[1, 2]。

豆粕的蛋白质含量较高, 供应稳定且低廉[3], 被认为是较好的鱼粉替代物。然而, 由于它含有许多抗营养因子, 包括蛋白酶抑制剂、凝集素、植酸、皂角苷、植物雌激素、抗维生素和致敏原[4, 5], 在一定程度上会对营养物质的消化吸收产生抑制作

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用[6, 7]。此外, 研究表明, 豆粕替代鱼粉会诱导鱼类的肠道微生物群失调[8], 过度替代还会诱发鱼的肠炎、氧化应激和免疫功能障碍[9]等不良反应。而豆粕经微生物发酵后可以减少大多数抗营养因子, 降解大分子蛋白为小肽和水溶性化合物, 从而提高其营养价值并增强营养物质的消化率[10, 11]。同时也可以为动物提供益生菌、益生元[12], 且能增加具备抗氧化性能的游离氨基酸含量, 如组氨酸、赖氨酸、缬氨酸和丝氨酸[13], 及酚类化合物的浓度[14]。

菌种及发酵工艺是影响发酵豆粕质量的关键因素, 不同菌种或发酵工艺对豆粕营养成分的改善作用不同[15]。杨慧等[16]研究表明, 枯草芽孢杆菌在固态发酵时降解豆粕抗原蛋白和降低豆粕抗原性的效果优于植物乳杆菌、干酪乳杆菌、地衣芽孢杆菌和米曲霉。而叶耀辉等[17]分别以芽孢菌类、酵母菌类和乳酸菌类对豆粕进行不同类型的发酵, 结果表明, 接种芽孢类微生物的发酵豆粕小分子蛋白质含量最高, 发酵程度最好。本试验所用的发酵豆粕是以枯草芽孢杆菌为菌种, 将豆粕在有氧条件下充分发酵后的产品, 对大分子蛋白的去除率高于常见的乳酸菌厌氧发酵豆粕, 且保留了大量有益的芽孢杆菌[18], 其营养组成及抗营养因子含量如表 1, 氨基酸组成如表 2。关于枯草芽孢杆菌有氧发酵豆粕的研究目前主要集中在对豆类蛋白质水解及提高抗氧化活性方面[19, 20], 对饲喂效果方面的研究较少, 仅见虹鳟(*Oncorhynchus mykiss*)[21]、岩鱼(*Sebastes schlegeli*)[22]和日本尖吻鲈(*Lateolabrax japonicus*)[9]等几个品种, 而在大口黑鲈中的研究甚少, 且对肠道菌群影响的研究鲜有报道。本文旨在探讨枯草芽孢杆菌有氧发酵豆粕对大口黑鲈幼鱼生长、脂质代谢、血清非特异性免疫及肠道菌群的影响, 以期为大口黑鲈配合饲料的研发优化和大口黑鲈人工养殖业的健康发展提供理论依据。

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	44.60	56.90
粗脂肪Crude lipid	1.80	2.00
抗营养因子 Anti-nutritional factor		
胰蛋白酶抑制因子 Trypsin inhibitors	1.50	0.10
大豆球蛋白Glycinin	11.68	1.14
β-伴大豆球蛋白 β-Conglycinin	17.09	2.08
水苏糖Stachyose	4.20	0.10
棉籽糖Raffinose	5.40	0.05

表 2 豆粕和发酵豆粕氨基酸组成

Tab. 2 Amino acid composition of soybean meal and fermented soybean meal (%)

氨基酸 Amino acid	豆粕 Soybean meal	发酵豆粕 Fermented soybean meal
必需氨基酸Essential amino acid		
精氨酸Arg	3.30	4.06
组氨酸His	1.08	1.56
异亮氨酸Ile	1.92	2.70
亮氨酸Leu	3.24	4.50
赖氨酸Lys	2.28	3.25
蛋氨酸Met	0.49	0.76
苯丙氨酸Phe	2.32	2.93
苏氨酸Thr	1.77	2.16
色氨酸Typ	0.51	0.74
缬氨酸Val	2.02	2.79
非必需氨基酸Non-essential amino acid		
丙氨酸Ala	1.86	2.49
天冬氨酸Asp	5.36	6.43
胱氨酸Cys	0.43	0.76
谷氨酸Glu	7.85	9.60
甘氨酸Gly	1.98	2.36
脯氨酸Pro	2.17	2.82
丝氨酸Ser	2.26	2.45
酪氨酸Tyr	1.64	2.07
合计Total	42.48	54.43

1 材料与方法

1.1 试验鱼

试验用鱼购自于上海秦皇山渔业有限公司, 试验正式开始之前进行1个月的驯养, 驯养期间投喂加州鲈鱼1号料。

1.2 试验饲料

以鱼粉为主要蛋白源, 以鱼油、豆油及大豆磷脂油为主要脂肪源配制基础饲料。在基础饲料中用发酵豆粕替代0、10%、20%、30%和40%鱼粉, 另外补充晶体蛋氨酸与赖氨酸使之达到与基础组同等水平(相比于其他必需氨基酸, 蛋氨酸与赖氨酸是大口黑鲈的限制性氨基酸, 因此配方在满足其营养需求的同时保证各组蛋氨酸与赖氨酸水平一致), 将粉碎后过60目筛的所有原料按照配方的比例混合均匀, 配制成5种等氮等能(CP47%, GE19 MJ/kg)的试验饲料, 分别为FM、FSM10、FSM20、FSM30和FSM40。混合的原料经饲料机制成直径2 mm的颗粒, 60℃烘至水分低于10%, 密封于–20℃

冰箱保存备用。试验饲料配方及营养组成如表 3。

1.3 试验分组与管理

在试验开始时, 先对大口黑鲈进行24h的饥饿处理, 然后挑选体格健壮、规格均匀的鱼进行分组。5组, 每组3个重复, 随机放置初始体重为(22.05±0.09) g的鱼30尾于15个大小一致的网箱中。试验养殖周期为58d, 采取表观饱食方式进行投喂, 每日两次(8: 00和17: 00)。定期检测水质, 水温在26—32℃, 氨氮低于0.5 mg/L, pH为7.5—8.5, 不间断充气, 每隔两天换一次水, 换水量在1/3左右。

1.4 样品采集与指标测定

在养殖试验结束后, 大口黑鲈禁食24h, 然后统计每组鱼的存活数、摄食量并称重, 用于计算生长指标。每网箱随机取12尾鱼, 其中6尾分别测体长, 称体重、肝脏重和内脏重, 用于计算形体指标。其中3尾鱼于–80℃保存, 用于全鱼组分分析。另外3尾鱼, 尾静脉采血1 mL, 取血清于–80℃保存, 用于免疫等指标分析; 取肠道, 用75%酒精擦拭肠道外壁, 用生理盐水冲洗数次, 混合成一个样本收集于灭菌的EP管中, –80℃保存, 用于肠道微生物的检测。

饲料、全鱼水分含量采用105℃烘箱干燥恒重法(GB/T 6435-2014)测定, 粗蛋白质、粗脂肪和粗灰分含量依次采用凯氏定氮法(GB/T 6432-2018)、索氏抽提法(GB/T 6433-2006)和550℃灼烧法(GB/T 6438-2007)测定。

血清脂质代谢及免疫指标均采用南京建成生物工程研究所生产的试剂盒进行测定。其中血清甘油三酯(TG)含量采用GPO-PAP酶法测定; 血清总胆固醇(T-CHO)含量采用COD-PAP法测定; 血清低密度脂蛋白胆固醇(LDL-C)和高密度脂蛋白胆固醇(HDL-C)含量均采用直接法测定; 血清总蛋白(TP)含量采用考马斯亮蓝法测定; 碱性磷酸酶(AKP)和酸性磷酸酶(ACP)活性均采用磷酸苯二钠法测定; 超氧化物歧化酶(SOD)、溶菌酶(LZM)活性依次采用WST-1法、比浊法测定。

使用E.Z.N.A.® soil DNA kit(Omega 美国)从每组每个平行3尾鱼肠道混样中提取微生物群落总DNA。DNA的完整性由1%的琼脂糖凝胶电泳检测, DNA浓度和纯度由超微量分光光度计(NanoDrop2000)测定。以338F(5′-ACTCCTACGGGAGGCAGCAG-3′)和806R(5′-GGACTACHVGGGTWTCTAAT-3′)为引物, 扩增16S rRNA基因的V3—V4区。扩增程序为: 95℃预变性3min, 27个循环(95℃变性30s, 55℃退火30s, 72℃延伸30s), 72℃延伸10min (PCR仪: ABI GeneAmp® 9700型)。使用2%琼脂糖凝胶回收PCR产物, 再利用AxyPrep DNA

表 3 饲料组成及营养水平(%干饲料)

Tab. 3 Composition and nutrient levels of diets (% dry diet)

原料Ingredient	组别Group				
	FM	FSM10	FSM20	FSM30	FSM40
超级蒸汽鱼粉 Super steam fish meal	54.95	49.45	43.96	38.46	32.97
发酵豆粕 Fermented soybean meal	0.00	8.00	13.00	18.00	23.00
酵母粉Yeast	4.00	4.00	4.00	4.00	4.00
豆粕Soybean meal	2.00	2.00	2.00	2.00	2.00
玉米蛋白粉 Corn powder	3.50	3.30	3.45	3.40	3.30
谷朊粉Wheat gluten	3.00	3.00	3.00	3.00	3.00
血球粉Dried blood	0.30	0.00	0.65	1.80	3.00
酵母膏Yeast extract	1.00	1.00	1.00	1.00	1.00
鱿鱼膏Squid paste	3.30	3.30	3.30	3.30	3.30
鱼油Fish oil	3.50	3.50	3.46	3.50	3.50
豆油Soybean oil	4.50	3.80	4.30	4.00	1.82
大豆磷脂油 Soybean phospholipid oil	2.00	2.00	2.00	2.00	2.00
米糠Rice bran	1.62	1.00	0.00	0.00	0.00
a-淀粉α-starch	10.50	10.50	10.00	9.65	9.50
沸石粉Zeolite powder	2.83	2.06	2.67	2.58	4.21
蛋氨酸Met	0.00	0.05	0.10	0.16	0.22
赖氨酸Lys	0.00	0.04	0.11	0.15	0.18
维生素预混物 Vitamins premix[a]	0.70	0.70	0.70	0.70	0.70
矿物质预混物 Minerals premix[b]	0.75	0.75	0.75	0.75	0.75
磷酸二氢钙 $Ca(H_2PO_4)_2$	1.00	1.00	1.00	1.00	1.00
氯化胆碱 Choline chloride	0.50	0.50	0.50	0.50	0.50
三氧化二钇Y_2O_3	0.05	0.05	0.05	0.05	0.05
合计Total	100.00	100.00	100.00	100.00	100.00
营养水平 Nutrient level					
水分Moisture (%)	6.70	6.92	6.58	6.76	6.10
粗蛋白质 Crude protein (%)	47.14	47.45	47.18	47.27	47.27
粗脂肪 Crude lipid (%)	13.60	12.49	12.43	11.81	11.28
总能量 Gross energy (MJ/kg)	19.02	19.04	19.06	19.05	19.09

注: [a]维生素预混物(IU或者mg/kg干饲料). 维生素A, 56000 IU; 维生素D, 14000 IU; 维生素E, 350; 维生素K, 70; 维生素B1, 105; 维生素B2, 105; 维生素B6, 56; 维生素B12, 0.14; 烟酰胺, 280; D-泛酸钙, 175; 叶酸, 17.5; 生物素, 0.56; 肌醇, 700. [b]矿物质预混物(mg/kg干饲料). 钙, 78.75; 钾, 675; 镁, 90; 铁, 7.5; 铜, 22.5; 锌, 75; 锰, 28.5; 钴, 6; 硒, 0.15

Note: [a]Vitamins premix (IU or mg/kg dry diet). vitamin A, 56000 IU; vitamin D, 14000 IU; vitamin E, 350; vitamin K, 70; vitamin B1, 105; vitamin B2, 105; vitamin B6, 56; vitamin B12, 0.14; niacinamide, 280; calcium-D-pantothenate, 175; folic acid, 17.5; biotin, 0.56; inositol, 700. [b]Minerals premix (mg/kg dry diet). Ca, 78.75; K, 675; Mg, 90; Fe ,7.5; Cu, 22.5; Zn, 75; Mn, 28.5; Co, 6; Se, 0.15

Gel Extraction Kit (Axygen Biosciences, 美国)纯化, 2%琼脂糖凝胶电泳检测, 并用Quantus™ Fluorometer (Promega, 美国)进行检测定量。使用NEXTFLEX Rapid DNA-Seq Kit进行建库。利用Illumina公司的Miseq PE300平台进行测序(上海美吉生物医药科技有限公司)。

1.5　数据处理及分析

生长数据处理:

增重率(Weight gain rate, *WGR*, %)=100%×(试验鱼终末体重–试验鱼初始体重)/试验鱼初始体重;

特定生长率(Specific growth rate, *SGR*, %/d)=100%×(ln试验鱼终末体重–ln试验鱼初始体重)/试验天数;

成活率(Survival rate, *SR*, %)=100%×试验终末鱼尾数/试验初始鱼尾数;

饲料系数(Feed coefficient ratio, *FCR*)=摄食饲料质量/(试验鱼终末体重–试验鱼初始体重);

肥满度(Condition factor, *CF*, g/cm^3)=100×体重/体长^3;

肝体比(Hepatosomatic index, *HSI*)=100×肝脏重/体重;

脏体比(Viscerosomatic index, *VSI*)=100×内脏团重/体重;

饲料干物质表观消化率(Apparent digestibility of dry matter, *ADDM*, %)=100%×(1–饲料中三氧化二钇的含量/粪便中三氧化二钇的含量)。

Illumina Mi seq 测序数据处理:

使用Trimmomatic软件原始测序序列进行质控, 使用Flash软件进行拼接。使用Uparse软件, 根据97%的相似度对序列进行OTU聚类并剔除嵌合体。利用RDP Classifier对每条序列进行物种分类注释, 比对Silva数据库(SSU 128), 设置比对阈值为70%。对原始的OTU数据进行抽平处理。利用Mothur软件对肠道菌群组成进行Alpha多样性指数分析。使用R语言工具作Venn图、群落柱状图。基于样本中群落丰度数据, 做多物种差异检验柱形图。

使用SPSS 25.0软件对数据进行单因素方差分析, 差异显著则用Duncan氏法进行多重性比较, 结果以平均值±标准误(mean±SE)表示, 显著水平为$P<0.05$。

2　结果

2.1　发酵豆粕替代鱼粉对大口黑鲈幼鱼生长的影响

由表 4可知, 各试验组间*WGR*、*SGR*均无显著差异($P>0.05$); FM、FSM10、FSM20和FSM30等4组的*SR*无显著差异($P>0.05$), 但均显著高于FSM40组($P<0.05$); FSM10、FSM20、FSM30和FSM40等4组的*FCR*均无显著差异($P>0.05$), 但均显著高于FM组($P<0.05$)。

由表 5可知, 各试验组间*CF*、*HSI*和*VSI*均无显著差异($P>0.05$); FSM40组*ADDM*显著低于其余各组($P<0.05$), FSM10、FSM20和FSM30三组与FM组无显著差异($P>0.05$)。

2.2　发酵豆粕替代鱼粉对大口黑鲈幼鱼体成分的影响

由表 6可知, 各组间大口黑鲈的体成分中粗蛋白、粗脂肪和粗灰分差异不显著($P>0.05$); 全鱼的水分出现了差异, FSM10和FSM20组水分显著低于FM组($P<0.05$)。

2.3　发酵豆粕替代鱼粉对大口黑鲈幼鱼脂质代谢的影响

由表 7可知, FSM40组血清TG含量和FSM30和FSM40组血清T-CHO含量显著低于FM组($P<0.05$), 各组间LDL-C和HDL-C含量均无显著差异($P>0.05$)。

2.4　发酵豆粕替代鱼粉对大口黑鲈幼鱼血清非特异性免疫的影响

由表 8可知, 随着发酵豆粕替代鱼粉比例的增加, 大口黑鲈血清中TP含量、AKP和ACP活性均无显著变化($P>0.05$), 但FSM40组血清中LZM和SOD

表 4　发酵豆粕替代鱼粉对大口黑鲈幼鱼生长指标的影响

Tab. 4　Effects of replacing fish meal with fermented soybean meal on growth indices of juvenile largemouth bass (*n*=3)

组别 Group	初始体重 Initial weight (g)	终末体重 Final weight (g)	增重率 *WGR* (%)	特定生长率 *SGR* (%/d)	成活率 *SR* (%)	饲料系数 *FCR*
FM	22.37±0.18	74.54±1.56	233.19±5.29	2.07±0.03	96.67±1.93^{a}	0.89±0.03^{b}
FSM10	22.07±0.10	75.84±1.06	243.70±5.40	2.13±0.03	97.78±2.22^{a}	1.03±0.01^{a}
FSM20	21.86±0.22	72.53±2.72	231.67±10.62	2.07±0.05	98.89±1.11^{a}	1.01±0.03^{a}
FSM30	22.08±0.18	72.77±2.61	229.39±9.22	2.05±0.05	98.89±1.11^{a}	1.04±0.04^{a}
FSM40	21.88±0.13	73.66±1.88	236.51±6.67	2.09±0.03	87.78±1.11^{b}	1.06±0.03^{a}

注: 同列数据肩标无字母或字母相同表示差异不显著($P>0.05$), 字母不同表示差异显著($P<0.05$); 下同

Note: In the same column, values with no or the same letter superscripts mean no significant difference ($P>0.05$), while with different letter superscripts mean significant difference ($P<0.05$). The same applies below

表 5 发酵豆粕替代鱼粉对大口黑鲈幼鱼形体指标(*n*=18)及干物质表观消化率(*n*=3)的影响

Tab. 5 Effects of replacing fish meal with fermented soybean meal on body shape (*n*=18) and *ADDM* (*n*=3) of juvenile largemouth bass

组别 Group	肥满度*CF* (g/cm³)	肝体比 *HSI*	脏体比 *VSI*	干物质表观消化率 *ADDM* (%)
FM	1.40±0.07	2.27±0.29	7.89±0.47	80.51±0.76ᵃ
FSM10	1.34±0.02	2.20±0.22	7.71±0.29	80.27±0.78ᵃ
FSM20	1.30±0.04	2.15±0.14	7.60±0.17	80.29±0.79ᵃ
FSM30	1.27±0.03	1.98±0.14	7.53±0.20	80.50±0.77ᵃ
FSM40	1.29±0.02	1.98±0.09	7.46±0.24	76.55±1.10ᵇ

表 6 发酵豆粕替代鱼粉对大口黑鲈幼鱼体成分的影响

Tab. 6 Effects of replacing fish meal with fermented soybean meal on body composition of juvenile largemouth bass (%, *n*=3)

组别 Group	水分 Moisture	粗蛋白 Crude protein	粗脂肪 Crude lipid	粗灰分 Crude ash
FM	71.73±0.76[a]	15.80±0.43	6.97±0.50	3.75±0.14
FSM10	69.41±0.62[b]	15.94±0.76	7.98±0.51	4.12±0.06
FSM20	69.49±0.61[b]	16.01±0.71	7.90±0.64	3.82±0.09
FSM30	69.93±0.60[ab]	15.83±0.48	7.14±0.50	4.05±0.13
FSM40	70.66±0.68[ab]	15.76±0.57	6.90±0.28	3.95±0.10

活性均显著高于FM、FSM10、FSM20和FSM30组(P<0.05)。

2.5 发酵豆粕替代鱼粉对大口黑鲈幼鱼肠道菌群多样性和OTU数量的影响

根据各组样本OTU数量, 采用Sobs指数、Shannon指数、Simpson指数、Ace指数和Chao指数对肠道菌群进行Alpha多样性分析(表 9)。五组覆盖度在99.86%—99.93%, 表示测序量充足, 可以反映大口黑鲈肠道菌群的真实情况。另外, 各组之间Sobs指数、Shannon指数、Simpson指数、Ace指数和Chao指数虽有差异, 但差异均不显著(P>0.05)。

进一步分析各组样品中细菌多样性的相互关系, 并构建Venn图(图 1)。由图 1可知, 五组肠道样品一共有647个OTUs, 其中32个共同OTUs, 占五组OTU总数的4.95%; FM组和FSM10组共有73个OTU, 占FM组OTU数的70.87%, 占FSM10组OTU数的28.52%; FM组和FSM20组共有73个OTU, 占FM组OTU数的70.87%, 占FSM20组OTU数的33.64%; FM组和FSM30组共有66个OTU, 占FM组OTU数的64.08%, 占FSM30组OTU数的16.84%; FM组和FSM40组共有65个OTU, 占FM组OTU数的63.11%, 占FSM40组OTU数的21.59%。

表 7 发酵豆粕替代鱼粉对大口黑鲈幼鱼脂质代谢的影响

Tab. 7 Effects of replacing fish meal with fermented soybean meal on lipid metabolism of juvenile largemouth bass (mmol/L, *n*=9)

组别 Group	甘油三酯 TG	总胆固醇 T-CHO	低密度脂蛋白胆固醇 LDL-C	高密度脂蛋白胆固醇 HDL-C
FM	3.57±0.62[ab]	9.42±0.09[a]	2.02±0.61	2.26±0.27
FSM10	4.02±0.75[a]	9.85±0.07[a]	2.60±0.27	2.17±0.34
FSM20	4.61±0.81[a]	10.04±0.22[a]	2.74±0.54	2.21±0.43
FSM30	2.80±0.03[ab]	8.27±0.05[b]	2.36±0.23	2.30±0.20
FSM40	1.84±0.01[b]	7.64±0.64[b]	1.88±0.24	2.23±0.36

表 8 发酵豆粕替代鱼粉对大口黑鲈幼鱼血清非特异性免疫的影响

Tab. 8 Effects of replacing fish meal with fermented soybean meal on serum nonspecific immunity of juvenile largemouth bass (*n*=9)

组别 Group	总蛋白含量 TP content (mg/mL)	碱性磷酸酶活性 AKP activity (U/L)	酸性磷酸酶活性 ACP activity (U/L)	溶菌酶活性 LZM activity (μg/mL)	超氧化物歧化酶活性 SOD activity (U/mL)
FM	21.14±2.30	132.42±16.11	102.01±36.08	7.83±2.03[b]	59.08±11.17[b]
FSM10	21.71±1.41	108.18±7.80	68.34±5.89	8.31±0.06[b]	68.37±1.61[b]
FSM20	22.02±1.14	130.68±29.62	87.77±28.63	11.58±2.84[b]	76.58±6.59[b]
FSM30	22.72±0.51	90.22±7.15	60.80±11.36	14.00±3.21[b]	76.98±7.77[b]
FSM40	23.73±0.57	111.21±12.55	68.51±8.40	44.08±7.29[a]	109.46±6.06[a]

表 9 样品的Alpha多样性指数

Tab. 9 Alpha-diversity of samples

组别 Group	Sobs指数 Sobs index	Shannon指数 Shannon index	Simpson指数 Simpson index	Ace指数 Ace index	Chao指数 Chao index	覆盖度 Coverage (%)
FM	43.00±17.95	0.80±0.19	0.54±0.10	144.81±41.90	75.39±28.46	99.93±0.03
FSM10	104.33±64.95	1.06±0.44	0.51±0.16	155.21±48.87	126.55±63.98	99.92±0.03
FSM20	102.33±16.97	0.53±0.16	0.75±0.12	124.31±14.15	123.08±15.15	99.92±0.00
FSM30	157.33±37.55	1.00±0.45	0.58±0.19	184.80±36.60	195.67±33.21	99.89±0.02
FSM40	120.67±26.41	0.46±0.07	0.84±0.04	229.84±26.87	179.71±4.76	99.86±0.01

2.6　发酵豆粕替代鱼粉对大口黑鲈幼鱼肠道菌群组成的影响

如图 2所示，从肠道中共检测出软壁菌门(Tenericutes)、变形菌门(Proteobacteria)、梭杆菌门(Fusobacteria)、厚壁菌门(Firmicutes)、拟杆菌门(Bacteroidetes)五个门及其他未被分类的细菌。其中, 梭杆菌门(63.87%)、变形菌门(22.18%)和软壁菌门(13.53%)为FM组的优势菌门。FSM10、FSM30和FSM40组的优势门相同, 为变形菌门(71.98%、36.51%和62.38%)、软壁菌门(18.84%、55.61%和34.63%)。而FSM20组的优势门为软壁菌门(83.09%)、梭杆菌门(11.82%), 变形菌门(3.97%)为劣势门, 其相对丰度低于其余各组。

如图 3所示，从肠道中共检测出支原菌属(*Mycoplama*)、邻单胞菌属(*Plesiomonas*)、鲸杆菌属(*Cetobacterium*)、气单胞菌属(*Aeromonas*)、罗姆布茨菌属(*Romboutsia*)、紫单胞菌科中某一属(*Dysgonomonas*)6个属及其他未被分类的细菌。其中, 鲸杆菌属(63.87%)、邻单胞菌属(21.82%)和支原菌属(13.53%)为FM组的优势属。FSM10、FSM30和FSM40组的优势属相同, 为邻单胞菌属(61.51%、34.51%和60.32%)和支原菌属(18.83%、55.61%和34.63%)。而FSM20组的优势属为支原菌属(83.09%)和鲸杆菌属(11.82%), 邻单胞菌属(3.39%)为劣势属, 其相对丰度低于其余各组。

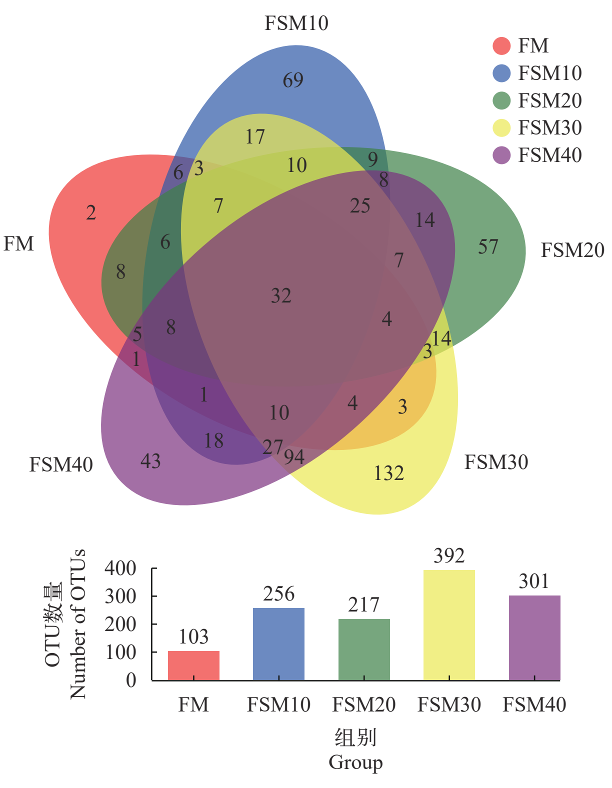

图 1　OTU数量对比维恩图

Fig. 1　Venn diagram representing shared OTUs

2.7　微生物群落差异分析

基于样本中群落丰度数据, 进行组间差异显著性检验。肠道菌群门水平上的单因素方差分析图如图 4所示, 各组间软壁菌门、变形菌门差异显著

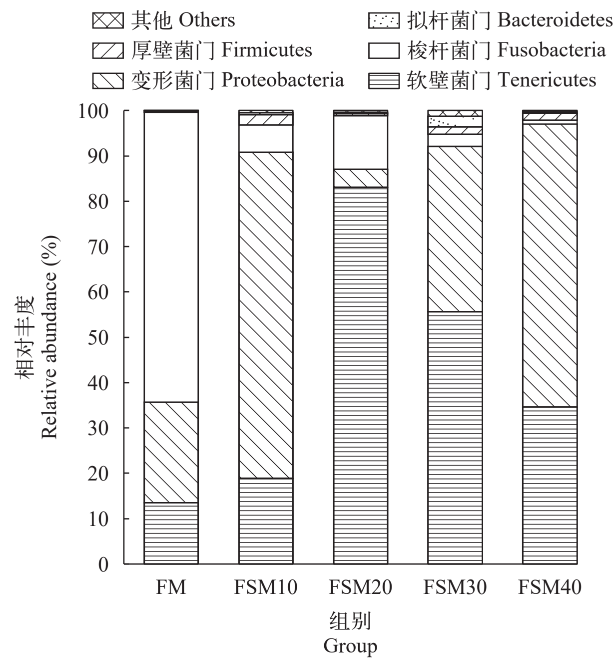

图 2　肠道菌群组成(门水平)

Fig. 2　Composition of intestinal flora (phylum level)

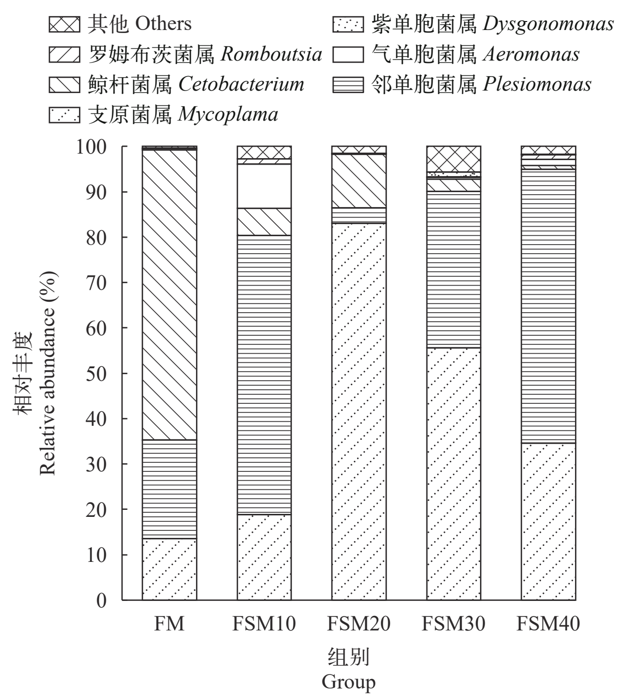

图 3　肠道菌群组成(属水平)

Fig. 3　Composition of intestinal flora (genus level)

(P<0.05)。肠道菌群属水平上的单因素方差分析图如图 5所示, 各组间支原菌属、邻单胞菌属同样也具有显著差异(P<0.05)。

3 讨论

3.1 发酵豆粕替代鱼粉对大口黑鲈幼鱼生长的影响

本研究结果表明, 发酵豆粕替代20%—30%的鱼粉不会对大口黑鲈生长产生负面影响。已有的研究中也出现了类似结果。Choi等[23]研究发现, 发酵豆粕替代40%的鱼粉, 不会对虹鳟的*WGR*产生影响。Luo等[24]发现发酵豆粕替代斜带石斑鱼(*Epinephelus coioides*)饲料中10%的鱼粉, 鱼的生长和

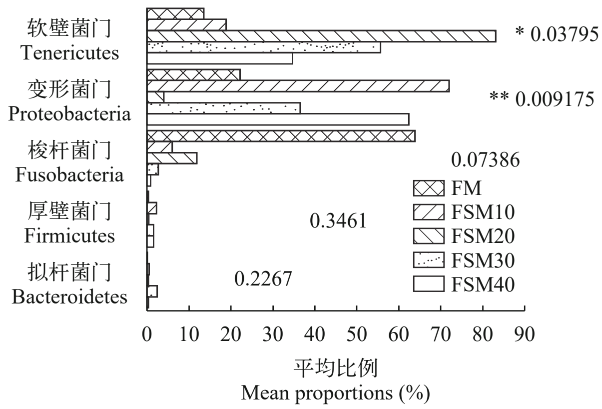

图 4 单因素方差分析条形图(门水平)

Fig. 4 One-way ANOVA bar plots (phylum level)

Y轴表示某一分类学水平下的物种名, X轴表示物种不同分组中平均相对丰度; 最右边为P值, * 0.01<P≤0.05, ** 0.001<P≤0.01, *** P≤ 0.001; 下同

The Y-axis represents the species name under a certain taxonomic level, the X-axis represents the average relative abundance in different groups of species; the rightmost is the P value, * 0.01<P≤0.05, ** 0.001<P≤0.01, *** P≤0.001. The same applies below

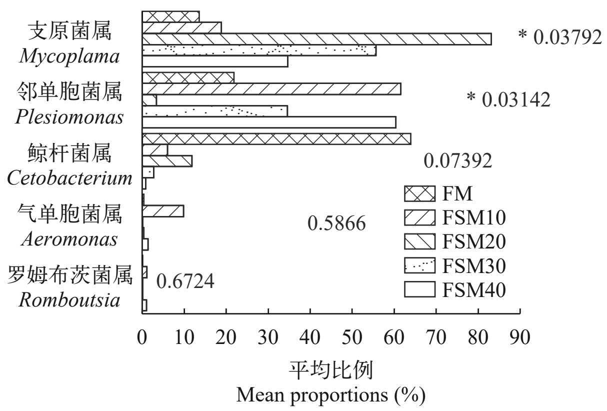

图 5 单因素方差分析条形图(属水平)

Fig. 5 One-way ANOVA bar plots (genus level)

鱼体组成没有显著变化。He等[25]研究表明, 在保持限制性氨基酸水平一致的条件下, 发酵豆粕替代不超过30%的鱼粉, 大口黑鲈的*SGR*、*SR*和*CF*与对照组相比均无显著差异。在饲料中大豆蛋白产品的含量超过一定水平, 会导致生长性能受损和饲料利用效率低下, 这与几个因素有关, 包括饲料中可消化碳水化合物水平的增加[26], 抗营养因子的存在和饲料适口性降低[4], 及氨基酸浓度不平衡[27]。在本研究中, 低于40%替代比例的组别其生长性能均未和FM对照组产生显著差异, 这可能是因为发酵消除了豆粕中大量的抗营养因子, 降解了多数大分子蛋白, 从而提高了鱼类对发酵豆粕的消化吸收能力[28]。另外, 也可能是因为在饲料中添加了适量的晶体氨基酸(赖氨酸和蛋氨酸)从而消除了限制性氨基酸的不良影响[29]。此外, 本研究结果还显示, 随着发酵豆粕替代比例的上升, 试验鱼*HSI*和*VSI*呈现逐渐降低的趋势, 这表示在一定程度上发酵豆粕可以促进脂肪的吸收, 但效果并不显著。这与He等[25]在大口黑鲈饲料中用发酵豆粕替代45%及以上比例的鱼粉可以显著降低试验鱼*HSI*的研究结果有所不同, 可能是由于发酵豆粕和饲料配方不同所致。

鱼类的消化率除了受到饲料中抗营养因子的影响还和其肠道消化酶活性有关。有研究表明, 用豆粕代替饲料中60%的鱼粉显著降低了乌鳢(*Channa argus*)的肠道消化酶活性[30], 发酵可以通过降解饲料中的大分子蛋白及提高石斑鱼(*Epinephelus coioides*)的肠道消化酶活性来提高营养物质的消化率[31]。在本研究中, 发酵豆粕中胰蛋白酶抑制因子、水苏糖和棉籽糖含量大大降低, 因此即使替代水平达到30%时, 试验鱼的*ADDM*也并未和对照组产生显著差异。而FSM40组*ADDM*的显著下降则可能和鱼类肠道消化酶活性降低有关, 有待进一步研究。

3.2 发酵豆粕替代鱼粉对大口黑鲈幼鱼体成分的影响

植物蛋白替代鱼粉对鱼体成分的影响与鱼的种类、大小和替代水平等因素相关。据报道, 植物蛋白替代鱼粉不会对虹鳟[21]、佛罗里达鲳鲹(*Trachinotus carolinus*)[32]、岩鱼[22]等的体组成造成显著影响, 但也有研究认为高水平(80%和75%)替代会显著增加石斑鱼(*Epinephelus lanceolatus*)[33]和日本尖吻鲈[34]的水分, 降低其粗蛋白和粗脂肪含量。本研究结果表明, 低水平替代组(10%和20%)与对照组相比显著降低了水分含量, 而粗蛋白和粗脂肪含量却有升高。而Daniela等[35]用豆制品替代鱼粉饲养锯盖鱼(*Centropomus viridis*)的研究中也

得到了类似的结果。

3.3　发酵豆粕替代鱼粉对大口黑鲈幼鱼脂质代谢的影响

血清TG和T-CHO是鱼类肝脏脂质代谢的重要指标[36], 而且两者在动物机体的变化趋势也基本一致。在本研究中, 随着发酵豆粕替代比例的增加, 血清TG和T-CHO含量先增后减, FSM40组的TG含量显著低于FM对照组, FSM30和FSM40组的T-CHO含量显著低于FM对照组。这种情况说明适量的发酵豆粕替代鱼粉后可以改善大口黑鲈肝脏脂质代谢状况, 而LDL-C水平的变化趋势也与之相互印证。另外高水平替代组血清TG和T-CHO含量的减少可能是大豆中的异黄酮[37]或者植物固醇[38]起的作用, 至于脂质代谢的其他分子机制还待进一步研究。

3.4　发酵豆粕替代鱼粉对大口黑鲈幼鱼血清非特异性免疫的影响

血清TP含量是衡量机体蛋白质和氨基酸的利用率的重要指标之一[39]。在本研究中, 随着发酵豆粕替代鱼粉水平的增加, 血清TP水平逐渐升高但并无显著变化。这表明在饲料中添加发酵豆粕在一定程度上可以促进血清蛋白质的合成, 从而提高机体免疫力。非特异性免疫系统作为防御系统的第一道防线, 对鱼类的抗病性尤为重要[40]。而LZM是其重要组成部分, 可以通过裂解细菌细胞壁而杀灭细菌[41]。在本研究中, 血清LZM活性随着发酵豆粕替代鱼粉比例的增加而逐渐上升并且在替代比例达到40%时与对照组产生显著差异, 这说明在本试验条件下发酵豆粕可以增强大口黑鲈的非特异性免疫力。益生菌在宿主胃肠道中既能作为免疫兴奋剂、改变酶活又能调节微生物群落结构, 有益于宿主健康[42]。推测原因可能是发酵豆粕中益生菌芽孢杆菌起到的免疫刺激作用。类似的结果, 在罗非鱼(*Oreochromis niloticus*)[43]、卵形鲳鲹(*Trachinotus ovatus*)[44]、异育银鲫(*Carassius auratus gibelio* var. CAS III)[45]中均有发现。SOD是抗氧化酶防御系统的第一道防线[46]。Zhang等[45]用发酵辣木叶替代鱼粉饲喂异育银鲫, 结果表明替代组血清SOD活性均显著升高。Choi等[23]用枯草芽孢杆菌、乳酸菌和酵母菌混合发酵的豆粕替代鱼粉, 研究发现虹鳟血清SOD活性随着发酵豆粕替代水平上升而上升。本研究结果显示, 发酵豆粕替代鱼粉可提高血清SOD活性, 替代水平为40%时与对照组产生显著差异。芽孢杆菌可以刺激抗氧化酶和抗氧化剂的产生, 从而起到清除机体自由基、维持稳态、提高抗氧化能力的作用[46, 47]。推测本研究结果可能和发酵豆粕中的枯草芽孢杆菌有关, 也可能和发酵豆粕中某些具有提高机体抗氧化能力的游离氨基酸有关[13]。

3.5　发酵豆粕替代鱼粉对大口黑鲈幼鱼肠道菌群多样性及组成的影响

鱼类肠道内的菌群种类丰富、数量庞大[48]。稳定且有益的肠道菌群对宿主的生长、健康起重要作用[49]。一旦肠道菌群失调, 宿主的免疫系统就有可能受到影响, 条件性致病菌还可能转移或危害宿主的其他组织器官, 导致细菌性疾病的爆发[50]。近几年, 研究人员广泛报道了富含营养物质(包括益生元、益生菌、小肽、游离氨基酸)的发酵豆粕是如何改善鱼类肠道微生物的[51, 52]。和Wang等[52]研究结果类似, 在本研究中, 发酵豆粕替代鱼粉并未显著改变大口黑鲈肠道菌群的多样性。这样看来肠道菌群的多样性并非是决定鱼类生长性能的关键因素。而鱼类肠道菌群组成, 在门的分类水平上, 软壁菌门、变形菌门和梭杆菌门占绝对优势, 在属的分类水平上, 支原菌属、邻单胞菌属和鲸杆菌属占绝对优势, 推测这些菌群可能是在鱼类苗种时期定值下来的[53], 大口黑鲈肠道存在一个以支原菌属、邻单胞菌属和鲸杆菌属为主的本土微生物群落。这与Larsen等[54]在大口黑鲈上的研究结果类似。而门和属水平上细菌相对丰度的差异可能与养殖环境、鱼类大小和饲料营养组成有关。

在通常情况下, 条件性致病菌是动物肠道菌群的正常组成部分, 只有在某些特定情况下, 才会引起疾病[50]。支原体隶属支原体科、柔膜菌纲, 是一类广泛存在于人和动物体内, 呈革兰氏阴性的原核细胞型微生物[55]。支原体属中的某些物种还是哺乳动物肠道中的潜在致病菌[56], 但已有研究证实支原体是鱼类肠道中正常菌群的一部分, 且其在健康和生理上可能发挥着某种未知的作用[57]。支原体的生长及繁殖需要葡萄糖和果糖, 而豆粕发酵会产生葡萄糖和果糖[58], 所以本研究替代组鱼类肠道中支原体属相对丰度均高于对照组。邻单胞菌属是养殖水体和淡水鱼体中常见的细菌, 其中的志贺氏邻单胞菌(*Plesiomonas shigelloides*)则不仅是人类肠道疾病的病原菌[59], 同时也是草鱼(*Ctenopharyngodon idellus*)[60]、银鲤(*Hypophthalmichthys molitrix*)[61]和罗非鱼[62]等鱼类的致病菌。有研究表明, 高水平发酵豆粕替代组受某些抗营养因子(如大豆球蛋白等)的影响, 会打破肠道菌群的稳态, 阻碍益生菌的生长, 使得变形菌门物种丰度增加[63]。而本研究, 饲料中大豆球蛋白和β-伴大豆球蛋白并未被降解完全, 因此组间变形菌门及该门内邻单胞菌属的相对丰度的变化(20%替代组显著低于40%替代

组)可能是由于饲料中残留的大豆球蛋白和β-伴大豆球蛋白引起的。这与何娇娇等[64]在大黄鱼上的研究结果类似, 与高水平(75%)发酵豆粕替代组相比, 中水平(45%)添加组幼鱼肠道内水栖菌属(*Enhyd-robacter*)和对照组副球菌属(*Paracoccus*)物种丰度均显著降低。

4 结论

发酵豆粕与豆粕相比, 抗营养因子含量显著降低, 氨基酸含量有所提升, 其替代鱼粉水平较低时不会对大口黑鲈幼鱼的生长、脂质代谢、血清非特异性免疫及肠道菌群产生负面影响, 且在一定程度上可以促进鱼类对脂肪的吸收, 降低肝体比和脏体比。而替代水平过高则会显著降低试验鱼成活率、干物质表观消化率、血清甘油三酯和总胆固醇含量, 引起鱼类免疫应激, 肠道菌群失衡。因此综合各方面的结果, 在此试验条件下, 鱼粉含量为54.95%的饲料中发酵豆粕替代鱼粉的适宜比例为20%。

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FERMENTED SOYBEAN MEAL INSTEAD OF FISH MEAL ON GROWTH, LIPID METABOLISM, SERUM NON-SPECIFIC IMMUNITY AND INTESTINAL FLORA OF JUVENILE LARGEMOUTH BASS

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Abstract: The global fishmeal is lacking, and the search for fishmeal substitutes has become a hot issue in the research of aquatic animal nutrition, which is of great practical significance. Soybean meal has a high protein content, a stable and low supply, and is considered as a good fish meal substitute, however, because it contains many anti-nutritional factors, including protease inhibitors, lectins, phytic acid, saponin, phytoestrogens, anti-vitamins and allergens, it will inhibit the digestion and absorption of nutrients to a certain extent. In addition, studies have shown that the replacement of fish meal with soybean meal can induce disorders of the intestinal microbiota of fish, and excessive replacement can also induce adverse reactions such as enteritis, oxidative stress and immune dysfunction in fish. However, soybean meal can reduce most anti-nutritional factors after microbial fermentation, and degrade macromolecular proteins into small peptides and water-soluble compounds, thereby increasing its nutritional value and enhancing the digestibility of nutrients. At the same time, it can also provide animals with probiotics and prebiotics, and can increase the content of free amino acids with antioxidant properties, such as histidine, serine, valine and lysine, and the concentration of phenolic compounds. In this context, the soybean meal was fully fermented under aerobic conditions with *Bacillus subtilis* as the bacterial source, and the fermented soybean meal used in this study was obtained. This product removed most of the anti-nutritional factors and degraded most of the molecular protein, increased amino acid content, has a good potential to replace fish meal. At present, the research on soybean meal fermented by *Bacillus subtilis* mainly focuses on the hydrolysis of soybean protein and the improvement of antioxidant activity, and there is very little research on the effect of fish feeding. Largemouth bass (*Micropterus salmoides*) has been introduced into China since it has been widely cultivated due to its delicious meat, few intermuscular spines, and strong disease resistance. After years of breeding development, it has become one of the important species of freshwater aquaculture in China. Therefore, the largemouth bass was used as the research object, and proved the feasibility of *Bacillus subtilis* fermented soybean meal to replace fish meal through experimental research, hoping to provide some references for the optimization of largemouth bass feed formula and the sustainable development of artificial breeding industry. To explore the effects of fermentation of soybean meal instead of fish meal on the growth, lipid metabolism, serum non-specific immunity and intestinal flora of juvenile largemouth bass, fish meal was replaced with fermentation of soybean meal at 0, 10%, 20%, 30%, and 40%, respectively. Crystal amino acids were used to balance the difference between methionine and lysine in each group, and 5 isonitrogen equal energy diets (CP47%, GE19 MJ/kg) referred to as FM, FSM10, FSM20, FSM30 and FSM40 were used to feed Largemouth bass with an initial weight of (22.05±0.09) g with three replicates for 58 days. The results showed that there were no significant differences in the weight gain rate (*WGR*), specific growth rate (*SGR*), condition factor (*CF*), hepatosomatic index (*HSI*) and viscerosomatic index (*VSI*) among groups (P>0.05), but soybean meal significantly increased the feed coefficient ratio (*FCR*) (P<0.05). The survival rate (*SR*) and apparent dry matter digestibility (*ADDM*) of the FSM40 group were significantly lower than other groups (P<0.05). The serum triglyceride (TG) levels of the FSM30 and FSM40 groups were significantly lower than the FM control group (P<0.05), and the serum total cholesterol (T-CHO) level of the FSM40 group was significantly lower than the FM control group (P<0.05). The activities of lysozyme (LZM) and superoxide dismutase (SOD) in the FSM40 group increased significantly (P<0.05). The Alpha diversity indexes (Sobs, Shannon, Simpson, Ace and Chao) of of intestinal flora had no significant difference among groups (P>0.05), but the number of OTUs were different. The main phyla in intestine of fish were Tenericutes, Proteobacteria and Fusobacteria in soybean meal groups; the dominant genera were *Mycoplama*, *Plesiomon*as and *Cetobacterium*. Compared with the FM control group, the relative abundances of Tenericutes and *Mycoplama* in the FSM20 group increased significantly (P<0.05), and the relative abundances of Proteobacteria and *Plesiomonas* significantly reduced (P<0.05). Overall, under the experimental conditions, the replacement of 20% fish meal with fermented soybean meal in largemouth bass feed did not have a negative impact on the growth, lipid metabolism, serum non-specific immunity and intestinal flora diversity of largemouth bass juveniles, and It significantly reduced the relative abundance of harmful bacteria including the *Plesiomonas* in intestine, so the appropriate ratio of fermented soybean meal to replace fish meal was 20%.

Key words: Fermented soybean meal; Growth; Lipid metabolism; Serum non-specific immunity; Intestinal flora; Largemouth bass