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生物絮团养殖模式下益生菌添加对异育银鲫生长、消化酶活性及肠道组织结构的影响

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摘要: 前期研究表明, 生物絮团技术(biofloc technology, BFT)适于异育银鲫(*Carassius auratus gibelio*)养殖。为进一步优化 BFT 养殖模式, 本研究设置 3 个实验组: BFT 模式下 EM 菌添加组(BB 组)、枯草芽孢杆菌(*Bacillus subtilis*)添加组(BI 组)和 BFT 对照组(B 组), 以均体重(1.60±0.50) g 的异育银鲫为研究对象, 探讨 BFT 模式下外源添加益生菌对养殖动物生长、消化酶活性及肠道组织结构的影响。结果表明: (1)益生菌添加组异育银鲫增重率和特定生长率显著高于对照组(P<0.05), BB 和 BI 组的增重率分别提高了 216.70%和 184.04%, 特定生长率分别提高了 141.18%和 125.49%, BB 和 BI 组间差异不显著(P>0.05); (2)益生菌添加组(BB 组和 BI 组)的消化酶(淀粉酶、脂肪酶和胃蛋白酶)活性均显著高于对照组(B 组)(P<0.05)。益生菌添加组间, BB 组淀粉酶活性显著高于 BI 组(P<0.05), 脂肪酶和胃蛋白酶活性亦高于 BI 组, 但差异不显著(P>0.05); (3)益生菌添加组肠道肌层厚度和黏膜下层厚度显著高于对照组(B 组)(P<0.05), BB 组异育银鲫肠道黏膜皱襞高度和皱襞间质宽度与 BI 和对照组相比, 均无显著差异(P>0.05)。研究表明, BFT 养殖模式下外源添加益生菌可以更好地促进异育银鲫生长。

关键词: 生物絮团技术; 益生菌; 异育银鲫; 生长; 消化酶; 肠道结构
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异育银鲫(*Carassius auratus gibelio*)是中国重要的经济养殖对象, 2016 年其年产量约为 291 万 t[1-2]。与其他水产养殖动物的发展历程相似, 随着养殖密度的增加和集约化程度的逐步提升, 养殖水环境污染日益严重, 导致异育银鲫病害频发, 如疱疹病毒Ⅱ型(CyHV-2)引起的鳃出血病[3-5]和粘孢子虫病, 已经严重影响了异育银鲫养殖业的可持续发展和养殖效益[6-8]。此外, 疾病防治过程中抗生素的滥用对环境和人类健康均产生了一定危害, 如菌株耐药、药物残留以及免疫抑制[9-10]。为此, 越来越多的水产科技工作者开始寻求异育银鲫疾病防治新思路和健康养殖的新模式。

生物絮团技术(biofloc technology, BFT)是一种新兴的零排放高效生态养殖技术, 其中发挥核心作用的是水体中的土著微生物。通过向养殖水体中外源添加碳源促进异养细菌等土著微生物繁殖, 将水体中细菌、原生动物、藻类和残饵粪便等絮凝成养殖动物可摄食的生物絮团, 从而起到净化水质、促进养殖动物生长和增强机体免疫的作用, 如日本对虾(*Marsupenaeus japonicus*)、吉富罗非鱼(*Oreochromis niloticus*)和凡纳滨对虾(*Litopenaeus vannamei*)等[11-16]。前期研究表明, BFT 模式适于异育银鲫养殖, 可促进鱼体生长, 增强其抗应激能力和抗病力[17]。

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近年来, 益生菌作为抗生素的替代品在水产养殖中的应用受到更多青睐[18-19]。益生菌(probiotics)是包括细菌、酵母和真菌在内的活的微生物, 其在一定浓度范围内有益于宿主健康[20-22]。Kozasa[23]于 20 世纪 80 年代首次提出将益生菌应用于水产养殖[24]。其中常用的益生菌有 EM(effective microorganisms)菌、芽孢杆菌属细菌(*Bacillus* sp.)和乳酸杆菌属细菌(*Lactobacillus* sp.)[25-27]。大量研究表明, 益生菌添加对海参(*Apostichopus japonicus*)、虾蟹及淡、海水鱼类等养殖动物的生长和免疫均具有一定的促进作用[28-31]。Yu 等[2]研究凝结芽孢杆菌(*Bacillus coagulans*)添加对异育银鲫的影响, 发现适当添加凝结芽孢杆菌可促进异育银鲫生长和抗氧化能力。因此, 为进一步优化异育银鲫的 BFT 养殖模式, 本研究首次尝试在 BFT 养殖模式下外源添加益生菌(EM 菌和枯草芽孢杆菌)来探讨在土著微生物作用的基础上外源添加益生菌对异育银鲫生长、消化酶活性和肠道组织结构的影响, 旨在为 BFT 的高效推广及应用奠定理论基础。

1　材料与方法

1.1　实验材料及暂养管理

异育银鲫购自盐城市龙辰水产苗种养殖基地。选取体重为(1.60±0.50) g, 体表无损伤、活力好的实验用鱼于盐城工学院实验基地暂养 5 d。养殖池使用高锰酸钾泼洒消毒, 养殖用水曝气过滤后用于实验。水温(25.0±0.5)℃, 溶解氧>5 mg/L。每日投喂商业饲料(粗蛋白 32.25%, 粗脂肪 5.90%, 总钙 1.17%和总磷 1.23%, 通威股份有限公司)3 次, 投喂时间为 6:30、11:30 和 19:00, 日投饵量为鱼体重的 3%。每日换水 1 次, 日换水量为 1/3~1/2, 每天清理 1 次残饵和粪便。

EM 菌购自江苏绿科生物技术有限公司, 枯草芽孢杆菌(*Bacillus subtilis*)自行分离于生物絮团养殖水体中, 糖蜜购自广西中孚信糖业有限公司。

1.2　实验设计

本研究在盐城工学院水产养殖实验基地进行。暂养结束后, 将 1800 尾体质健康、大小均一的异育银鲫随机分入 9 个室内水泥池(3.0 m×1.0 m×0.8 m), 放养密度为 200 尾/池。共设置 3 个实验组, 即 BFT 养殖模式下 EM 菌添加组(BB 组)、BFT 养殖模式下枯草芽孢杆菌添加组(BI 组)以及 BFT 养殖对照组(B 组), 每组设置 3 个重复, 每个养殖池均 24 h 不间断供氧, 循环。

实验期间, 水温维持在 26~28℃, pH 7.5~8.5, 溶解氧>5.0 mg/L, 投喂时间和方式与暂养一致, 每周根据异育银鲫摄食和生长情况适当调整投喂量。养殖期间所有实验组均不换水, 仅补充渗漏、蒸发及采样丢失的水量, 并通过外源添加糖蜜调控 C/N = 20。养殖用水取自室外蓄水池, 曝气过滤后用于实验, 养殖周期为 35 d, 期间, 每日观察实验鱼摄食及死亡情况, 发现死鱼及时捞出, 称重、计数并检查死亡原因。实验结束时, 记录存活率。

1.3　测定指标与方法

1.3.1　生长指标测定　养殖 35 d 后, 禁食 24 h, 每池随机采取 10 尾鱼, 经 MS-222 快速麻醉后, 测量记录其终末体质量, 计算异育银鲫增重率、特定生长率和存活率。计算公式如下:

增重率(weight gain, WG, %)=$(W_t-W_0)/W_0\times100$

特定生长率(specific growth rate, SGR, %) = $(\ln W_t-\ln W_0)/t\times100$

存活率(survival rate, SR,%) = $N_f/N_i\times100$

式中, W_t 为实验第 t 天时鱼体重(g); W_0 为初始鱼体重(g); t 为养殖周期(d); N_f 为实验结束时异育银鲫的尾数; N_i 为实验开始时异育银鲫的尾数。

1.3.2　消化指标测定　养殖 35 d 后, 禁食 24 h, 每个重复随机采取 5 尾鱼, 同 **1.3.1** 麻醉后取其肠道, 用预冷的无菌生理盐水洗去肠中杂物, 滤纸吸去组织样品表面水分后, 剪碎, 称重, 按组织样品重量的 10%加入 4℃预冷的 0.85%的生理盐水, 然后用组织匀浆器匀浆, 离心, 4℃, 2000 r/min, 15 min, 取上清, 4℃保存待用(< 3 d), 以测定淀粉酶(amylase, AMS)、脂肪酶(lipase, LPS)和胃蛋白酶(protease)活性。AMS、LPS、胃蛋白酶和总蛋白定量试剂盒均购自南京建成生物工程研究所, 并按照试剂盒说明书进行测定。

1.3.3　肠道组织结构观察　通过石蜡切片、H&E 染色进行组织结构观察。取样和麻醉方法同 **1.3.1**。解剖, 从内脏团中小心分离出肠道, 去除肠

道表面的脂肪等物质, 用 0.9%生理盐水洗去肠道上的血液、粪便等杂质, 放入 Bouin 氏液中固定。然后在浓度梯度为 70%、85%、90%、95%以及 100%的乙醇中分级充分脱水, 并依次置于二甲苯∶酒精(1∶1)溶液和纯二甲苯中充分透明, 最后包埋于经过反复冻融的低熔点石蜡中。使用 KD-2508 型切片机横向连续切片, 厚度为 6 μm, 然后展片于涂有甘油蛋白的载玻片上, 脱蜡复水后, 采用 H&E 染色, 中性树胶封片。在 Nikon Eclipse Ni M570 E 光学显微镜下观察, 通过 NIS-Elements D 软件测量。

1.4 数据统计分析

实验结果均以平均值±标准差($\bar{x}$±SD)表示, 原始数据使用 Excel 2016 进行初步整理后, 使用统计分析软件 SPSS 24.0 的单因素方差分析(One-Way ANOVA)进行统计分析, 并用 Duncan 法进行多重比较, P<0.05 为差异显著。

2 结果与分析

2.1 BFT 养殖模式下益生菌添加对异育银鲫生长性能的影响

经 35 d 养殖后, BFT 养殖模式下益生菌添加显著影响异育银鲫的生长性能(表 1)。益生菌添加组(BB 组和 BI 组)异育银鲫增重率和特定生长率显著高于对照组(B 组)(P<0.05), 增重率较对照组分别提高了 216.70%和 184.04%; 特定生长率较对照组分别提高了 141.18%和 125.49%。不同益生菌间, BB 组增重率和特定生长率高于 BI 组, 但无显著差异(P>0.05)。不同实验组间存活率差异不显著(P>0.05)。

表 1 生物絮团(BFT)养殖模式下益生菌添加对异育银鲫生长性能的影响

Tab. 1 Effects of probiotics addition on the growth performance of gibel carp (*Carassius auratus gibelio*) cultured in biofloc technology (BFT) system

n=10; $\bar{x}$±SD

组别 group	初始均重/g initial body weight	终末均重/g final body weight	增重率/% weight gain rate	特定生长率/% special growth rate	存活率/% survival rate
BB	1.61±0.01	3.81±0.09[a]	136.34±5.76[a]	2.46±0.07[a]	91.8±2.7
BI	1.63±0.02	3.65±0.28[a]	124.43±17.14[a]	2.30±0.22[a]	92.7±1.6
B	1.63±0.01	2.33±0.13[b]	43.05±7.92[b]	1.02±0.16[b]	91.2±2.4

注: BB 为 BFT 养殖模式下 EM 菌添加组, BI 为 BFT 养殖模式下枯草芽孢杆菌添加组, B 为 BFT 养殖组. 同列上标小写字母不同表示差异显著(P<0.05).

Note: BB, effective microorganisms addition group in BFT system; BI, *Bacillus subtilis* addition group in BFT system; B, BFT group. Values with different superscripts in the same column are significantly different (P<0.05).

2.2 BFT 养殖模式下益生菌添加对异育银鲫消化酶活性的影响

由表 2 可知, 益生菌添加组(BB 组和 BI 组)的消化酶(淀粉酶、脂肪酶和胃蛋白酶)活性均显著高于对照组(B 组)(P<0.05)。益生菌添加组间, BB 组淀粉酶活性显著高于 BI 组(P<0.05), 脂肪酶和胃蛋白酶活性亦高于 BI 组, 但差异不显著(P>0.05)。

2.3 BFT 养殖模式下益生菌添加对异育银鲫肠道组织结构的影响

异育银鲫肠道包括肌层、黏膜层、黏膜下层以及浆膜层 4 个部分, 其中黏膜向肠腔突起形成皱襞, 皱襞内的腔隙即皱襞间质, 如图 1 所示。

表 2 生物絮团(BFT)养殖模式下益生菌添加对异育银鲫肠道消化酶活性的影响

Tab. 2 Effects of probiotics addition on digestive enzyme activities of gibel carp (*Carassius auratus gibelio*) cultured in biofloc technology (BFT) system

n=5; $\bar{x}$±SD; U/mg (protein)

组别 group	淀粉酶 amylase	脂肪酶 lipase	胃蛋白酶 protease
BB	192.40±26.84[a]	19.58±10.29[a]	44.29±10.50[a]
BI	186.31±22.19[b]	19.12±12.09[a]	44.23±14.16[a]
B	177.51±26.30[c]	17.06±5.15[b]	36.14±6.18[b]

注: BB 为 BFT 养殖模式下 EM 菌添加组, BI 为 BFT 养殖模式下枯草芽孢杆菌添加组, B 为 BFT 养殖组. 同列上标小写字母不同表示差异显著(P<0.05).

Note: BB, effective microorganisms addition group in BFT system; BI, *Bacillus subtilis* addition group in BFT system; B, BFT group. Values with different superscripts in the same column are significantly different (P<0.05).

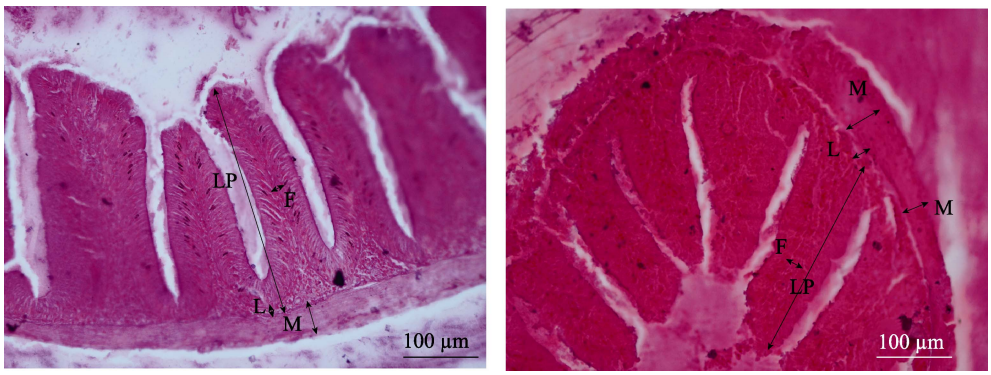

图 1　异育银鲫肠道组织结构

M: 肌层; L: 黏膜下层; F: 皱襞间质; LP: 黏膜皱襞.

Fig. 1　Gut histological structure of *Carassius auratus gibelio*

M: muscularis; L: submucosa; F: fold mesenchyme; LP: mucosal fold.

益生菌添加对异育银鲫肠道组织结构的影响见表 3。益生菌添加组(BB 和 BI 组)肠道肌层厚度和黏膜下层厚度显著高于对照组(B 组)(P<0.05); BB 组异育银鲫肠道黏膜皱襞高度和皱襞间质宽度与 BI 和对照组相比, 均无显著差异(P>0.05)。

表 3　生物絮团(BFT)养殖模式下益生菌添加对异育银鲫肠道组织结构的影响

Tab. 3　Effects of probiotics addition on gut structure of gibel carp (*Carassius auratus gibelio*) cultured in biofloc technology (BFT) system

n=3; $\bar{x}$±SD

组别 group	肌层 muscularis	黏膜皱襞 mucosal fold	皱襞间质 fold mesenchyme	黏膜下层 submucosa
BB	30.03±1.04^{a}	223.59±10.15	11.75±0.71	13.93±0.38^{a}
BI	30.02±1.01^{a}	223.07±12.46	11.75±0.80	12.26±0.65^{b}
B	25.17±1.27^{b}	223.07±14.70	11.38±0.34	12.26±0.19^{b}

注: BB 为 BFT 养殖模式下 EM 菌添加组, BI 为 BFT 养殖模式下枯草芽孢杆菌添加组, B 为 BFT 养殖组. 同列上标小写字母不同表示差异显著(P<0.05).

Note: BB, effective microorganisms addition group in BFT system; BI, *Bacillus subtilis* addition group in BFT system; B, BFT group. Values with different superscripts in the same column are significantly different (P<0.05).

3　讨论

3.1　BFT 养殖模式下益生菌添加对异育银鲫生长的影响

BFT 可以促进养殖动物生长并提高其存活率[10-16]。朱学宝[32]和 Kuhn 等[33]研究发现, 应用 BFT 养殖罗非鱼(*Oreochromis* spp.)和凡纳滨对虾(*Litopenaeus vannamei*)可显著提高其增重率。前期研究表明, BFT 组异育银鲫个体增重率和特定生长率显著高于对照组[17]。本研究进一步表明, BFT 养殖模式下, 外源添加 EM 菌和芽孢杆菌可更好地促进异育银鲫生长。EM 菌是一种由光合细菌、酵母菌、乳酸菌、放线菌和芽孢杆菌等微生物复合培养而成的活性菌剂[34]。枯草芽孢杆菌在使用上具有无毒、无残留和不产生耐药性等优点[35], 是一种绿色、无污染的益生菌, 在肠道内能够迅速繁殖, 抑制肠道有害菌的繁殖并促进有益菌的增殖[36]。本研究显示, 益生菌添加组异育银鲫增重率和特定生长率显著高于对照组, 这与胡毅等[37]、武鹏等[38]、Lee 等[39]以及李莎等[40]的研究结果相类似。并且, 复合菌株——EM 菌添加组的增重率和特定生长率高于单一菌株——枯草芽孢杆菌添加组, 说明复合菌株的促生长效果优于单一菌株[41-42]; 但两者差异不显著, 其可能与养殖周期较短有关。此外, 益生菌添加组异育银鲫肠道消化酶活性均显著高于对照组, 这表明其消化吸收能力有所提高。其促生长机理可能为: (1) 外源添加的益生菌含有蛋白质、维生素等大量的营养物质, 为养殖动物提供更加充足的营养; (2) 益生菌在代谢过程中可产生多种酶类, 促进动物的消化、吸收。

3.2　BFT 养殖模式下益生菌添加对异育银鲫消化酶活性的影响

消化酶是反映机体营养生理的重要指标, 与其生长发育密切相关, 消化酶活性的高低直接影

响到动物对饲料的利用程度[40]。与常规每日换水 1/4~1/3 的养殖模式相比, BFT 可提高异育银鲫肠道 AMS、LPS 和胃蛋白酶活性[17]。本研究进一步表明, BFT 养殖模式下, 外源添加 EM 菌和芽孢杆菌可提高异育银鲫肠道消化酶活性, 其与普通养殖模式外源添加益生菌的作用效果类似。史东杰等[43]在锦鲤(*Cyprinus carpio*)饲料中添加微生态制剂发现, 其肠道消化酶活性增强且适宜添加量为 0.02%。Afrilasari 等[44]研究巨大芽孢杆菌(*Bacillus megaterium*)对鲇(*Clarias* sp.)生长、消化酶活和肠道菌群的影响, 发现 1%芽孢杆菌添加组鲇的蛋白酶和淀粉酶活性显著高于对照组和其他处理组。鱼类可产生蛋白酶、脂肪酶、淀粉酶、纤维酶等多种内源性酶来分解食物, 然而这些酶的数量和活性不足以使其摄食的食物完全代谢, 因此随着益生菌在养殖动物肠道内的定植、增殖和代谢, 其产生的多种胞外酶进入肠道的“酶池”, 从而提高养殖动物消化酶活性[30,42,45-47]。同时, 益生菌发酵产生短链脂肪酸可能降低肠内 pH 值, 提高肠道消化酶活力, 从而提高鱼类的消化能力[48-49]。

3.3 BFT 养殖模式下益生菌添加对异育银鲫肠道组织结构的影响

鱼类肠道是其营养物质消化吸收的主要场所[50]。张锦华[51]用不同微生态制剂饲喂鲤(*Cyprinus carpio*), 发现养殖中合理使用微生态制剂可在一定程度上提高鲤肠黏膜高度和肠壁厚度。本实验表明, 益生菌添加后, 异育银鲫肠道结构发生了一定的变化, 益生菌添加组肠道肌层厚度和黏膜下层厚度显著高于对照组。同时, Daniels 等[52]和 Asaduzzaman 等[53]在欧洲龙虾(*Homarus gammarus* L.)和结鱼(*Tor tambroides*)的研究过程中发现, 益生菌可显著提高养殖动物的肠黏膜高度。而本研究中益生菌组异育银鲫的黏膜高度和间质宽度较对照组均无显著差异。本研究结果和对这类研究的文献查阅表明, 关于益生菌添加诱导的鱼肠道组织结构潜在改变的复杂机制仍需进行更多的研究。

4 总结

BFT 养殖模式下益生菌添加对异育银鲫的生长有明显的促进作用, 同时, 其肠道消化酶活性和组织结构的变化进一步证实了益生菌对异育银鲫的促生长作用。本研究表明, 复合菌株(EM 菌)添加组机体在生长、消化酶活性和肠道吸收面积方面均展现出优于单一菌株(枯草芽孢杆菌)添加组的趋势, 这可能与 EM 菌本身为复合菌株制剂的特性有关, 不同细菌在肠道微生物区系中占有不同的生态位, 因而添加复合益生菌有比添加单一益生菌更好的免疫及促生长效果[41-42]。在无外源微生物添加的情况下, BFT 养殖模式中发挥核心调控作用的是水体中的土著微生物, 本研究表明, BFT 养殖模式下外源添加微生物对其有一定的影响, 其影响机制包括添加益生菌后水体及养殖动物肠道中微生物区系、生物絮团组份、代谢产物等有待进一步研究。

参考文献:

[1] Fan Y, Zhou Y, Zeng L, et al. Identification, structural characterization, and expression analysis of toll-like receptors 2 and 3 from gibel carp (*Carassius auratus gibelio*)[J]. Fish and Shellfish Immunology, 2018, 72: 629-638.

[2] Yu Y B, Wang C H, Wang A M, et al. Effects of various feeding patterns of *Bacillus coagulans* on growth performance, antioxidant response and Nrf2-Keap 1 signaling pathway in juvenile gibel carp (*Carassius auratus gibelio*)[J]. Fish and Shellfish Immunology, 2018, 73: 75-83.

[3] Wu T, Zhu C Y, Wang Y, et al. Prevention and control technology of gill hemorrhagic disease in silver prussian carp (*Carassius auratus gibelio*)[J]. China Fisheries, 2015(10): 92-93. [吴霆, 朱春艳, 王瑶, 等. 异育银鲫病毒性鳃出血病预防与控制技术[J]. 中国水产, 2015(10): 92-93.]

[4] Wu T, Ding Z F, Zhu C Y, et al. Epidemiological investigation and survey of gill hemorrhagic disease in silver prussian carp (*Carassius auratus gibelio*)[J]. Fisheries Science, 2014, 33(5): 283-287. [吴霆, 丁正峰, 朱春艳, 等. 异育银鲫鳃出血病流行病学调查和研究[J]. 水产科学, 2014, 33(5): 283-287.]

[5] Zhou Y, Jiang N, Ma J, et al. Protective immunity in gibel carp (*Carassius gibelio*) of the truncated proteins of cyprinid herpesvirus 2 expressed in *Pichia pastoris*[J]. Fish and Shellfish Immunology, 2015, 47(2): 1024-1031.

[6] Shan N. Effects of different environmental and biological stresses on biochemical indexes in the plasma of gibel carp (*Carassius auratus gibelio*)[D]. Shanghai: Shanghai Ocean University, 2016. [单娜. 不同环境和生物源性因素对异育银鲫血浆生化指标的影响[D]. 上海: 上海海洋大学, 2016.]

[7] Li D. Establishment and preliminary application of PCR detection methods for myxosporeans in *Carassius auratus*

gibelio[D]. Wuhan: Huazhong Agricultural University, 2016. [李丹. 异育银鲫粘孢子虫 PCR 检测方法的建立与初步应用[D]. 武汉: 华中农业大学, 2016.]

[8] Wu T, Ding Z F, Ren M, et al. The histo- and ultra-pathological studies on a fatal disease of Prussian carp (*Carassius gibelio*) in mainland China associated with cyprinid herpesvirus 2 (CyHV-2)[J]. Aquaculture, 2013, 412-413: 8-13.

[9] Hoseinifar S H, Esteban M Ã, Cuesta A, et al. Prebiotics and fish immune response: A review of current knowledge and future perspectives[J]. Reviews in Fisheries Science and Aquaculture, 2015, 23(4): 315-328.

[10] Mo W Y, Chen Z T, Leung H M L, et al. Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks[J]. Environmental Science Pollution Research International, 2017, 24(10): 8978-8989.

[11] Avnimelech Y, Kochba M. Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using 15N tracing[J]. Aquaculture, 2009, 287(1-2): 163-168.

[12] Azim M E, Little D C. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*)[J]. Aquaculture, 2008, 283(1-4): 29-35.

[13] Xu W J, Pan L Q. Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks[J]. Aquaculture, 2014, 426-427: 181-188.

[14] Kim S K, Pang Z G, Seo H C, et al. Effect of bioflocs on growth and immune activity of Pacific white shrimp (*Litopenaeus vannamei*) postlarvae[J]. Aquaculture Research, 2014, 45(2): 362-371.

[15] Zhao P, Huang J, Wang X H, et al. The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*[J]. Aquaculture, 2012, 354-355: 97-106.

[16] Zhao Z G, Xu Q Y, Luo L, et al. Effect of feed C/N ratio promoted bioflocs on water quality and production performance of bottom and filter feeder carp in minimum-water exchanged pond polyculture system[J]. Aquaculture, 2014, 434: 442-448.

[17] Zhang M M, Xu C, Zhao Z G, et al. Effects of biofloc technology on growth performance and disease resistance of gibel carp (*Carassius auratus gibelio*)[J]. Journal of Fishery Sciences of China, 2017, 24(3): 533-542. [张明明, 徐晨, 赵志刚, 等. 生物絮团技术对异育银鲫生长性能和抗性的影响[J]. 中国水产科学, 2017, 24(3): 533-542.]

[18] Nayak S K. Probiotics and immunity: A fish perspective[J]. Fish and Shellfish Immunology, 2010, 29(1): 2-14.

[19] Gupta A, Gupta P, Dhawan A. Dietary supplementation of probiotics affects growth, immune response and disease resistance of *Cyprinus carpio* fry[J]. Fish and Shellfish Immunology, 2014, 41(2): 113-119.

[20] Liu Z Y, Liu Y, Bao X B, et al. Research advances in aquaculture probiotics[J]. Fisheries Science, 2010, 29(8): 500-504. [刘忠颖, 刘洋, 鲍相渤, 等. 水产养殖益生菌的研究进展[J]. 水产科学, 2010, 29(8): 500-504.]

[21] Kechagia M, Basoulis D, Konstantopoulou S, et al. Health benefits of probiotics: A review[J]. International Scholarly Research Notices, 2013, 2013: 481651.

[22] Steenbergen L, Sellaro R, van Hemert S, et al. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood[J]. Brain, Behavior, and Immunity, 2015, 48: 258-264.

[23] Kozasa M. Toyocerin (*Bacillus toyoi*) as growth promotor for animal feeding[J]. Microbiologie Aliments Nutrition, 1986, 4(1): 121-135.

[24] Gatesoupe F J. The use of probiotics in aquaculture[J]. Aquaculture, 1999, 180(1-2): 147-165.

[25] Ma Y X, Wu Y, Liu C F, et al. The application of probiotic bacteria as biocontrol agents in aquaculture[J]. Journal of Dalian Fisheries University, 2003, 18(3): 180-185. [马悦欣, 吴垠, 刘长发, 等. 益生菌作为生物控制剂在水产养殖中的应用[J]. 大连水产学院学报, 2003, 18(3): 180-185.]

[26] Chen Y R, Wang H, Qi F L. Development of studies on microecological modulator as aquatic feed[J]. Fisheries Science & Technology, 2003(3): 4-8. [陈有容, 王华, 齐凤兰. 水产微生态调节剂研究进展[J]. 水产科技, 2003(3): 4-8.]

[27] Banerjee G, Ray A K. The advancement of probiotics research and its application in fish farming industries[J]. Research in Veterinary Science, 2017, 115: 66-77.

[28] Gatesoupe F J. Lactic acid bacteria increase the resistance of turbot larvae (*Scophthalnus maximus*), against pathogenic Vibrio[J]. Aquatic Living Resources, 1994, 7(4): 277-282.

[29] Mohapatra S, Chakraborty T, Prusty A K, et al. Use of different microbial probiotics in the diet of rohu (*Labeo rohita*) fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora[J]. Aquaculture Nutrition, 2012, 18(1): 1-11.

[30] Ziaei-Nejad S, Rezaei M H, Takami G A, et al. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*[J]. Aquaculture, 2006, 252(2-4): 516-524.

[31] Hu J. Effects of two kinds of EM probiotics on cultural water quality, growth performance and immunity of sea cucumber (*Apostichopus japonicus*)[D]. Dalian: Dalian Polytechnic University, 2015. [胡京. 两种 EM 菌剂对养殖水体水质、海参生长性能及免疫力的影响[D]. 大连: 大连工业大学, 2015.]

[32] Zhu X B. Bacteria floccule as diet of filter-feeding fishes[J]. Journal of Fisheries of China, 1989, 13(4): 339-345. [朱学宝. 细菌絮凝体对滤食性鱼类饵料效果的研究[J]. 水产学报, 1989, 13(4): 339-345.]

[33] Kuhn D D, Boardman G D, Lawrence A L, et al. Microbial

floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed[J]. Aquaculture, 2009, 296(1-2): 51-57.

[34] Lin J D. Study on treating municipal sewage with SBR strengthened by EM[D]. Xiamen: Xiamen University, 2008. [林金盾. 采用 EM 菌强化 SBR 处理生活污水的试验研究[D]. 厦门: 厦门大学, 2008.]

[35] Yu Y, Li H R, Li Y, et al. The application of probiotic preparation to aquaculture[J]. Journal of Fishery Sciences of China, 2001, 8(2): 92-96. [余勇, 李会荣, 李筠, 等. 益生菌制剂在水产养殖中的应用[J]. 中国水产科学, 2001, 8(2): 92-96.]

[36] Qi B, Wu S G, Wang J, et al. Effects of *Bacillus subtilis* on growth performance, intestinal morphology and bacterial enumeration of broilers[J]. Chinese Journal of Animal Nutrition, 2016, 28(6): 1748-1756. [齐博, 武书庚, 王晶, 等. 枯草芽孢杆菌对肉仔鸡生长性能、肠道形态和菌群数量的影响[J]. 动物营养学报, 2016, 28(6): 1748-1756.]

[37] Hu Y, Tan B P, Mai K S, et al. Effects of dietary probiotic on growth, immunity and intestinal bacteria of juvenile *Litopenaeus vannamei*[J]. Journal of Fishery Sciences of China, 2008, 15(2): 244-251. [胡毅, 谭北平, 麦康森, 等. 饲料中益生菌对凡纳滨对虾生长、肠道菌群及部分免疫指标的影响[J]. 中国水产科学, 2008, 15(2): 244-251.]

[38] Wu P, Zhao D Q, Cai H H, et al. Effects of three probiotics on water quality and growth in sea cucumber (*Apostichopus japonicus*)[J]. Journal of Dalian Ocean University, 2013, 28(1): 21-26. [武鹏, 赵大千, 蔡欢欢, 等. 3 种微生态制剂对水质及刺参幼参生长的影响[J]. 大连海洋大学学报, 2013, 28(1): 21-26.]

[39] Lee S, Katya K, Park Y, et al. Comparative evaluation of dietary probiotics *Bacillus subtilis* WB60 and *Lactobacillus plantarum* KCTC3928 on the growth performance, immunological parameters, gut morphology and disease resistance in Japanese eel (*Anguilla japonica*)[J]. Fish and Shelfish Immunology, 2017, 61: 201-210.

[40] Li S, Guan M, Guo W T, et al. Effects of probiotics on growth, and activities of digestive enzymes and enzymes related to non-specific immunity in Chinese sturgeon[J]. Chinese Journal of Fisheries, 2014, 27(1): 50-54. [李莎, 管敏, 郭文韬, 等. 微生物制剂对中华鲟生长、消化酶及非特异性免疫酶活性的影响[J]. 水产学杂志, 2014, 27(1): 50-54.]

[41] Salinas I, Cuesta A, Esteban M A, et al. Dietary administration of *Lactobacillus delbrùeckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses[J]. Fish and Shellfish Immunology, 2005, 19(1): 67-77.

[42] Wang Y B, Xu Z R. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities[J]. Animal Feed Science and Technology, 2006, 127(3-4): 283-292.

[43] Shi D J, Liang Y J, Xu J H, et al. Effects of probiotics on growth, digestive enzymes activity and apparent digestibility of *Cyprinus carpio*[J]. Jiangsu Agricultural Sciences, 2017, 45(13): 136-139. [史东杰, 梁拥军, 许金华, 等. 微生态制剂对锦鲤生长、消化酶活性和表观消化率的影响[J]. 江苏农业科学, 2017, 45(13): 136-139.]

[44] Afrilasari W, Widanarni, Meryandini A. Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.)[J]. Hayati Journal of Biosciences, 2016, 23(4): 168-172.

[45] Liu X Y, Zhang Y, Qi Q, et al. Effects of *Bacillus subtilis* on growth, digestive enzyme activity, and non-specific immunity in hybrid sturgeon (*Acipenser baeri* ♂×*Acipenser schrenkii* ♀) juveniles[J]. Journal of Fishery Sciences of China, 2011, 18(6): 1315-1320. [刘晓勇, 张颖, 齐茜, 等. 枯草芽孢杆菌对杂交鲟幼鱼生长性能、消化酶活性及非特异性免疫的影响[J]. 中国水产科学, 2011, 18(6): 1315-1320.]

[46] Sharifuzzaman S M, Al-Harbi A H, Austin B. Characteristics of growth, digestive system functionality, and stress factors of rainbow trout fed probiotics *Kocuria*, SM1 and *Rhodococcus*, SM2[J]. Aquaculture, 2014, 418-419: 55-61.

[47] Gao X. Effects of *Bacillus* spp. on the growth performance and intestine bacteria in juvenile *Cipenser baeri*[D]. Shijiazhuang: Hebei Normal University, 2008. [高欣. 芽孢杆菌对西伯利亚鲟幼鱼摄食生长及肠道菌群的影响[D]. 石家庄: 河北师范大学, 2008.]

[48] Suzer C, Çoban D, Kamaci H O, et al. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth performance and digestive enzyme activities[J]. Aquaculture, 2008, 280(1-4): 140-145.

[49] Duan Y F, Zhang Y, Dong H B, et al. Effect of dietary *Clostridium butyricum* on growth, intestine health status and resistance to ammonia stress in Pacific white shrimp (*Litopenaeus vannamei*)[J]. Fish and Shellfish Immunology, 2017, 65: 25-33.

[50] Lou Y D. Embryology[M]. Beijing: China Agriculture Press, 2000: 101-104. [楼允东. 组织胚胎学[M]. 北京: 中国农业出版社, 2000: 101-104.]

[51] Zhang J H. The application studies of different probiotics in caws cultivation[D]. Nanchang: Jiangxi Agricultural University, 2003. [张锦华. 不同微生态制剂在鲤鱼养殖中的应用研究[D]. 南昌: 江西农业大学, 2003.]

[52] Daniels C L, Merrifield D L, Boothroyd D P, et al. Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth, performance, gut morphology and gut microbiota[J]. Aquaculture, 2010, 304(1-4): 49-57.

[53] Asaduzzaman M, Iehata S, Akter S, et al. Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian mahseer (*Tor tambroides*)[J]. Aquaculture Reports, 2018, 9: 53-61.

Effects of probiotic addition on the growth performance, digestive enzyme activity, and intestinal morphology of gibel carp (*Carassius auratus gibelio*) cultured using biofloc technology

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Abstract: Probiotics have been defined as live microorganisms, which confer health benefits to the host when administered in adequate amounts. The probiotics used in aquaculture commonly include effective microorganisms (EM bacteria), *Bacillus* sp. and *Lactobacillus* sp. The biofloc technology (BFT) is a zero-water exchange and eco-friendly aquaculture system. The BFT can recycle nutrient by introducing additional carbon source to culture water in order to stimulate the growth of heterotrophic bacteria that convert ammonia into microbial biomass. The microbial biomass will further aggregate with other microorganisms and particles to form bioflocs. The bioflocs contain a heterogeneous mixture of diatoms, macroalgae, food and fecal remnants, exoskeletons, bacteria, invertebrates, and other microorganisms. The bioflocs can maintain good water quality, increase fish growth performance, reduce feed cost by recycling feed residues and fecal excrements, aid enzymatic activity, and enhance innate immunity and disease resistance. Meanwhile, the BFT can minimize water exchange to save labor and environmental costs, and reduce water usage and waste generated in aquaculture. It can also avoid drug abuse for disease control, because of the key microorganism in the bioflocs. The BFT has the potential to be used widely in aquaculture. Gibel carp (*Carassius auratus gibelio*) is one of important freshwater species farmed in China, and it is a representative species of mudflat cultured fish. However, with the rapid development of aquaculture, the waste of water resources, pollution, and diseases seriously affected the sustainable development and aquaculture efficiency of gibel carp. Our previous studies showed that the BFT can be used in gibel carp culture and that it has positive effects on the growth performance and immune response of gibel carp. To further optimize the BFT system in gibel carp culture, a 35-day feeding experiment was conducted to evaluate the effects of probiotics on the growth performance, digestive enzyme activities, and intestinal morphology of gibel carp cultured using the BFT. A total of 1800 normal gibel carps with a mean body weight of 1.60 g were randomly assigned to nine ponds (3.0 m ×1.0 m × 0.8 m) as three experimental treatments, including the EM bacteria addition group in BFT system (BB), *Bacillus subtilis* addition group in BFT system (BI), and BFT without any probiotics addition (B). The results revealed the followings (1) Compared with those of the control group, weight gain and specific growth of gibel carp in the BB and BI groups were significantly higher (P<0.05); the weight gain in the BB and BI groups increased by 216.70% and 184.04%, respectively. Furthermore, the specific growth increased by 141.18% and 125.49%, respectively. The weight gain and specific growth in BB group were higher but no significantly different from those in BI group (P>0.05). These results indicate that the addition of probiotics to BFT system can promote the growth of gibel carp. Additionally, compound bacteria (EM bacteria) addition showed better efficiency than the addition of single strain *B. subtilis*. (2) The activity of digestive enzymes (amylase, lipase and protease) in the probiotics addition groups (BB and BI) was significantly higher than those in control group (P<0.05), and the highest activities were observed in BB group. The activity of amylase in BB group was significantly higher than that in BI group (P<0.05). (3) The thickness of muscularis and submucosa of gibel carp gut in the probiotics addition groups was significantly higher than those in control group (P<0.05). The mucosal fold height and fold mesenchyme width of gibel carp gut were not significantly different among the BB, BI, and control groups (P>0.05). The results suggest that the addition of probiotics can promote growth of gibel carp and improve their intestinal digestive enzyme activities. Furthermore, the addition of probiotics can also affect the intestinal morphology of gibel carp cultured in BFT system. This study provides some valuable information to promote the usage of BFT in aquaculture.

Key words: biofloc technology; probiotics; *Carassius auratus gibelio*; growth performance; digestive enzyme; intestinal morphology

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