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饲料中复方中草药对大菱鲆生长、饲料利用及非特异性免疫的影响

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摘要: 研究探讨了不同剂量的复方中草药[党参(Codonopsis pilosula Nannf.)、白术(Atractylodes macrocephala Koidz.)、茯苓(Poria cocos Wolf.)、熟地黄(Rehmannia glutinosa Libosch.)、甘草(Glycyrrhiza uralensis Fisch.)、山楂(Crataegus pinnatifida Bunge.)、五倍子(Rhus chinensis Mill.)、栀子(Gardenia jasminoides Ellis.)和生姜(Zingiber officinale Roscoe.)9种中草药合煎而成]对大菱鲆(*Scophthalmus maximus* Linnaeus)幼鱼生长性能、饲料利用、体组成及相关免疫指标的影响。在基础饲料中以水煎剂的形式分别添加0、1%、2%和4%的复方中草药配制4种试验饲料(对照组、1%组、2%组、4%组), 大菱鲆幼鱼[(22.01±0.01) g]随机分为4组, 每组3个重复, 每个重复95尾鱼, 饱食投喂40d。结果表明, 复方中草药对大菱鲆的摄食率无显著影响(P≥0.05); 添加1%的复方中草药可显著提高大菱鲆的终末均重、增重率和特定生长率, 降低饲料系数(P<0.05); 与对照组相比, 1%的复方中草药显著提高了大菱鲆的肥满度和脾指数(P<0.05), 但复方中草药对大菱鲆的脏体比、肝体比和体组成均无显著影响(P≥0.05); 与对照组相比, 添加1%的复方中草药可显著提高血浆补体C3和溶菌酶的水平(P<0.05), 而对血浆免疫球蛋白M含量无显著影响(P≥0.05)。综上表明, 复方中草药提高了大菱鲆幼鱼的生长性能、饲料利用和非特异性免疫力, 在大菱鲆饲料中的推荐添加剂量为1%。

关键词: 复方中草药; 大菱鲆; 生长性能; 饲料利用; 体组成; 非特异性免疫

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大菱鲆(*Scophthalmus maximus* Linnaeus)又名多宝鱼, 属鲽形目、菱鲆科、菱鲆属, 是我国北方重要的工厂化海水养殖鱼类。近年来随着高密度集约化养殖模式的发展, 各种养殖环境胁迫导致鱼体免疫力降低[1], 大菱鲆养殖过程中如肠炎及腹水病等疾病频发, 进而带来药物使用问题, 2006年及2015年的2次大菱鲆药物残留事件也使得大菱鲆养殖业遭受重创。因此, 亟需开发水产绿色饲料免疫增强剂以提高大菱鲆的免疫力。

中草药富含多糖、脂肪、维生素和微量元素等多种动物机体所需要的营养成分, 及生物碱、皂苷、挥发油和色素等生物活性物质[2], 兼具营养与药效的双重作用, 具有无毒、无污染、无抗药性、无残留且经济易得等优点, 能够增强鱼类的抗氧化与免疫力, 并通过提高摄食及改善肠道消化功能而促进鱼类生长[3, 4]。复方中草药经合理配伍, 不同的活性成分能够表现出特定的协同效应, 比单一中草药更有效[5]。目前关于饲料中添加复方中草药对大菱鲆影响的研究报道较少, 且中草药配方和添加方式各异效果也有差别[6—9]。“四君子汤”是人参、白术、茯苓和甘草四味基本中草药为主的古方剂, 其具有健脾益气、治疗胃肠功能紊乱和改善肠道黏膜免疫功能的药理作用[10]。采用高效液相色谱/串联质谱法分析“四君子汤”的主要活性化学成分为人参皂甙(来自人参)、黄酮类和三萜化合物(来自甘草)[11]。本研究在传统方剂“四君子汤”的基础上, 筛选了9种中草药进行配伍, 旨在探讨这种复方中草药的不同剂量对大菱鲆幼鱼生长性能、饲料利用及相关免疫指标的影响, 为复方中草药在大菱鲆养殖中的应用提供理论依据。

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1 材料与方法

1.1 实验设计与实验饲料

本研究以9种中草药原材料配伍成复方中草药添加剂, 以水煎剂的形式在基础饲料中添加质量分数分别为0、1%、2%和4%的复方中草药制成4种试验饲料(对照组、1%组、2%组和4%组)。实验所用中草药购于河北省石家庄市康仁堂大药房。复方中草药由党参(*Codonopsis pilosula* Nannf.)、白术(*Atractylodes macrocephala* Koidz.)、茯苓(*Poria cocos* Wolf.)、熟地黄(*Rehmannia glutinosa* Libosch.)、甘草(*Glycyrrhiza uralensis* Fisch.)、山楂(*Crataegus pinnatifida* Bunge.)、五倍子(*Rhus chinensis* Mill.)、栀子(*Gardenia jasminoides* Ellis.)和生姜(*Zingiber officinale* Roscoe.)9种中草药组成, 配比为6∶5∶3∶3∶2∶2∶1∶1∶1。各中草药原材料经粉碎过40目筛, 按照方剂配伍置于不锈钢锅中逐级混匀, 加蒸馏水常温浸泡30min, 煮沸50min, 将药汁过滤倒出, 得到复方中草药的水煎剂, 使5 mL药液相当于1 g中草药。基础饲料以白鱼粉和鸡肉粉为主要蛋白源、α-淀粉为主要糖源配制而成, 饲料中蛋白质水平与脂肪水平的设定参考Li等[12]和彭墨[13]的结果, 基础饲料配方及营养成分见表 1。将中草药水煎剂以质量分数分别为0、1%、2%和4%的比例添加到基础饲料中形成4种试验饲料, 混合均匀后制成直径为3.0 mm的软颗粒饲料, 存放于−20℃的冰箱中备用。

表 1 基础饲料配方及营养组成

Tab. 1 Formulation and proximate composition of the control diet

原料 Ingredient	含量(%干物质) Content (% in dry matter)
鱼粉Fish meal	37.00
鸡肉粉Poultry meal	20.00
乌贼肝粉Squid liver powder	5.00
α-淀粉α-starch	16.00
谷朊粉Wheat gluten	6.00
酵母粉Yeast powder	4.00
鱼油Fish oil	5.00
磷酸氢钙Calcium hydrophosphate	3.00
预混料Vitamin and mineral premix	4.00
营养组成Proximate composition	
粗蛋白Crude protein	50.35
粗脂肪Crude lipid	10.40
粗灰分Crude ash	15.90
能量Gross energy (MJ/kg)	19.80

注: 预混料配比参考Zhang等[14]

Note: The composition of premix was same as Zhang, *et al*.[14]

1.2 实验用鱼与养殖管理

实验用大菱鲆购自天津盛亿水产有限公司。正式实验开始前进行14d驯化, 使大菱鲆幼鱼能适应实验养殖环境。驯化期间每天2次(8:00和18:00)饱食投喂基础饲料。在驯化结束后, 禁食24h称重并随机分组。1140尾大小均匀, 体质健康的大菱鲆幼鱼[(22.01±0.01) g]随机放入12个长方体玻璃纤维缸(长2 m、宽1.2 m和高1 m), 每组3个重复, 每个重复95尾。

养殖用水为沙滤后的自然海水, 每日换水2次, 每次换水1/2。光照采用自然光照加人工光照, 光周期为14L∶10D。每天8:00和18:00进行饱食投喂。投喂30min后收集残饵并烘干称重, 以校正摄食量。试验期间保证溶氧充足, 水质条件如下: 溶氧大于6.0 mg/L, 盐度25‰—28‰, 水温15—18℃, 氨氮浓度小于0.05 mg/L。正式养殖实验期为40d。

1.3 样品采集及测定

样品采集 在养殖试验结束后, 对大菱鲆禁食24h, 称量并记录各缸中大菱鲆的总尾数和总体重。每缸随机选取5尾鱼, 称重并测量体长, 装入密封袋中于−20℃冰箱中保存, 用于进行鱼体营养组成的分析。每缸随机取5尾鱼进行解剖, 取出完整内脏团称重并记录, 然后从内脏团中剥离肝脏、脾脏并称重以计算脏体比、肝体比和脾指数等指标。另外每缸再随机取5尾鱼, 用经肝素钠润洗过的注射器尾静脉取血, 3000×*g*离心15min, 获得血浆用于非特异性免疫指标测定。

样品分析 饲料与鱼体营养组成: 饲料与鱼体水分采用105℃直接干燥法(GB/T6435)测定。粗蛋白采用Foss全自动凯氏定氮仪Kjeltec8420测定。粗脂肪采用索氏提取法(GB/T6433)测定。粗灰分采用马弗炉灼烧法(GB/T6438)测定。能量采用Parr6200全自动氧弹热量仪测定。

非特异性免疫指标: 采用比浊法测定血浆溶菌酶(LZM)的活力; 利用免疫比浊法测定血浆免疫球蛋白(IgM)和补体C3的含量。以上指标均采用自南京建成生物技术研究所提供试剂盒。

指标计算 增重率(*WGR*, %)=100 ×(终末体重–初始体重)/终末体重

特定生长率(*SGR*, %/d)=100×(ln终末体重–ln初始体重)/d

摄食率(*FR*, %/d)=100×摄食量/ [*t*×(终末体重+初始体重)/2]

饲料系数(*FCR*)=摄食量/(终末体重–初始体重)

存活率(*SR*, %)=100×终末鱼尾数/初始鱼尾数

肥满度(*CF*, g/cm^3)=100×体重/体长^3

脏体比(*VSI*)=100×内脏团重/体重

肝体比(*HSI*)=100×肝脏重/体重

脾指数(*SI*)=100×脾脏重/体重

1.4　数据分析

实验结果以平均值±标准差(mean±SD)表示, 采用Statistica10.0软件对实验数据进行单因素方差分析(One-way ANOVA), 若差异显著时进行Duncan's多重比较, 显著水平为$P<0.05$。

2　结果

2.1　复方中草药对大菱鲆生长与饲料利用的影响

复方中草药对大菱鲆幼鱼的摄食率无显著影响($P\geq0.05$), 但显著影响了大菱鲆的终末体重、增重率、特定生长率和饲料系数($P<0.05$)。1%组大菱鲆幼鱼终末均重、增重率、特定生长率显著高于其他各组($P<0.05$), 而4%组的生长显著低于其他各组($P<0.05$)。1%组的饲料系数显著低于其他各组($P<0.05$), 其他各组间无显著差异($P\geq0.05$; 表 2)。

2.2　复方中草药对大菱鲆形体指标的影响

结果表明复方中草药对脏体比及肝体比没有显著影响, 但显著影响了大菱鲆的肥满度与脾指数, 随着添加水平的升高, 呈现出先升高后降低的趋势(表 3)。1%组大菱鲆幼鱼肥满度显著高于对照组和4%组($P<0.05$), 1%组的脾指数显著高于其他各组($P<0.05$), 其余各组之间均无显著差异($P\geq0.05$)。

2.3　复方中草药对大菱鲆鱼体营养组成的影响

由表 4可知, 复方中草药对大菱鲆幼鱼的水分、粗蛋白、粗脂肪、粗灰分和能量均无显著影响($P\geq0.05$)。

2.4　复方中草药对大菱鲆非特异性免疫指标的影响

由表 5可见, 复方中草药显著影响了大菱鲆幼鱼血浆中补体C3和LZM的含量($P<0.05$), 但对IgM含量没有显著影响($P\geq0.05$)。1%组大菱鲆幼鱼血浆中补体C3和LZM含量显著高于对照组($P<0.05$); 2%组大菱鲆幼鱼血浆中LZM含量显著高于对照组($P<0.05$), 而补体C3含量与对照组无显著性差异($P\geq0.05$)。

3　讨论

3.1　复方中草药对大菱鲆摄食生长与饲料利用的影响

本研究所采用9种中草药活性成分物质包括多糖类、黄酮类、萜类化合物(单萜类、三萜类、倍半萜烯类、环烯醚萜)及各中草药特有的活性化学成分(党参-聚乙炔、多烯; 甘草-甘草甜素; 五倍子-没食子酸, 生姜-姜辣素、姜酮酚, 栀子-藏红花素)[15—23]。在本实验条件下, 添加1%的复方中草药可显著提高大菱鲆的生长性能和饲料利用, 且此复方中草药对摄食率无显著影响, 因此, 本实验复方中草药可能是通过其所富含的多种营养成分来增强机体新陈代谢, 促进蛋白质合成来提高鱼体饲料利用能力, 从而达到促进生长性能的作用。前人研究表明中草药活性成分可提高鱼体消化酶活性[24]、调节肠道微生物[25]; 中草药多酚类、黄酮类、多糖类和萜类化合物可提高鱼体抗氧化能力和抗病力, 从而间接促进鱼体生长[26]; 另外, 中草药有效成分可促进DNA、RNA和蛋白质合成及刺激生长激素-胰岛素样生长因子轴1(GH-IGF-1轴)[27, 28]。在克氏原螯虾(*Procambarus clarkii*)的研究中, 饲料中添加0.05%—0.30%的党参多糖可显著促进其生长和饲料利用[29]。同样, 在大头亮鲃(*Luciobarbus capito*)的饲料中添加0.1%—0.4%熟地黄多糖显著提高特定生长率和降低饲料系数[28]。使用0.8%—1.0%的干姜来饲喂南亚野鲮(*Labeo rohita*)幼鱼60d, 可显著提高特定生长率[30]。在水产动物中使用复方中草药也可发现类似现象。由白术、茯苓和甘草等组成的复方中草药也提高了大菱鲆的增重率, 且降低了饲料系数[9]。另外, 陈辉辉等[31]在饲料中添加甘草、山楂和茯苓等11味中草药饲喂凡纳滨对虾(*Litopenaeus vannamei*), 降低了饲料系数, 提高了特定生长率和增重率。在花鲈(*Lateolabrax japonicus*)的研究中, 饲喂含有 2%复方中草药的特定生长率和增重率却显著低于0.8%组[24], 这与本实验结果相一致, 本实验中高剂量复方中草药会导致特定生长

表 2　复方中草药对大菱鲆生长与饲料利用的影响

Tab. 2　The effect of Chinese herbal medicine on growth and feed utilization of turbot

组别 Group	终末均重 FBW (g)	摄食率 *FR* (%/d)	增重率 *WGR* (%)	特定生长率 *SGR* (%/d)	饲料系数 *FCR*	存活率 *SR* (%)
对照组Control	38.32±0.09^{b}	1.43±0.01	74.01±0.42^{b}	1.46±0.01^{b}	1.10±0.03^{a}	95.44±0.61
1%	41.10±0.50^{c}	1.38±0.09	86.80±2.34^{c}	1.64±0.03^{c}	0.94±0.08^{b}	96.84±1.82
2%	39.25±0.59^{b}	1.45±0.07	78.25±2.83^{b}	1.52±0.04^{b}	1.10±0.07^{a}	96.49±2.19
4%	37.02±0.62^{a}	1.36±0.09	68.24±2.85^{a}	1.37±0.05^{a}	1.16±0.08^{a}	95.79±1.05

注: 同一列中数值后的上标字母不同代表差异显著($P<0.05$); 下同

Note: Means with different letters in the same column are significantly different ($P<0.05$), the same applies below

表 3 复方中草药对大菱鲆形体指标的影响

Tab. 3 The effect of Chinese herbal medicine on physical indicators of turbot

组别 Group	肥满度 *CF* (g/cm^3)	脏体比 *VSI*	肝体比 *HSI*	脾指数 *SI*
对照组 Control	3.32±0.22^a	6.13±0.54	1.70±0.21	0.14±0.04^a
1%	3.66±0.24^b	6.20±0.57	1.76±0.24	0.20±0.06^b
2%	3.52±0.33^{ab}	6.17±0.51	1.73±0.15	0.15±0.05^a
4%	3.33±0.23^a	6.04±0.40	1.71±0.25	0.14±0.03^a

表 4 复方中草药对大菱鲆鱼体营养组成的影响

Tab. 4 The effect of Chinese herbal medicine on body composition of turbot

组别 Group	水分 Moisture (%)	粗蛋白 Crude protein (%)	粗脂肪 Lipid (%)	粗灰分 Ash (%)	能量 Energy (MJ/kg)
对照组 Control	76.49± 0.56	16.33± 0.56	2.31± 0.15	3.15± 0.16	22.87± 0.97
1%	76.78± 0.91	16.82± 0.66	2.27± 0.09	3.10± 0.17	22.22± 0.94
2%	76.70± 0.44	16.60± 0.23	2.34± 0.05	3.26± 0.09	22.43± 0.41
4%	76.67± 0.78	16.36± 0.50	2.48± 0.24	3.30± 0.12	22.50± 1.00

表 5 复方中草药对大菱鲆非特异性免疫指标的影响

Tab. 5 The effect of Chinese herbal medicine on nonspecific immune indicators of turbot

组别Group	IgM (μg/mL)	补体C3 (g/L)	LZM (μg/mL)
对照组Control	17.17±2.77	3.58±0.15^a	4.01±0.14^a
1%	22.30±5.87	3.85±0.25^b	4.53±0.14^c
2%	20.13±1.96	3.71±0.15^{ab}	4.33±0.23^b
4%	19.39±4.49	3.68±0.09^{ab}	4.13±0.12^a

率下降可能是由于中草药导致大菱鲆过度免疫反应, 从而消耗了更多的能量。

复方中草药的配方与添加形式会对中草药的药效发挥和适宜添加量产生影响[32]。单味药或单一活性成分存在作用不全面的缺点, 复合方剂也不应是2种以上中草药的简单叠加, 传统方剂中某些固定配伍性的中草药可能并不适用于水生动物, 制约着中草药作为鱼类饲料添加剂的发展。本实验所用方剂是通过已知渔用中草药的显著疗效与中药学“君臣辅使”理论的结合, 适当加减药剂药量进行的合理配伍, 以达到促进生长发育和提高免疫的目的。本实验添加1%的复方中草药显著提高了大菱鲆的肥满度, 2%组和4%组鱼的肥满度也有所提高, 这与中草药促进鲤、斜带石斑鱼(*Epinephelus coioides*)的肥满度升高的结果一致[33, 34], 肥满度与鱼类的营养状况直接相关[35], 也进一步说明本实验中1%的复方中草药对大菱鲆的机体生理和营养状况产生了良好效果。

3.2 复方中草药对大菱鲆非特异性免疫的影响

非特异性免疫系统是鱼类抵御病原侵袭的第一道防线。中草药及其提取物含有多糖、黄酮类、皂苷、挥发油、多酚类、生物碱、萜类、凝集素和多种氨基酸等活性成分, 能够刺激补体和溶菌酶等的活性和抗体反应, 促进吞噬细胞的功能, 提高杀菌活性, 来对抗由细菌、真菌、病毒和寄生虫等引起疾病[36]。以前研究发现, 党参对大黄鱼(*Larimichthys crocea* Richardson.)[37]、鲫[38]和仿刺参(*Apostichopus japonicus*)[39], 含白术和山楂的复方对黄鳝(*Monopterus albus*)[40], 茯苓对施氏鲟(*Acipenser schrencki* Brandt.)[41], 熟地黄和甘草对鲤[42, 43], 五倍子对鳙(*Aristichthys nobilis*)[44]和牙鲆(*Paralichthys olivaceus*)[45], 栀子对克氏原螯虾(*Procambarus clarkia* Girard.)[46], 生姜对尖吻鲈(*Lates calcarifer* Bloch.)[47]和斑马鱼(*Danio rerio*)[48]等均显示出提高免疫功能和增强抗炎抗菌能力的作用。复方中草药经合理配伍, 不同的活性成分能够表现出特定的协同效应, 可能比单一草药更有效[5]。本研究中的复方采用了具有健脾益气、清热解毒、收湿敛疮和抗炎杀菌等功效的中草药, 结果表明其可显著提高大菱鲆的补体C3和溶菌酶活性, 这也与李华等[7]和路晶晶等[9]的结果相似, 能够提高大菱鲆免疫功能。

脾脏作为鱼体内重要的免疫器官, 其增殖和发育与鱼类的免疫功能密切相关[49]。在草鱼、罗非鱼和黄颡鱼的研究中发现, 中草药可显著促进脾指数升高, 增强免疫力[50—52]。在本实验中1%组大菱鲆的脾指数显著提高, 另两组也较对照组略有提高, 说明本实验中的复方中草药有利于大菱鲆的脾脏发育, 具有一定的免疫调节作用。

以前研究结果表明免疫增强剂对鱼类免疫力的作用受给药时间与添加剂量的综合影响, 添加剂量与免疫增强作用之间并非总呈正相关[53, 54]。罗非鱼的研究发现在饲料中添加0.025%—0.2%剂量的苦参(*Sophora flavescens* Ait.)提取物时, 罗非鱼的非特异性免疫指标在0.1%时的增强作用最大[55]。本试验也表现出相似的结果, 添加2%和4%的高浓度组的免疫指标均低于1%组, 说明过高浓度的复方中草药对鱼类的免疫功能反而有一定的抑制作用。此外, 在对凡纳滨对虾连续投喂复方中草药21d后发现, 其消化酶指标及抗氧化指标分别在7d和14d达到最高值, 表明复方中草药的作用与投喂时间长短也有着直接联系[56]。然而, 本研究仅在40d时分析了免疫指标, 是否还存在更好的投喂时

间和方式还需进一步探讨。

4　结论

本研究中的复方中草药合煎剂在饲喂大菱鲆40d后显著提高了大菱鲆的特定生长率, 降低了饲料系数, 显著提高了肥满度和脾指数, 并通过提高大菱鲆血浆补体C3含量和溶菌酶活力增强了大菱鲆的非特异性免疫力。综合分析, 本复方中草药在大菱鲆配合饲料中的推荐添加量为1%。

参考文献:

[1] Wang X Y, Wang J Y, Ma J J, *et al*. Effects of dietary Vitamin E and L-carnosine on growth performance, antioxidant status, non-specific immunity and serum biochemical indices in juvenile turbot (*Scophthalmus maximus*) [J]. *Acta Hydrobiologica Sinica*, 2017, **41**(1): 86-94. [王晓艳, 王际英, 马晶晶, 等. VE和L-肌肽对大菱鲆幼鱼生长、抗氧化、非特异性免疫及血清生化指标的影响 [J]. 水生生物学报, 2017, **41**(1): 86-94.]

[2] Hai N V. The use of medicinal plants as immunostimulants in aquaculture: A review [J]. *Aquaculture*, 2015(446): 88-96.

[3] Wu Y S, Chen Y Y, Ueng P S, *et al*. Effects of medicinal herbs "*Plantago asiatica*", "*Houttuynia cordata*" and "*Mentha haplocalyx*" on non-specific immune responses of cobia (*Rachycentron canadum*) [J]. *Fish & Shellfish Immunology*, 2016(58): 406-414.

[4] Bilen S, Özkan O, Alagöz K, *et al*. Effect of dill (*Anethum graveolens*) and garden cress (*Lepidium sativum*) dietary supplementation on growth performance, digestive enzyme activities and immune responses of juvenile common carp (*Cyprinus carpio*) [J]. *Aquaculture*, 2018(495): 611-616.

[5] Xu A L, Shangguan J, Li Z, *et al*. Effects of dietary Chinese herbal medicines mixture on feeding attraction activity, growth performance, nonspecific immunity and digestive enzyme activity of Japanese seabass (*Lateolabrax japonicus*) [J]. *Aquaculture Reports*, 2020(17): 100304.

[6] Guo P P, Chen Z S, Hu F G, *et al*. Effect of compound Chinese herbal medicine feed additives on growth of turbot *Scophthalmus maximus* Linnaeus [J]. *Fishery Modernization*, 2013, **40**(5): 64-68. [郭萍萍, 陈增生, 胡凡光, 等. 复合中草药饲料添加剂对大菱鲆生长的影响 [J]. 渔业现代化, 2013, **40**(5): 64-68.]

[7] Li H, Zhang T E, Li Q. Influence of compound Chinese herbal medicine on non-specific immunity of turbot *Scophthalmus maximus* [J]. *Journal of Dalian Ocean University*, 2013, **28**(2): 115-120. [李华, 张太娥, 李强. 复方中草药对大菱鲆非特异性免疫力的影响 [J]. 大连海洋大学学报, 2013, **28**(2): 115-120.]

[8] Diao J, Li L, Ye H B, *et al*. In vitro bacteriostasis and influences of one compound and 11 kinds of Chinese herbal medicine on intestinal microflora in turbot [J]. *Fisheries Science*, 2018, **37**(4): 433-439. [刁菁, 李乐, 叶海斌, 等. 1种中草药复方和11种单方的体外抑菌效果及对大菱鲆肠道菌群的影响 [J]. 水产科学, 2018, **37**(4): 433-439.]

[9] Lu J J, Guo R, Qi G S, *et al*. Effects of Chinese herbal medicine compounds on growth and non-specific immunity of turbot *Scophthalmus maximus* [J]. *Journal of Dalian Ocean University*, 2018, **33**(6): 722-728. [路晶晶, 郭冉, 齐国山, 等. 复方中草药对大菱鲆幼鱼生长性能及非特异性免疫指标的影响 [J]. 大连海洋大学学报, 2018, **33**(6): 722-728.]

[10] Huang X Y, Sun X, Yu X, *et al*. Efficacy and safety of Sijunzi decoction for peptic ulcers: A systematic review and meta-analysis [J]. *Journal of Traditional Chinese Medical Sciences*, 2018(5): 237-254.

[11] Guan Z B, Wang M, Cai Y, *et al*. Rapid characterization of the chemical constituents of Sijunzi decoction by UHPLC coupled with Fourier transform ion cyclotron resonance mass spectrometry [J]. *Journal of Chromatography B*, 2018(1086): 11-22.

[12] Li Y, Jiang K Y, Sun G X, *et al*. The eco-nutrition requirements for dietary protein and its rhomb characteristics in juvenile turbot (*Scophthalmus maximus* L.) [J]. *Chinese Journal of Oceanology and Limnology*, 2011, **29**(5): 1002-1008.

[13] Peng M. The effects of dietary lipid level and fatty acids composition on lipid deposition in juvenile turbot (*Scophthalmus maximus* L.) [D]. *Ocean University of China*, 2014: 1-156. [彭墨. 饲料脂肪水平和脂肪酸组成对大菱鲆幼鱼脂沉积的影响 [D]. 中国海洋大学, 2014: 1-156.]

[14] Zhang H, Sun J, Liu H, *et al*. The effect of dietary lipid levels on growth performance, lipid deposition, and antioxidant status of juvenile turbot, *Scophthalmus maximus*, fed isonitrogenous and isoenergetics diets [J]. *Israeli Journal of Aquaculture-Bamidgeh*, 2015(67): 1142.

[15] Ali B H, Blunden G, Tanira M O, *et al*. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research [J]. *Food and Chemical Toxicology*, 2008, **46**(2): 409-420.

[16] Zhang R X, Li M X, Jia Z P. *Rehmannia glutinosa*: Review of botany, chemistry and pharmacology [J]. *Journal of Ethnopharmacology*, 2008, **117**(2): 199-214.

[17] Djakpo O, Yao W. *Rhus chinensis* and *Galla Chinensis*-folklore to modern evidence: review [J]. *Phytotherapy Research*, 2010, **24**(12): 1739-1747.

[18] Ríos J L. Chemical constituents and pharmacological properties of *Poria cocos* [J]. *Planta Medica*, 2011, **77**(7): 681-691.

[19] Liu H, Chen Y, Li F, *et al*. *Fructus gardenia* (*Gardenia jasminoides* J. Ellis) phytochemistry, pharmacology of

cardiovascular, and safety with the perspective of new drugs development [J]. *Journal of Asian Natural Products Research*, 2013, **15**(1): 94-110.

[20] Wu J, Peng W, Qin R, *et al*. *Crataegus pinnatifida*: chemical constituents, pharmacology, and potential applications [J]. *Molecules*, 2014, **19**(2): 1685-1712.

[21] Gao S, Liu J, Wang M, *et al*. Traditional uses, phytochemistry, pharmacology and toxicology of Codonopsis: A review [J]. *Journal of Ethnopharmacology*, 2018(219): 50-70.

[22] Karkanis A, Martins N, Petropoulos S A, *et al*. Phytochemical composition, health effects, and crop management of liquorice (*Glycyrrhiza glabra* L.): A medicinal plant [J]. *Food Reviews International*, 2018, **34**(2): 182-203.

[23] Zhu B, Zhang Q, Hua J, *et al*. The traditional uses, phytochemistry, and pharmacology of *Atractylodes macrocephala* Koidz.: A review [J]. *Journal of Ethnopharmacology*, 2018(226): 143-167.

[24] Wang C Y, Li Z, Sun Y Z, *et al*. Effects of Chinese herbal medicines mixture on growth performance digestive enzyme activity immune response of juvenile Japanese seabass, *Lateolabrax japonicus* [J]. *Aquaculture Nutrition*, 2018, **24**(2): 683-693.

[25] Büyükdeveci M E, Balcazar J L, Demirkale I, *et al*. Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*) [J]. *Aquaculture*, 2018(486): 170-174.

[26] Chakraborty S B, Horn P, Hancz C, *et al*. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture [J]. *Reviews in Aquaculture*, 2014, **6**(1): 1-19.

[27] Citarasu T. Herbal biomedicines: a new opportunity for aquaculture industry [J]. *Aquaculture International*, 2010, **18**(3): 403-414.

[28] Wu C, Shan J, Feng J, *et al*. Effects of dietary *Radix Rehmanniae Preparata* polysaccharides on the growth performance, immune response and disease resistance of *Luciobarbus capito* [J]. *Fish & Shellfish Immunology*, 2019(89): 641-646.

[29] Liu F, Geng C, Qu Y K, *et al*. The feeding of dietary *Codonopsis pilosula* polysaccharide enhances the immune responses, the expression of immune-related genes and the growth performance of red swamp crayfish (*Procambarus clarkii*) [J]. *Fish & Shellfish Immunology*, 2020(103): 321-331.

[30] Sukumaran V, Park S C, Giri S S, *et al*. Role of dietary ginger *Zingiber officinale* in improving growth performances and immune functions of *Labeo rohita* fingerlings [J]. *Fish & Shellfish Immunology*, 2016(57): 362-370.

[31] Chen H H, Tu C L, Tang Y, *et al*. Effects of compound Chinese herbs on growth, digestive enzyme, immunologic factors and WSSV-resistant capacity of *Litopenaeus vannamei* [J]. *Journal of Fisheries of China*, 2017, **41**(11): 1766-1778. [陈辉辉, 涂晨凌, 唐杨, 等. 复方中草药对凡纳滨对虾生长、消化酶和免疫因子活性及抗WSSV的影响 [J]. 水产学报, 2017, **41**(11): 1766-1778.]

[32] Dong X H, Li M, Ye J D. Effect of Chinese herbal medicines on growth and non-specific immunity in Pacific white shrimp *Litopenaeus vannamei* [J]. *Journal of Dalian Ocean University*, 2009, **24**(2): 162-165. [董晓慧, 李明, 叶继丹, 等. 复方中草药对凡纳滨对虾生长性能和血清非特异免疫因子的影响 [J]. 大连水产学院学报, 2009, **24**(2): 162-165.]

[33] Zhang Y W, Cai B, Chen W G, *et al*. Effect of compound Chinese herbal medicine on growth performance and muscle quality of *Cyprinus carpio* [J]. *Scientific Fish Farming*, 2012(12): 72-73. [张耀武, 蔡冰, 陈万光, 等. 复方中草药制剂对黄河鲤鱼生长性能和肌肉品质的影响 [J]. 科学养鱼, 2012(12): 72-73.]

[34] Kong J H. Effect of Chinese herbal medicines on growth and non-specific immunity of orange-spotted grouper, *Epinphelus coioides* [D]. Xiamen: Jimei University, 2011: 1-62. [孔江红. 复方中草药对斜带石斑鱼生长性能及非特异性免疫功能的影响 [D]. 厦门: 集美大学, 2011: 1-62.]

[35] Dai Q, Dai J H, Li C, *et al*. Discussion on relative fatness [J]. *Chinese Journal of Applied and Environmental Biology*, 2006(5): 715-718. [戴强, 戴建洪, 李成, 等. 关于肥满度指数的讨论 [J]. 应用与环境生物学报, 2006(5): 715-718.]

[36] Harikrishnan R, Balasundaram C, Heo M, *et al*. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish [J]. *Aquaculture*, 2011, **317**(1): 1-15.

[37] Li M F, Zhang W J, Mao Z J, *et al*. Influence of oral administration of Chinese herbal medicines on activity of several immunity related enzymes from large yellow croaker *Pseudosciaena crocea* [J]. *Fisheries Science*, 2014, **33**(11): 718-722. [李梅芳, 张文杰, 毛芝娟, 等. 投喂中草药对大黄鱼几种免疫酶活性的影响 [J]. 水产科学, 2014, **33**(11): 718-722.]

[38] Ai C H, Zou J H, Yu Y Z, *et al*. Effect of six kinds of Chinese medicines on non-specific immunity of *Carassius auratus* [J]. *Freshwater Fisheries*, 2009, **39**(1): 76-79. [艾春汉, 邹金虎, 喻运珍, 等. 6种中药对鲫非特异性免疫效果的影响 [J]. 淡水渔业, 2009, **39**(1): 76-79.]

[39] Fan Y, Li T B, Diao J, *et al*. Effects of *Codonopsis pilosula* on the immunity and digestion of *Apostichopus japonicas* [J]. *China Feed*, 2017(7): 20-25. [樊英, 李天保, 刁菁, 等. 党参对仿刺参免疫和消化功能的影响 [J]. 中国饲料, 2017(7): 20-25.]

[40] Li J L, Hu Y, Dai Z Y, *et al*. Effects of feeding modes of Chinese herbal medicine on growth and physiological-biochemical indices of *Monopterus albus* [J]. *Journal of*

Hydroecology, 2013, **34**(2): 90-95. [李金龙, 胡毅, 戴振炎, 等. 中草药不同投喂模式对黄鳝生长及生理生化指标的影响 [J]. 水生态学杂志, 2013, **34**(2): 90-95.]

[41] Xian T, Wang D, Liu H B. Effects of Chinese herbal medicines *Astragalus mongholicus*, *Glycyrrhiza uralensis* and *Poria cocos* on non-specific immunity in Amur sturgeon [J]. *Journal of Dalian Ocean University*, 2018, **33**(3): 365-369. [线婷, 王荻, 刘红柏. 黄芪、甘草、茯苓对施氏鲟非特异性免疫功能的影响 [J]. 大连海洋大学学报, 2018, **33**(3): 365-369.]

[42] Wang J L, Meng X L, Lu R H, *et al*. Effects of *Rehmannia glutinosa* on growth performance, immunological parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.) [J]. *Aquaculture*, 2015(435): 293-300.

[43] Han Y F, Wu T, Jia Z R, *et al*. Study on the effect of compound gallnut and licorice on non-specific immunity of yellow river carp [J]. *China Fisheries*, 2018(9): 91-93. [韩永峰, 吴桃, 贾振荣, 等. 甘草与复方五倍子对黄河鲤非特异性免疫力影响的研究 [J]. 中国水产, 2018(9): 91-93.]

[44] Liu L. Effects of Chinese gall on digestive function and immune function of *Aristichthys nobilis* [D]. Changchun: Jilin Agricultural University, 2014: 1-51. [刘蕾. 五倍子对鳙鱼消化机能及免疫机能的影响 [D]. 长春: 吉林农业大学, 2014: 1-51.]

[45] Zhu Z C, Shi X G, Zhang S J, *et al*. *In vitro* antibacterial activity of Chinese herbs to pathogenic *Edwardsiella tarda* isolated from flounder (*Paralichthys olivaceus*) [J]. *Fisheries Science*, 2007(5): 278-281. [朱壮春, 史相国, 张淑杰, 等. 中草药对牙鲆病原迟钝爱德华氏菌的体外抑制作用研究 [J]. 水产科学, 2007(5): 278-281.]

[46] Huang A, Tu X, Qi X, *et al*. *Gardenia jasminoides* Ellis inhibit white spot syndrome virus replication in red swamp crayfish *Procambarus clarkii* [J]. *Aquaculture*, 2019(504): 239-247.

[47] Talpur A D, Ikhwanuddin M, Bolong A A, *et al*. Nutritional effects of ginger (*Zingiber officinale* Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi* [J]. *Aquaculture*, 2013(400): 46-52.

[48] Ahmadifar E, Sheikhzadeh N, Roshanaei K, *et al*. Can dietary ginger (*Zingiber officinale*) alter biochemical and immunological parameters and gene expression related to growth, immunity and antioxidant system in zebrafish (*Danio rerio*) [J]. *Aquaculture*, 2019(507): 341-348.

[49] Wang H H, Huang J F, Sheng Y P, *et al*. A review on the traditional Chinese medicinal immunopotentiators for fishery [J]. *Chinese Journal of Veterinary Drug*, 2005, **39**(1): 41-44. [王海华, 黄江峰, 盛银平, 等. 渔用中药免疫增强剂研究进展 [J]. 中国兽药杂志, 2005, **39**(1): 41-44.]

[50] Li C, Zhang Q Z, Yang Y Y, *et al*. Effects of compound Chinese herbal medicine on growth and blood cell number in grass carp (*Ctenopharyngodon idellus*) [J]. *Fisheries Science*, 2012, **31**(1): 7-11. [李超, 张其中, 杨莹莹, 等. 复方中草药对草鱼生长和血细胞数量的影响 [J]. 水产科学, 2012, **31**(1): 7-11.]

[51] Zhang Z H. Effects of Chinese herbal compound on growth performance and non-specific immune function of tilapia (*Oreochromis niloticus*) and *Ctenopharyngodon idellus* [D]. Fuzhou: Fujian Agriculture and Forestry University, 2011: 1-45. [张照红. 复方中草药对罗非鱼、草鱼生长性能和非特异免疫功能的影响 [D]. 福州: 福建农林大学, 2011: 1-45.]

[52] Jiang G L. Effect of Chinese herbal medicine extract on production performance and immune function of *Pelteobrus fulvidraco* [J]. *Feed Research*, 2015(9): 56-60. [姜光丽. 中草药提取物对黄颡鱼生产性能及免疫功能的影响 [J]. 饲料研究, 2015(9): 56-60.]

[53] Awad E, Awaad A S. Role of medicinal plants on growth performance and immune status in fish [J]. *Fish and Shellfish Immunology*, 2017(67): 40-54.

[54] Harikrishnan R, Kim J S, Kim M C, *et al*. *Prunella vulgaris* enhances the non-specific immune response and disease resistance of *Paralichthys olivaceus* against *Uronema marinum* [J]. *Aquaculture*, 2011, **318**(1): 61-66.

[55] Wu Y R, Gong Q F, Fang H, *et al*. Effect of *Sophora flavescens* on non-specific immune response of tilapia (GIFT *Oreochromis niloticus*) and disease resistance against *Streptococcus agalactiae* [J]. *Fish and Shellfish Immunology*, 2013, **34**(1): 220-227.

[56] Lin H Z, Li Z J, Lu X, *et al*. Effects of feeding time of dietary Chinese herbs on digestive and immune enzymes of *Litopenaus vannamei* [J]. *Feed Industry*, 2011, **32**(2): 11-14. [林黑着, 李卓佳, 陆鑫, 等. 复方中草药饲喂时间对凡纳滨对虾消化和免疫酶活性的影响 [J]. 饲料工业, 2011, **32**(2): 11-14.]

EFFECTS OF DIFFERENT LEVELS OF CHINESE HERBAL MEDICINE ON THE GROWTH PERFORMANCE, FEED UTILIZATION AND NON-SPECIFIC IMMUNITY OF JUVENILE TURBOT (*SCOPHTHALMUS MAXIMUS* L.)

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Abstract: Turbot (*Scophthalmus maximus* L.) is an important industrial mariculture species in China. With the development of high-density and intensive culture, turbot always suffer from various kinds of environmental stresses, which led to the reduction of fish immunity and brought out many diseases. Therefore, it is urgent to find the green feed additives to improve the immunity of fish to avoid the disease. Chinese herbal medicine is rich in carbohydrate, lipid, vitamin and minerals, and other bioactive substances such as alkaloids, saponins, polyphenols, flavonoids, polysaccharides and terpenoids, which could stimulate the activities of complement, lysozyme and antibody reaction and promote the function of phagocytes to improve the bactericidal activity. And The herbal medicine has the advantages of non-toxic, no drug resistance, no residue and environmental friendly. "Sijunzi Decoction" is an ancient prescription in China, and mainly composed of *Panax ginseng*, *Atractylodes macrocephala* Koidz, *Wolfiporia cocos* and *Glycyrrhiza uralensis*. The main active chemical components analyzed in this Decoction are ginsenosides (from *Panax ginseng*), flavonoids and triterpenoids (from *Glycyrrhiza uralensis*). It has the pharmacological activities including improving the gastrointestinal function, invigorating spleen-reinforcing Qi and immunomodulatory activity for human. However, there is no report about the application of this Decoction in fish. Based on the components of the traditional prescription of "Sijunzi Decoction", nine kinds of Chinese herbal medicines was chose to apply in the feed of fish in this study. To investigate the effects of different levels of a Chinese herbal medicines mixture (CHMM: decoction of *Codonopsis pilosula*, *Atractylodes macrocephala* Koidz, *Wolfiporia cocos*, *Rehmannia glutinosa* Libosc, *Glycyrrhiza uralensis* Fisch, *Crataegus pinnatifida* Bunge, *Rhus chinensis* Mill, *Gardenia jasminoides* Ellis and *Zingiber officinale* Roscoe) on growth performance, feed utilization, body composition and related immune indices of juvenile turbot, basal diets were supplemented with 0, 1%, 2% or 4% CHMM in the form of water decoction to feed juvenile turbot [initial mean body weight was (22.01±0.01) g] for 40d. The fish were fed two times every day (8: 00 and 18: 00), the light period was 14h light and 10h dark, the water dissolved oxygen was greater than 6.0 mg/L, salinity was 25‰—28‰, water temperature was 15—18℃, and ammonia nitrogen concentration was less than 0.05 mg/L. The results indicated that dietary supplementation of CHMM had no significant influence on feeding rate ($P \geqslant 0.05$). However, the dietary CHMM significantly influenced the growth performance and feed utilization efficiency of turbot ($P<0.05$), the specific growth rate (*SGR*) and weight gain rate (*WGR*) of turbot in 1% group were significantly higher than those in other groups ($P<0.05$), no significant difference was observed between 2% group and control group ($P \geqslant 0.05$). The feed coefficient (*FCR*) in 1% group was significantly lower than other groups ($P<0.05$), and there were no significant differences among other groups ($P \geqslant 0.05$). The supplementation of CHMM in diet significantly affected the condition factor (*CF*) and spleen index (*SI*) of turbot ($P<0.05$), and the highest *CF* and *SI* were observed in turbot fed 1% CHMM diet ($P<0.05$). Meanwhile, the supplementation of CHMM had no significant effect on the visceral index (*VSI*), hepatosomatic index (*HSI*) and body composition of turbot ($P \geqslant 0.05$). The contents of C3 and activities of lysozyme in plasma were significantly affected by the dietary supplementation of CHMM ($P<0.05$), while no significant difference was observed in the contents of plasma IgM among all treatments. The contents of plasma C3 in 1% group were significantly higher than that in control group ($P<0.05$), the activities of plasma LZM in 1% group were significantly higher than those in other groups ($P<0.05$), and no significant differences were observed among other groups ($P \geqslant 0.05$). In conclusion, the supplement of CHMM in diet significantly improved the growth performance, feed utilization and non-specific immunity of turbot. The optimum supplemental level of this compound Chinese herbal medicine in the feed of turbot was 1%.

Key words: Chinese herbal medicine mixture; Turbot; Growth performance; Feed utilization; Body composition; Non-specific Immunity