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卵形鲳鲹配合饲料中酶解鱼浆蛋白和陆生复合蛋白替代鱼粉的研究*

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摘要 为了评估卵形鲳鲹(*Trachinotus ovatus*)配合饲料中酶解鱼浆蛋白和陆生复合蛋白替代鱼粉的可行性，本研究设计了 4 种等蛋(42%)等脂(12%)配合饲料(D1~D4)，其中，D1(对照组)含 30%鱼粉，D2~D4(处理组)都含 14%陆生复合蛋白且还分别含有 16%、11%、6%鱼粉和 0、5%、10%酶解鱼浆蛋白；各处理组都补充蛋氨酸和赖氨酸。将 360 尾初始体重为(7.28±0.10) g 的卵形鲳鲹幼鱼随机分配到 12 个海上网箱中，每个网箱 30 尾鱼，每种饲料设 3 个网箱。将鱼以上述 4 种饲料饲养 62 d 后，测定其生长性能、体组成、血清生化指标、肠道消化酶活性与组织抗氧化指标。结果显示，各实验组鱼的增重率(WGR)、特定生长率(SGR)、蛋白质效率(PER)、饲料系数(FC)、胃蛋白酶(PEP)、脂肪酶(LPS)和淀粉酶(AMS)活性均无显著差异(P>0.05)。D3 和 D4 组全鱼粗蛋白含量显著高于 D1 和 D2 组，D4 组鱼肌肉脂肪含量显著低于 D1~D3 组(P<0.05)；D2~D4 组鱼血清谷草转氨酶(AST)及 D2 和 D3 组鱼谷丙转氨酶(ALT)活性都显著低于 D1 组(P<0.05)，D1 和 D4 组血清总蛋白(TP)和球蛋白(GLO)水平显著高于 D2 和 D3 组(P<0.05)；D2~D4 组肝脏过氧化氢酶(CAT)活性、总抗氧化能力(T-AOC)和肌肉超氧化物歧化酶(SOD)活性显著高于 D1 组(P<0.05)，且肝脏丙二醛(MDA)含量低于 D1 组(P<0.05)。研究表明，含 6%鱼粉的 D4 组鱼的生长性能与 30%鱼粉 D1 组无差异，且全鱼蛋白含量及肝脏和肌肉抗氧化能力显著提高，说明 14%陆生复合蛋白配合 10%酶解鱼浆蛋白可有效替代卵形鲳鲹饲料中 80%鱼粉，使饲料鱼粉使用量低至 6%。本研究是首次探讨酶解鱼浆蛋白在卵形鲳鲹配合饲料中应用的可行性，结果可为研发高效低成本配合饲料提供参考依据。

关键词 卵形鲳鲹；鱼粉替代；酶解鱼浆；生长性能；抗氧化指标

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鱼粉因其蛋白含量高、氨基酸组分较平衡等优点而在水产饲料行业备受青睐。然而，随着水产养殖业的迅猛发展，饲料需求量骤增而带来的鱼粉短缺、价格上涨问题日益凸显。为解决这一问题，寻找适宜的

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鱼粉替代蛋白源已成为当务之急。目前，已有大量关于单一动植物蛋白源替代鱼粉的研究，且在开发新型饲料蛋白源、降低生产成本和保护环境等方面初见成效(易新文等, 2019; 胡海滨等, 2019; Chen *et al*, 2021; He *et al*, 2021)。但对于多数肉食或杂食性鱼类来说，单一蛋白源替代鱼粉的比例一般不超过 50%，替代率过高容易导致生长性能和营养品质下降(周晖等, 2012; 王婧瑶等, 2021; 潘世会等, 2020)。因此，利用不同类型蛋白源互补组合，提高其在水产配合饲料中的利用率，已成为提高鱼粉替代比例的重要途径(林一帆等, 2019; 朱旺明等, 2019; 胡鹏莉等, 2019; Ma *et al*, 2020)。酶解鱼浆蛋白是一种由渔获物加工而成的新型蛋白源，鱼粉不同的是在其生产过程中有一道酶解步骤，因此，其含有较多寡肽、游离氨基酸及一些鱼粉所没有的促生长因子而被业界广泛关注(周露阳等, 2019; 吴代武等, 2015; Shi *et al*, 2019; Halim *et al*, 2016)。在黄颡鱼(*Pelteobagrus fulvidraco*)(吴代武等, 2018)、银鲳鱼(*Trachinotus blochii*)(Tejpal *et al*, 2021)和大菱鲆(*Scophthalmus maximus*)(Wei *et al*, 2020)等品种中的研究表明，酶解鱼浆蛋白可有效替代饲料中的鱼粉，不仅对鱼体生长无负面影响，还会显著提高其免疫力和抗氧化力。同时，酶解鱼浆蛋白也被认为是一种功能性添加剂，具有提高水产动物蛋白利用率的效果(Egerton *et al*, 2020; Wei *et al*, 2019)。然而，它在卵形鲳鲹上的应用研究尚未见报道。

卵形鲳鲹(*Trachinotus ovatus*)俗称金鲳，是我国主要的海水养殖品种之一，属于肉食鱼类，具有生长速度快、产量高、肉味鲜美的特点(李远友等, 2019; Wang *et al*, 2017)。本课题组近年的研究表明，以几种陆生动植物蛋白按一定比例配制的复合蛋白可替代其配合饲料中 80%鱼粉(Ma *et al*, 2020)。基于此，本研究利用该复合蛋白与酶解鱼浆蛋白搭配来替代卵形鲳鲹幼鱼配合饲料中不同水平的鱼粉，通过生长性能、体组成、血清生化指标、消化酶活性及抗氧化能力的比较，评估替代可行性，获得适宜替代比例。所获结果将为卵形鲳鲹低鱼粉配合饲料开发提供理论依据。

1 材料与方法

1.1 实验饲料

以鱼粉、复合蛋白和酶解鱼浆蛋白为主要蛋白源，以鱼油、亚麻籽油、豆油、磷脂油等按一定比例配制的金鲳复合油为脂肪源，制备 4 种等蛋(42%)等脂(12%)配合饲料(D1~D4)，其中，D1(对照组)含 30%鱼粉，D2~D4 分别含 16%、11%和 6%鱼粉及 0、5%、10%酶解鱼浆蛋白，饲料配方见表 1。此外，各组饲料氨基酸和脂肪酸组成分别见表 2 和表 3。

1.2 实验设计

实验所用卵形鲳鲹幼鱼购自福建某金鲳养殖场，用鱼船运至汕头大学南澳临海实验站后，以商品饲料暂养于实验站附近的海上网箱中 14 d 后，将大小基本一致的鱼按每个网箱 40 尾左右放养在 12 个实验网箱(1.0 m×1.0 m×1.5 m)中，以 4 种实验饲料等量混合进行小网箱中适应性养殖 14 d，然后开始正式养殖实验。分组前，将鱼禁食 24 h，挑选规格整齐、体格健康的幼鱼[初始均重为(7.28±0.10) g]集中到一个大网箱中，然后随机给每个网箱分配 30 尾并称重，每种饲料设 3 个重复网箱，养殖 62 d。每天近饱食投喂 2 次(08:00 和 17:00)，记录鱼死亡情况和投饵量；期间，水温为 26.9~33.3℃，盐度为 27~33。

1.3 实验鱼称重、采样

养殖实验结束时，将鱼停喂 24 h 后，对各网箱中的鱼称重并清点数量。从每个网箱随机取 3 尾鱼，用含 0.01% 2-苯氧基乙醇的海水麻醉后，通过尾静脉取血。将采集到的血液移入 1.5 mL 离心管中，在 4℃下静置 2~4 h 后于 3000 r/min 离心 10 min (4℃)；将上清液转移到新的离心管中后，放入液氮中速冻，然后置于−80℃保存，待测相应指标。此外，将取血后的鱼解剖，称肝脏重和内脏重；取适量肝脏、胃、小肠和肌肉样品放入离心管中，液氮中速冻后置于−80℃冰箱中保存备用。最后，从每网箱取 2 尾鱼用于全鱼营养成分测定。

1.4 计算公式及样品分析方法

存活率(survival rate, SR, %) = $100\times N_f/N_i$；

增重率(weight gain rate, WGR, %) = $100\times(W_f-W_i)/W_i$；

特定生长率(specific growth rate, SGR, %/d) = $100\times(\ln W_f-\ln W_i)/t$；

饲料系数(feed coefficient，FC) = $D_a/(W_f-W_i)$；

日摄食率(feed intake, FI, %/d) = $100\times D_a/[(W_i+W_f)/2]/t$；

蛋白质效率(protein efficiency ratio, PER, %) = $100\times(W_f-W_i)/(D_a\times P_d)$；

表 1　饲料组成和营养水平(%干物质)

Tab.1　Feed formulation and nutrient component (% dry matter)

项目 Items	组别 Groups			
	D1	D2	D3	D4
鱼粉 Fish meal	30.00	16.00	11.00	6.00
酶解鱼浆蛋白 Fish protein hydrolysate	–	–	5.00	10.00
复合蛋白 Compound protein[a]	–	14.00	14.00	14.00
基础蛋白 Basic protein[b]	28.50	32.00	33.00	34.00
复合油 Blend oil[c]	8.20	8.50	8.60	8.80
高筋面粉 Wheat meal	17.00	17.00	17.00	17.00
氯化胆碱 Choline chloride	0.50	0.50	0.50	0.50
维生素预混料 Vitamin premix[d]	1.00	1.00	1.00	1.00
矿物质预混料 Mineral premix[e]	1.00	1.00	1.00	1.00
磷酸二氢钙 Calcium dihydrogen phosphate	0.50	0.50	0.50	0.50
蛋氨酸 Methionine	–	0.14	0.15	0.16
赖氨酸 Lysine	–	0.29	0.33	0.32
麸皮 Wheat bran	13.30	9.07	7.92	6.72
合计 Total	100.00	100.00	100.00	100.00
饲料中营养水平 Nutrient level/%				
水分 Moisture	6.91	7.13	7.42	6.88
粗蛋白质 Crude protein	42.18	42.24	42.13	42.22
粗脂肪 Crude lipid	12.02	12.01	12.01	11.98
灰分 Ash	8.24	7.77	7.22	7.73

注：a: 由几种陆生动植物蛋白按一定比例组成，因其涉及专利不便公布具体配比；b: 由鸡肉粉、大豆浓缩蛋白和玉米蛋白按一定比例组成；c: 由鱼油、亚麻籽油、豆油、磷脂油等组成；d: 维生素预混料(mg 或 IU/kg)：维生素 A 2000 IU，维生素 D 3700 IU，维生素 E 10 mg，维生素 K_3 2.5 mg，硫胺素 2.5 mg，核黄素 5 mg；e: 矿物质预混料(mg 或 g/kg)：钙 230 g，钾 36 g，镁 9 g，铁 10 g，锌 8 g，锰 1.9 g，铜 1.5 g，钴 250 mg，碘 32 mg，硒 50 mg

Note: a: Consists of several terrestrial animal and plant proteins; b: Consists of by-poultry meal, soy protein concentrate and corn gluten meal; c: Consists of fish oil, flaxseed oil, soybean oil and phospholipid oil; d: vitamin premix (mg or IU/kg): Vitamin A 2000 IU, vitamin D 3700 IU, vitamin E 10 mg, vitamin K_3 2.5 mg, thiamin 2.5 mg, riboflavin 5 mg; e: Mineral premix (mg or g/kg): Ca 230 g, K 36 g, Mg 9 g, Fe 10 g, Zn 8 g, Mn 1.9 g, Cu 1.5 g, Co 250 mg, I 32 mg, Se 50 mg

表 2　实验饲料的氨基酸组成(%)

Tab.2　Amino acid composition of the experimental diets (%)

氨基酸 Amino acid	组别 Groups				氨基酸 Amino acid	组别 Groups			
	D1	D2	D3	D4		D1	D2	D3	D4
必需氨基酸 EAA					非必需氨基酸 NEAA				
苏氨酸 Thr	3.01	2.87	2.92	2.86	天冬氨酸 Asp	3.94	3.94	3.96	3.91
缬氨酸 Val	1.70	1.71	1.73	1.68	丝氨酸 Ser	1.99	2.05	2.11	2.08
蛋氨酸 Met	1.13	0.98	0.98	0.95	谷氨酸 Glu	6.63	6.99	7.05	7.08
异亮氨酸 Ile	1.58	1.57	1.52	1.56	甘氨酸 Gly	2.56	2.61	2.77	2.72
亮氨酸 Leu	3.93	3.98	4.01	3.99	丙氨酸 Ala	2.46	2.42	2.47	2.37
苯丙氨酸 Phe	1.90	1.99	2.02	1.98	半胱氨酸 Cys	0.27	0.26	0.29	0.28
赖氨酸 Lys	2.16	2.08	2.02	2.07	酪氨酸 Tyr	1.52	1.54	1.62	1.52
					组氨酸 His	1.19	1.17	1.15	1.12
					精氨酸 Arg	2.22	2.34	2.33	2.27
					脯氨酸 Pro	1.52	1.60	1.63	1.61

表 3 实验饲料的脂肪酸组成(%总脂肪酸)

Tab.3 Fatty acid composition of the experimental diets (% total fatty acids)

脂肪酸 Fatty acid	组别 Groups			
	D1	D2	D3	D4
14:0	3.69	3.44	3.32	3.34
15:0	0.37	0.41	0.36	0.38
16:0	29.97	30.46	29.84	29.61
17:0	0.59	0.54	0.61	0.64
18:0	4.86	5.50	5.82	5.34
20:0	0.55	0.58	0.59	0.56
21:0	0.68	0.70	0.70	0.65
22:0	0.33	0.33	0.35	0.32
SFA	41.05	41.97	41.58	40.83
16:1	2.91	2.65	2.84	3.01
17:1	0.27	0.28	0.25	0.26
18:1t	22.41	23.64	23.35	23.49
18:1c	3.40	2.76	3.27	3.50
22:1n9	0.40	0.38	0.46	0.53
MUFA	29.38	29.71	30.16	30.78
18:2n-6 LNA	14.03	14.56	14.05	13.39
20:2n-6	0.16	0.13	0.14	0.14
20:4n-6 ARA	0.18	0.22	0.21	0.22
n-6 PUFA	14.37	14.92	14.40	13.75
18:3n-3 ALA	2.03	1.96	2.05	2.17
20:3n3	0.74	0.67	0.75	0.82
20:5n-3 EPA	4.32	3.64	3.51	3.58
22:5n-3 DPA	0.71	0.58	0.55	0.57
22:6n-3 DHA	5.15	4.24	4.46	5.01
n-3 PUFA	12.96	11.09	11.32	12.15
n-3/n-6 PUFA	0.90	0.74	0.79	0.88

肥满度(condition factor, CF) = $100\times W_b/L_b^3$；

肝体比(hepatosomatic index, HSI, %) = $100\times W_l/W_b$；

脏体比(viscerosomatic index, VSI, %) = $100\times W_v/W_b$。

式中，N_i、N_f 分别为初始、终末鱼的数量；W_i、W_f 分别为初始、终末平均鱼体体重(g)；W_i、W_f 分别为初始、终末鱼体总重(g)；t 为养殖天数(d)；D_a 为总摄入饲量(干物质基础，g)；P_d 为饲料(干物质基础)的粗蛋白质含量(%)；W_b 为鱼体重(g)；W_l、W_v 分别为肝脏、内脏团重量(g)；L_b 为鱼体长(cm)。

饲料和组织常规营养成分分析：水分采用常压干燥法测定，依照 GT/T6435-2014；灰分采用马弗炉灼烧法测定，依照 GB/T6438-2007；粗蛋白采用凯氏定氮法测定，依照 GB/T6433-2006；粗脂肪采用索氏抽提法测定，依照 GB/T6433-2006。

脂肪提取及脂肪酸组成分析：将待测样品干燥粉碎后，称取粉碎样品 200 mg 放入 15 mL 玻璃离心管中，加入 6 mL 氯仿：甲醇(2∶1)溶液后充分混合，然后置于 4℃中浸泡 48 h，期间充分混匀几次；加入蒸馏水 2.7 mL 后混合，再于 3000 r/min 离心 5 min；吸取下层脂肪到新的离心管中，45℃氮气吹干。向脂肪干品中加入 0.5 mmol/L KOH-甲醇溶液 2 mL，于 65℃水浴皂化至油滴消失。冷却后加入 2 mL 15%三氟化硼甲醇液，在 70℃水浴加热 30 min 后加入 2 mL 正己烷和 2 mL 饱和氯化钠溶液，充分混合后，于 3000 r/min 下离心 5 min 后，取上层样用气相色谱仪(Agilent 7890B GC)分析脂肪酸组成。

氨基酸组成测定：将待测样品干燥粉碎后，称取粉碎样品 30 mg 放入水解管中，加入 6 mol/L 盐酸 10 mL，封住水解管管口，在 110℃恒温下干燥水解 22 h，取出冷却；打开水解管，将水溶液过滤，使用 0.02 mol/L 盐酸定容至 50 mL。取 0.1 mL 定容液与 0.9 mL 0.02 mol/L 盐酸混匀，60℃水浴蒸干后加入 1 mL 0.02 mol/L 盐酸复溶，取样用全自动氨基酸分析仪(SYKAM S-433D)分析氨基酸组成。

生化指标测定：从–80℃冰箱取出血清样品放置冰上解冻溶化，根据试剂盒说明书要求测定相关指标；从–80℃冰箱取出肌肉、胃、小肠和肝脏样品后，取适量样品于研磨管中研磨，加入生理盐水或指定溶液，离心后取上层样根据试剂盒说明书要求测定相关指标。血清总蛋白(total protein, TP)、白蛋白(albumin, ALB)、甘油三酯(triglyceride, TG)、总胆固醇(total cholesterol, T-CHO)、尿素氮(blood urea nitrogen, BUN)、血氨(serum ammonia, SA)、谷草转氨酶(aspartate aminotransferase, AST)和谷丙转氨酶(alanine aminotransferase, ALT)含量，肝脏和肌肉总抗氧化能力(total antioxidant capacity, T-AOC)、还原性谷胱甘肽(reduced glutathione, GSH)含量、过氧化氢酶(catalase, CAT)活性、超氧化物歧化酶(superoxide dismutase, SOD)活性和丙二醛(malondialdehyde, MDA)含量，胃蛋白酶(pepsin, PEP)、脂肪酶(lipase, LPS)和淀粉酶(amylase, AMS)活性均采用南京建成生物技术研究所生产的试剂盒进行测定。

1.5 数据处理

结果以平均值±标准误(Mean±SE)表示。用 SPSS 16.0 对数据进行单因素方差分析(one-way ANOVA)；数据差异显著时，采用 Turkey′s 多重检验法进行比较，$P<0.05$ 时认为差异显著。

2　结果

2.1　各饲料投喂组鱼的生长性能与饲料利用情况比较

实验结束时，卵形鲳鲹幼鱼的生长性能与饲料利用指标见表 4。结果显示，各饲料投喂组鱼的生长性能指标，增重率(WGR)、特定生长率(SGR)、蛋白质效率(PER)、日摄食率(FI)、饲料系数(FC)、肝体比(HSI)和脏体比(VSI)相互间均无显著差异(P>0.05)。

2.2　各饲料投喂组全鱼与肌肉营养成分比较

各饲料投喂组全鱼及肌肉营养成分比较结果见表 5。各饲料组全鱼的水分和灰分、肌肉的水分、粗蛋白和灰分均无显著差异(P>0.05)。但 D3 和 D4 组全鱼粗蛋白含量显著高于 D1 和 D2 组鱼(P<0.05)；D2 组全鱼粗脂肪含量显著高于 D4 组鱼(P<0.05)，但与其他实验组鱼无显著差异(P>0.05)；D4 组鱼肌肉粗脂肪含量显著低于其他实验组鱼(P<0.05)。

2.3　各饲料投喂组鱼血清生化指标的比较

由表 6 可知，血清总蛋白(TP)和球蛋白(GLO)含量随着鱼粉替代比例上升而显著增加(P<0.05)。D1 组鱼血清谷丙转氨酶(ALT)和谷草转氨酶(AST)显著高于 D2 和 D3 组鱼(P<0.05)，但其与 D4 组 ALT 无显著差异(P>0.05)。各饲料组鱼血清白蛋白(ALB)、尿

表 4　各饲料投喂组卵形鲳鲹的生长性能与饲料利用情况

Tab.4　Growth performance and feed utilization of *T. ovatus* fed different diets

项目 Items	组别 Groups			
	D1	D2	D3	D4
初重 Initial body weight/g	7.17±0.25	7.22±0.22	7.44±0.22	7.28±0.15
末重 Final body weight/g	56.23±3.66	56.12±2.76	60.03±2.05	52.91±1.40
增重率 WGR/%	683.82±36.96	679.53±53.34	709.11±49.60	627.46±22.34
特定生长率 SGR/(%/d)	3.32±0.08	3.30±0.11	3.37±0.10	3.20±0.05
蛋白质效率 PER/%	5.32±0.28	5.14±0.19	5.25±0.20	5.03±0.13
日摄食率 FI/(%/d)	3.80±0.25	4.00±0.19	3.87±0.17	3.85±0.07
饲料系数 FC	1.32±0.08	1.37±0.04	1.33±0.04	1.36±0.03
肝体指数 HSI/%	1.74±0.15	1.76±0.17	1.76±0.10	1.50±0.09
脏体指数 VSI/%	6.98±0.30	6.91±0.37	7.40±0.15	7.65±0.52
肥满度 CF	3.59±0.08	3.89±0.12	4.08±0.16	3.79±0.14
存活率 SR/%	97.78±2.22	95.56±4.44	97.78±2.22	100.00±0.00

注：数据为平均值±标准误(Mean±SE) (n=3)。

Note: Values are Mean±SE (n=3).

表 5　各饲料投喂组卵形鲳鲹全鱼及肌肉的营养成分

Tab.5　Nutritional composition in whole fish and muscle of *T. ovatus* fed different diets

项目 Items	组别 Groups			
	D1	D2	D3	D4
全鱼 Whole body				
水分 Moisture /%	65.57±0.27	65.19±0.23	66.75±0.36	66.76±0.26
粗蛋白 Crude protein /%	49.45±0.23^{a}	48.52±0.21^{a}	51.30±0.68^{b}	51.49±0.29^{b}
粗脂肪 Crude lipid /%	32.82±0.38^{ab}	35.37±1.04^{b}	34.60±1.36^{ab}	31.00±0.50^{a}
灰分 Crude ash /%	11.92±0.62	10.84±0.18	11.54±0.33	11.91±0.35
肌肉 Muscle				
水分 Moisture /%	71.80±0.13	74.50±0.68	74.07±0.71	74.11±0.72
粗蛋白 Crude protein /%	70.14±0.51	71.30±0.50	74.20±2.20	74.54±1.40
粗脂肪 Crude lipid /%	23.23±0.70^{b}	23.08±0.96^{b}	21.69±0.79^{b}	17.69±0.12^{a}
灰分 Crude ash /%	3.31±0.39	3.41±0.12	3.59±0.04	3.40±0.10

注：数据为平均值±标准误(n=3)。同一行中，无相同小写字母标注者表示相互间有显著差异(P<0.05)，下同。

Note: Values are Mean±SE (n=3). In the same row, values without sharing a common superscript letter are significantly different (P<0.05), the same as below.

表 6 不同处理组饲料对卵形鲳鲹血清生化指标的影响

Tab.6 Serum biochemical parameters of *T. ovatus* fed different diets

项目 Items	组别 Groups			
	D1	D2	D3	D4
总蛋白 TP /(g/L)	37.40±0.22^{c}	26.71±0.14^{a}	33.25±0.60^{b}	37.26±0.28^{c}
白蛋白 ALB /(g/L)	11.25±0.38	11.84±0.32	11.47±0.19	10.35±0.59
球蛋白 GLO /(g/L)	25.16±0.23^{bc}	18.94±1.06^{a}	23.12±0.66^{b}	27.00±0.64^{c}
尿素氮 BUN /(mmol/L)	2.56±0.09	2.89±0.22	2.33±0.07	2.72±0.08
血氨 SA /(μmol/L)	414.29±3.58	389.95±31.79	398.41±2.00	443.12±6.25
谷丙转氨酶 ALT /(U/L)	32.48±1.37^{b}	24.13±0.42^{a}	23.55±1.93^{a}	27.15±2.17^{ab}
谷草转氨酶 AST /(U/L)	61.34±2.94^{b}	22.22±3.35^{a}	19.57±0.97^{a}	24.05±3.10^{a}

素氮(BUN)和血氨(SA)均无显著差异(P>0.05)。

2.4 各饲料投喂组鱼消化酶活性的比较

不同饲料投喂组鱼胃和肠的消化酶活性见表 7。各饲料组鱼胃蛋白酶(PEP)、脂肪酶(LPS)和淀粉酶(AMS)活性均无显著差异(P>0.05)。

2.5 各饲料投喂组鱼肝脏与肌肉抗氧化指标的比较

图 1 呈现各饲料投喂组鱼肝脏与肌肉抗氧化指标结果。D2~D4 组鱼肝脏过氧化氢酶(CAT)活性和总抗氧化能力(T-AOC)，肌肉超氧化物歧化酶(SOD)活性显著高于 D1 组(P<0.05)，而肝脏丙二醛(MDA)含量正好相反。此外，肝脏还原性谷胱甘肽(GSH)、肌肉 CAT 活性随着鱼粉替代比例升高而增加。肝脏 SOD 活性，肌肉 GSH 活性和丙二醛(MDA)含量各组间均无显著差异(P>0.05)。

3 讨论

3.1 复合蛋白搭配酶解鱼浆蛋白可高比例替代鱼粉的原因分析

本研究中，各饲料投喂组鱼的增重率(WGR)、特

表 7 各饲料投喂组卵形鲳鲹的消化酶活性

Tab.7 The digestive enzyme activity of *T. ovatus* fed different diets

项目 Items	组别 Groups			
	D1	D2	D3	D4
胃蛋白酶 PEP /(U/mg prot)	8.33±0.85	7.13±1.01	8.91±0.90	7.40±0.63
肠脂肪酶 LPS /(U/mg prot)	3.58±0.50	4.45±0.27	4.15±0.30	3.35±0.23
肠淀粉酶 AMS /(U/mg prot)	5.46±0.21	5.93±0.16	4.80±0.45	5.73±0.20

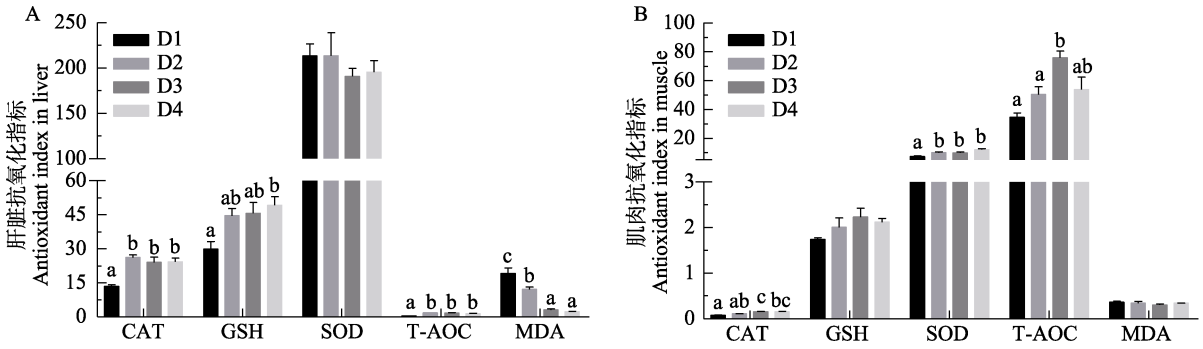

图 1 各饲料投喂组鱼肝脏(A)和肌肉(B)的抗氧化指标

Fig.1 The antioxidant index in liver (A) and muscle (B) of *T. ovatus* fed different diets

过氧化氢酶(CAT, U/mg prot)；还原性谷胱甘肽(GSH, μmol/g prot)；超氧化物歧化酶(SOD, U/mg prot)；总抗氧化能力(T-AOC, mmol/g)；丙二醛(MDA, nmol/mg prot)。数据为平均值±标准误(n=3)，各指标柱上无相同小写字母标注者表示相互间有显著差异(P<0.05)。

Catalase (CAT, U/mg prot); Reduced glutathione (GSH, μmol/g prot); Superoxide dismutase (SOD, U/mg prot); Total antioxidant capacity (T-AOC, mmol/g); Malondialdehyde (MDA, nmol/mg prot). Values are Mean±SE (n=3), for each index, columns without sharing a common superscript letter are significantly different (P<0.05).

定生长率(SGR)、蛋白质效率(PER)、饲料系数(FC)和日摄食率(FI)等指标无差异，说明复合蛋白搭配酶解鱼浆蛋白可有效替代配合饲料中 80%鱼粉，使饲料鱼粉用量降低至 6%。相似地，利用酶解鱼浆蛋白搭配植物蛋白可替代红鳍东方鲀(*Takifugu rubripes*)饲料中 60%鱼粉(Wei *et al*, 2021)。在银鲳鱼中，随着酶解鱼浆蛋白替代鱼粉比例增加，生长性能和饲料利用都显著提高(Tejpal *et al*, 2021)。酶解鱼浆蛋白可以有效替代鱼粉的可能原因有：(1) 经酶解的鱼浆蛋白会含有更多易于消化吸收的蛋白质、小肽和游离氨基酸等营养物质(周露阳等, 2019; Hien *et al*, 2014; Juliano *et al*, 2019)，饲料中小肽和游离氨基酸比例的改变将上调肠道小肽和氨基酸转运蛋白的基因表达水平，提高肠道对营养物质的吸收能力(Wu *et al*, 2018)，从而缓解因鱼粉替代对鱼体生长造成的负面影响；(2)酶解鱼浆蛋白具有足量的精氨酸和亮氨酸，会促进雷帕霉素靶蛋白 1 (mammalian target of rapamycin 1, mTOR1)的活化，从而有助于鱼体的生长(Robert *et al*, 2017)。

3.2　酶解鱼浆蛋白影响卵形鲳鲹全鱼和肌肉营养成分的原因探讨

全鱼和肌肉粗蛋白含量直接反映饲料中蛋白质在机体中的沉积情况(Hernández *et al*, 2021)。本研究中，全鱼和肌肉粗蛋白含量随鱼粉替代水平的增加呈现上升趋势，表明酶解鱼浆蛋白能够增加鱼体对饲料中蛋白质的沉积效率。这可能是由于酶解鱼浆蛋白中足量的精氨酸和亮氨酸会激活 mTOR1，进而通过磷酸化使其下游靶蛋白核糖体 S6 蛋白激酶 1 (ribosomal protein S6 kinase1, S6K1)及真核细胞始动因子 4E 结合蛋白 1(eukaryotic initiation factor4E binding protein1, 4EBP1)激活，促进鱼体蛋白质的合成(Robert *et al*, 2017; Tang *et al*, 2013; Liang *et al*, 2016; Zhou *et al*, 2019)。与卵形鲳鲹结果不同的是，在红鳍东方鲀(Wei *et al*, 2021)、石斑鱼(*Epinephelus fuscoguttatus*)(Mamauag *et al*, 2017)和大菱鲆(Wei *et al*, 2016)的研究中，尽管酶解鱼浆蛋白替代饲料中的鱼粉会显著提高生长性能，但全鱼或肌肉的蛋白质含量都无显著变化，这可能与物种间对酶解鱼浆蛋白利用的差异所致。

3.3　酶解鱼浆蛋白影响卵形鲳鲹血清生化指标的原因探讨

血清总蛋白(TP)是血清中白蛋白(ALB)和球蛋白(GLO)之和。血清中 TP 含量可直接反映机体营养状态及蛋白质的吸收和代谢情况，其含量增加，表明蛋白质的合成代谢加强。GLO 是机体内参与免疫反应的蛋白质，其含量增加，表明机体的抗体水平增加(杨宏波等, 2015; Yu *et al*, 2020; Mu *et al*, 2015; Marono *et al*, 2017)。此外，正常情况下，谷丙转氨酶(ALT)与谷草转氨酶(AST)大量存在于肝细胞中，当肝细胞受损时其会被释放到血清中，造成血清 ALT 和 AST 活性升高(陈晨等，2010)。本研究中，卵形鲳鲹血清总蛋白(TP)和球蛋白(GLO)含量随鱼粉被酶解鱼浆蛋白替代水平的增加呈上升趋势，而谷丙转氨酶(ALT)和谷草转氨酶(AST)活性降低，说明复合蛋白搭配酶解鱼浆蛋白能够增强卵形鲳鲹的蛋白代谢水平和免疫功能，同时降低肝细胞受损风险。在一些海洋肉食性鱼类的研究发现，饲料中添加酶解鱼浆蛋白可以有效增强银鲳鱼对鳗弧菌(*Vibrio anguillarum*)(Tejpal *et al*, 2021)，尖吻鲈(*Lates calcarifer*)对海豚链球菌(*Streptococcus iniae*)(Siddik *et al*, 2018)和褐牙鲆(*Paralichthys olivaceus*)对爱德华氏菌(*Edwardsiella tarda*)(Khosravi *et al*, 2018)的抗菌力。以上结果提示，酶解鱼浆蛋白的使用可以增强养殖鱼类的抵抗力，更好地适应复杂的养殖环境，从而提高养殖效益。

3.4　复合蛋白配合酶解鱼浆蛋白对卵形鲳鲹消化酶活性无影响的原因探讨

鱼类的生长及饲料利用率与其肠道功能密切相关。其中，鱼类肠道酶的活性决定了其肠道的消化吸收功能，并能够直接反映鱼体获得和利用饲料营养成分的能力。本研究中，各替代组鱼体消化酶活性与鱼粉组鱼无显著差异，提示复合蛋白配合酶解鱼浆替代饲料中鱼粉未对金鲳消化功能产生负面影响。其与黄金鲫(*Carassius auratus*)中的研究结果相反，即利用豆粕替代饲料中 34%鱼粉时，肠道蛋白酶活性显著下降(王婧瑶等, 2021)。这可能是由于豆粕中存在的抗营养因子损伤消化道黏膜结构，进而降低肠腺体分泌消化酶的功能(Fuertes *et al*, 2013)。此外，抗营养因子会与消化酶形成稳定的化合物，使其失去酶解能力，导致酶活性被抑制(徐奇友等, 2006)。本研究所用的复合蛋白是一种酶解植物蛋白和动物蛋白按一定比例配合后的产品(Ma *et al*, 2020)，其抗营养因子被有效降解，从而替代饲料中鱼粉未对鱼体消化功能产生负面影响。

3.5　不同处理组饲料影响卵形鲳鲹肝脏和肌肉抗氧化指标的原因探讨

生物有机体在氧化代谢过程中不断产生自由基，具有强氧化性，机体对自由基的清除主要依赖于机体

完整的抗氧化防御系统的预防性或阻断性控制，从而保护机体组织和细胞免受自由基的氧化损伤(Su *et al*, 2018; Zhao *et al*, 2017、2020)。本研究中，鱼粉替代组肝脏过氧化氢酶(CAT)活性和总抗氧化能力(T-AOC)，肌肉超氧化物歧化酶(SOD)活性和总抗氧化能力(T-AOC)显著高于 D1 组。此外，肝脏 GSH、肌肉 CAT 活性随着鱼粉替代比例上升而显著增加，而肝脏和肌肉丙二醛(MDA)含量变化趋势与之相反。这些结果表明，复合蛋白搭配酶解鱼浆蛋白替代鱼粉能够增强卵形鲳鲹肝脏和肌肉组织的抗氧化能力。这可能由于复合蛋白中含一定量的植物蛋白，其富含的维生素 E、槲皮素和姜黄素具有增加抗氧化能力的作用(Mahmoud *et al*, 2017; Qiang *et al*, 2019)。此外，机体抗氧化能力与饲料中 n-3 PUFA 含量密切相关，一方面，n-3 PUFA 作为一种高不饱和脂肪酸，自身极易被氧化，机体会增强内生抗氧化系统来抵御这种情况，这是一种负反馈现象；另一方面，n-3 PUFA 的生理作用直接增加了人体抗氧化系统成分的活力(Li *et al*, 2020; Freneau *et al*, 2001)。因此，饲料中增加的 n-3 PUFA 会显著促进机体抗氧化能力，但本实验 D1 组饲料 n-3 PUFA 含量为最高，抗氧化值则最低，这可能与复合蛋白的使用有关，具体原因有待进一步研究。

4 结论

综上，本研究利用复合蛋白配合酶解鱼浆蛋白替代卵形鲳鲹饲料中 80%鱼粉后，其生长性能与 30%鱼粉组一样，且全鱼蛋白含量、肝脏和肌肉抗氧化能力增加，说明 80%鱼粉替代水平可行，使饲料中鱼粉用量降低至 6%。研究结果为研发高效低鱼粉卵形鲳鲹配合饲料提供了参考依据。

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Replacing Fishmeal with Fish Protein Hydrolysate and Terrestrial Complex Protein in the Compound Feed of *Trachinotus ovatus*

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Abstract Golden pompano *Trachinotus ovatus* is one of the most commercially important carnivorous marine species cultured in China. In recent years, the large-scale development of the aquaculture industry of *T. ovatus* has been rapid because of its fast growth rate and high flesh quality, and annual output has exceeded 100 000 tons. Currently, commercial formula feed for this fish still contains over 20%~30% of fishmeal (FM), the use of which precludes a sustainable aquaculture industry. As an alternative protein source, terrestrial compound proteins, which are a combination of several easily available and relatively low-priced terrestrial animal proteins and enzymatic hydrolysis or fermentation of terrestrial plant protein in a certain proportion, can alleviate the amino acid imbalance caused by a single alternative source. Terrestrial compound proteins are known to reduce the use of FM in marine carnivorous fish diets. In addition, fish protein hydrolysate is a new protein source that is processed from fish catches. In contrast to fish meal, it has an essential enzymatic hydrolysis step in the production process, so it contains more oligopeptides and free amino acids, which has been widely concerned in the industry. However, there are no reports on the application of fish protein hydrolysate in the feed of golden pompano. In order to decrease the use of FM in *T. ovatus* diets and evaluate the feasibility of substitution by terrestrial compound proteins and fish protein hydrolysate, this study was conducted to investigate the effects of replacing FM with compound protein and fish protein hydrolysate on growth performance, serum biochemical indexes, digestive enzyme activity, and tissue antioxidant capacity of *T. ovatus*. Four diets containing 42% crude protein and 12% crude fat were formulated, including D1 (control group), containing 30% fish meal; D2~D4 containing 14% terrestrial compound protein, 16%, 11%, and 6% fish meal, and 0%, 5%, and 10% fish protein hydrolysate, respectively. Methionine and lysine were added to the D2~D4 groups. *T. ovatus* juveniles (approximately 4 g mean initial weight) were bought from a local fish farm and then maintained for 2 months in two sea cages (2.0 m × 2.0 m × 2.0 m) at the coast near Nanao Marine Biology Station of Shantou University. A total of 360 fish (average body weight: [(7.28±0.10)g] were randomly distributed into 12 cages (1.0 m × 1.0 m × 1.5 m) and fed one of the four diets for 62 d. During the 62-d feeding trial, fish were fed to apparent satiation twice daily at 6:00 and 17:00, and the seawater temperature was 26.9℃~33.3℃, salinity was 27~33. The dead fish were collected over time and the day of death recorded. There were no significant differences in weight gain rate, specific growth rate, protein efficiency rate, feed conversion, feed rate, hepatosomatic index, viscerosomatic index, and condition factor, as well as pepsin, lipase, and amylase activity among all treatment groups (P>0.05). The whole-body protein content of groups D3 and D4 was significantly higher than those of groups D1 and D2 (P<0.05), and muscle fat content of groups D1~D3 was significantly higher than that of group D4 (P<0.05). The activity rate of aspartate aminotransferase in groups D2~D4 and alanine aminotransferase

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in groups D2 and D3 were significantly lower than those in group D1 (P<0.05). Total protein and globulin levels of groups D1 and D4 were significantly higher than those of groups D2 and D3 (P<0.05). Liver catalase activity, total antioxidant capacity, and muscle superoxide dismutase activity of groups D2~D4 were significantly higher than those of group D1 (P<0.05), but liver malondialdehyde content in groups D2~D4 was lower than that of group D1 (P<0.05). Overall, the whole-body protein content and tissue (liver and muscle) antioxidant capacity of group D4 were significantly higher than those of other groups, and no significant differences in growth performance were found between groups D4 and D1. The use of fish protein hydrolysate increased the level of protein metabolism and immune function of the fish and reduced the degree of liver cell damage. The results indicate that 14% terrestrial compound protein combined with 10% fish protein hydrolysate can effectively replace 80% fish meal in the diet of golden pompano when the amount of fish meal is reduced to 6%. This study is the first to investigate the feasibility of the application of fish protein hydrolysate in the formula feed of golden pompano, and the results will be relevant to the research for high-quality and inexpensive protein sources in the artificial formula feed of juvenile golden pompano.

Key words *Trachinotus ovatus*; Fish meal replacement; Fish protein hydrolysate; Growth performance; Antioxidant enzyme activity