



河南师范大学

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Full length article

Effects of dietary *Panax notoginseng* extract on growth performance, fish composition, immune responses, intestinal histology and immune related genes expression of hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) fed high lipid diets



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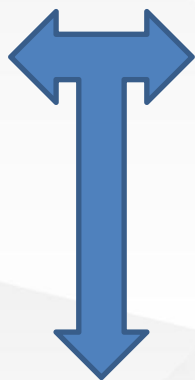
研究背景-杂交石斑鱼



棕点石斑鱼 ♀
生长缓慢但寿命长，抗病性强

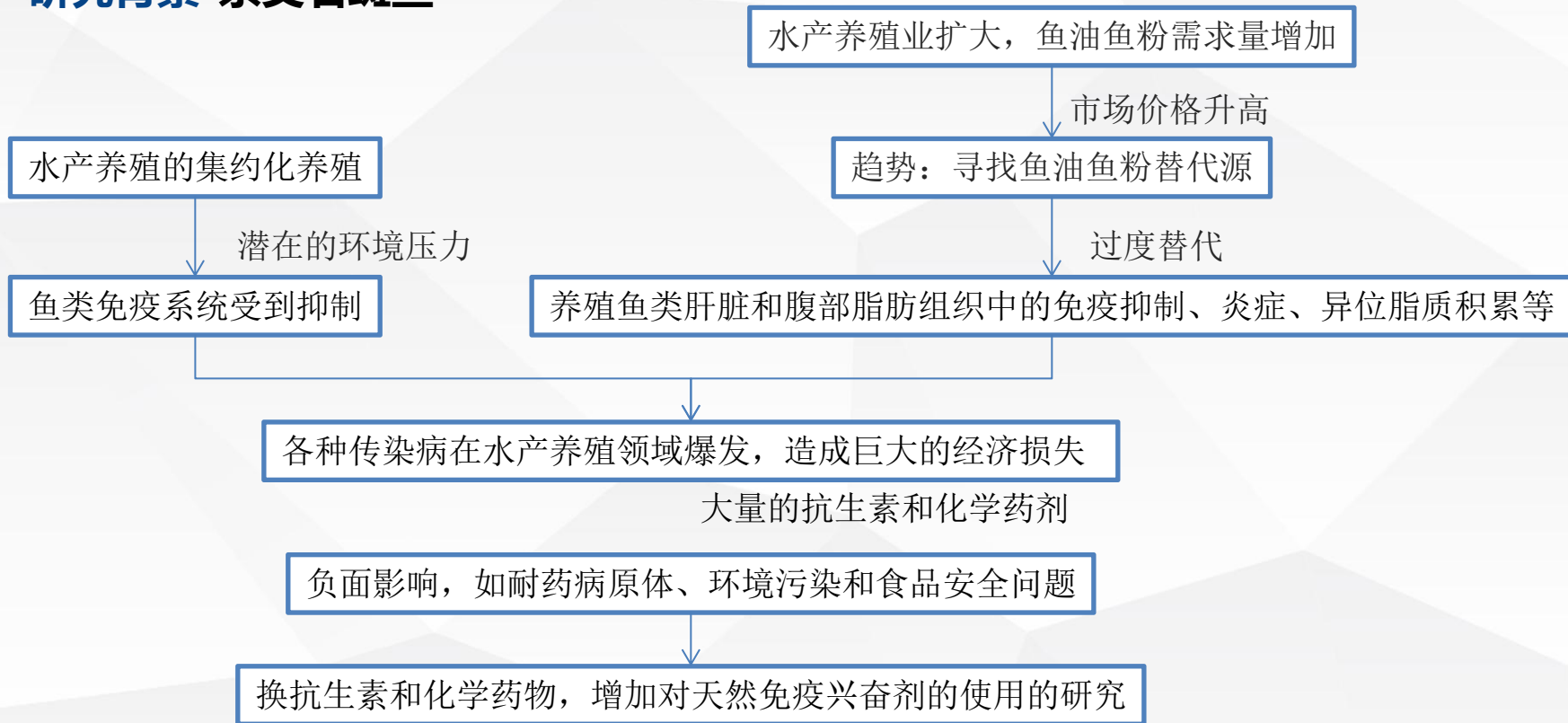


鞍带石斑鱼 ♂
生长快速



杂交石斑鱼
肉质好、生长快、抗病性强

研究背景-杂交石斑鱼



研究背景-三七

三七，俗称三七，是一种著名的中药，属于山茱萸科。

从三七中可分离得到了各种化合物，如皂苷、聚乙炔、植物甾醇、黄酮类化合物和多糖。**三七皂苷（PNE）**是从三七根中提取的一种成分，是由二十多种达玛烷类皂苷的混合物，包括三七皂苷R1、人参皂苷Rg1、Rd、Re、Rb1组成。三七多年来被广泛应用于中药止血、止血、活血、散瘀、消肿止痛等方面。

近年来研究表明，三七具有抗氧化、抗炎、降血脂、保肝、免疫调节等多种有益作用。迄今为止，还没有关于在石斑鱼饲料中使用三七提取物(PNE)作为补充的报道。

本文研究了PNE对喂食高脂饲料的杂交石斑鱼生长性能、血浆生化参数、鱼的组成、免疫反应、肠道组织学和免疫相关基因表达的影响，这将促进高脂饲料在高脂血症养殖中的应用。

材料与方法

◆ 饲料

◆ 实验动物和取样

◆ 生长性能

◆ 常规生长指标

◆ 生化分析

◆ 免疫分析

◆ 肠道组成分析

◆ RNA提取和基因表达分析

◆ 数据分析

表 1. 饲料配方

饲料	PNE添加量 (g/kg)
Diet 1	0
Diet 2	0.50
Diet 3	1.00
Diet 4	2.00
Diet 5	4.00
Diet 6	10.00

Table 1

Composition and nutrient levels of basal diets (g kg^{-1}).

Ingredients	Diet 1
Fish meal	450
Soybean meal	130
Flour	237.4
Beer yeast powder	50
Fish oil	50
Soybean oil	50
Lecithin	10
Vitamin premix ¹	2
Mineral premix ²	5
Choline chloride (50%)	5
Antioxidant	0.1
Vitamin C	0.5
Monocalcium phosphate	10
Nutrient levels (%)	
Moisture	5.12
Crude protein	46.63
Crude lipid	14.81
Ash	10.36

结果

表3: 饲料中添加PNE对杂交石斑鱼生长性能、饲料利用率和体指数的影响

Table 3

Effects of dietary *Panax notoginseng* extract on growth performance, feed utilization and body indices of hybrid grouper.

Diets (g kg ⁻¹)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	0	0.50	1.00	2.00	4.00	10.00
IBW (g)	124.46 ± 1.57	121.67 ± 3.51	121.61 ± 1.39	124.69 ± 2.10	123.16 ± 1.52	122.96 ± 2.33
FBW (g)	303.53 ± 3.04 ^a	317.06 ± 2.38 ^{bc}	309.82 ± 4.77 ^{ab}	324.62 ± 2.96 ^{cd}	334.91 ± 10.32 ^d	328.88 ± 6.82 ^{cd}
WGR (%)	143.92 ± 4.00 ^a	166.80 ± 3.37 ^c	154.81 ± 6.79 ^b	157.53 ± 2.67 ^{bc}	167.10 ± 8.30 ^c	162.55 ± 4.57 ^{bc}
SGR (%/d)	1.59 ± 0.03 ^a	1.75 ± 0.03 ^c	1.67 ± 0.04 ^b	1.69 ± 0.02 ^{bc}	1.75 ± 0.06 ^c	1.76 ± 0.06 ^c
FER (%)	0.91 ± 0.01 ^a	1.03 ± 0.01 ^c	1.06 ± 0.05 ^c	1.02 ± 0.05 ^c	1.00 ± 0.03 ^{bc}	0.93 ± 0.02 ^{ab}
FI (g/fish)	196.15 ± 2.47 ^{ab}	208.76 ± 18.85 ^{abc}	187.51 ± 13.46 ^a	192.40 ± 6.89 ^{ab}	213.89 ± 12.42 ^{bc}	221.54 ± 4.42 ^c
PER	2.06 ± 0.02 ^a	2.34 ± 0.02 ^b	2.39 ± 0.11 ^b	2.33 ± 0.12 ^b	2.28 ± 0.06 ^b	2.11 ± 0.05 ^a
PDR (%)	42.73 ± 3.04 ^a	51.70 ± 3.71 ^c	47.28 ± 0.99 ^{abc}	50.01 ± 2.13 ^{bc}	42.01 ± 0.90 ^a	44.66 ± 2.35 ^{ab}
CF (g/cm ³)	2.70 ± 0.26	2.66 ± 0.07	2.68 ± 0.24	2.67 ± 0.12	2.63 ± 0.16	2.78 ± 0.17
VSI (%)	9.35 ± 0.64 ^b	8.84 ± 1.19 ^{ab}	8.72 ± 0.59 ^{ab}	8.65 ± 0.94 ^{ab}	8.19 ± 0.59 ^a	8.33 ± 0.72 ^{ab}
HSI (%)	2.33 ± 0.40	2.39 ± 0.65	2.52 ± 0.27	2.24 ± 0.36	2.32 ± 0.32	1.95 ± 0.31
SI (%)	0.27 ± 0.07	0.28 ± 0.10	0.32 ± 0.10	0.31 ± 0.08	0.20 ± 0.03	0.24 ± 0.16
AFP (%)	2.28 ± 0.63 ^b	2.01 ± 0.46 ^{ab}	1.67 ± 0.39 ^{ab}	1.77 ± 0.52 ^{ab}	1.52 ± 0.55 ^a	2.00 ± 0.43 ^{ab}
RGL (%)	120.23 ± 9.35 ^a	140.70 ± 12.53 ^b	132.73 ± 10.06 ^{ab}	142.91 ± 16.18 ^b	129.82 ± 12.43 ^{ab}	134.17 ± 11.91 ^{ab}
Survival (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

注: 体重增加率比生长速率(SGR)饲料效率比(FER)蛋白质效率比(PER)蛋白质沉积率(PDR)条件因素(CF)Viscerosomatic指数(VSI %) Hepatosomatic指数(HSI)脾脏指数(SI,%)腹部脂肪百分比(AFR)相对肠道长度(RGL)存活率(Survival)

表4: 饲料中添加PNE对杂交石斑鱼全身、肌肉和肝成分的影响

Table 4
Effects of dietary *Panax notoginseng* extract on whole body, muscle and liver proximate composition of hybrid grouper.

Diets (g kg ⁻¹)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	0	0.50	1.00	2.00	4.00	10.00
Whole body						
Moisture	66.22 ± 1.27	67.84 ± 1.26	67.65 ± 1.08	66.98 ± 1.64	67.84 ± 1.51	65.97 ± 0.68
Crude protein	56.18 ± 0.38 ^b	57.10 ± 0.74 ^c	57.13 ± 0.50 ^c	57.13 ± 0.18 ^c	55.79 ± 0.06 ^{ab}	55.21 ± 0.47 ^a
Crude lipid	18.86 ± 0.01 ^a	21.81 ± 0.85 ^b	22.26 ± 1.58 ^b	22.32 ± 0.62 ^b	26.98 ± 0.89 ^c	22.62 ± 0.86 ^b
Ash	13.90 ± 0.53 ^a	14.40 ± 0.45 ^{ab}	14.57 ± 0.46 ^{ab}	15.17 ± 0.39 ^b	14.79 ± 0.29 ^b	15.00 ± 0.39 ^b
Muscle						
Moisture	74.90 ± 0.20 ^a	75.12 ± 0.04 ^{ab}	75.54 ± 0.22 ^b	75.49 ± 0.37 ^{ab}	75.24 ± 0.17 ^{ab}	75.17 ± 0.44 ^{ab}
Crude protein	78.18 ± 0.63 ^a	81.97 ± 0.51 ^b	82.08 ± 1.64 ^b	81.99 ± 1.61 ^b	81.87 ± 1.14 ^b	82.02 ± 1.36 ^b
Crude lipid	11.10 ± 0.78 ^c	11.06 ± 0.88 ^c	7.66 ± 0.76 ^a	9.49 ± 0.39 ^b	8.56 ± 0.52 ^{ab}	8.70 ± 0.11 ^{ab}
Ash	5.50 ± 0.16	5.55 ± 0.05	5.70 ± 0.18	5.65 ± 0.02	5.73 ± 0.02	5.66 ± 0.08
Liver						
Moisture	58.46 ± 1.55	54.92 ± 3.50	58.31 ± 0.95	58.98 ± 1.23	58.17 ± 2.36	55.44 ± 2.25
Crude protein	20.30 ± 0.38 ^b	18.99 ± 0.51 ^a	19.36 ± 0.11 ^a	20.84 ± 0.14 ^c	20.27 ± 0.16 ^b	20.66 ± 0.07 ^{bc}
Crude lipid	24.28 ± 0.28 ^b	25.84 ± 0.50 ^c	24.01 ± 0.42 ^b	24.52 ± 0.26 ^b	22.89 ± 0.13 ^a	25.42 ± 0.26 ^c

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < 0.05$).

结果

表5: 饲料中添加PNE对杂交石斑鱼血浆生化指标的影响

Table 5
Effects of dietary *Panax notoginseng* extract on plasma biochemical indices of hybrid grouper.

Diets (g kg ⁻¹)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	0	0.50	1.00	2.00	4.00	10.00
CHO/(mmol L ⁻¹)	3.11 ± 0.06 ^D	2.49 ± 0.16 ^a	2.51 ± 0.11 ^a	2.51 ± 0.15 ^a	2.57 ± 0.37 ^a	2.67 ± 0.06 ^a
TG/(mmol L ⁻¹)	3.36 ± 0.18 ^b	2.78 ± 0.10 ^a	2.49 ± 0.28 ^a	2.67 ± 0.06 ^a	2.35 ± 0.35 ^a	2.77 ± 0.28 ^a
GLU/(mmol L ⁻¹)	2.73 ± 0.06 ^{bc}	2.58 ± 0.14 ^{bc}	2.32 ± 0.37 ^b	2.38 ± 0.52 ^b	1.46 ± 0.37 ^a	3.25 ± 0.23 ^c
TP/(g L ⁻¹)	41.80 ± 0.71 ^a	47.95 ± 1.91 ^D	46.40 ± 0.96 ^D	47.95 ± 0.64 ^D	45.35 ± 3.32 ^{abD}	47.45 ± 0.78 ^D
ALP/(U L ⁻¹)	85.00 ± 8.49 ^a	108.50 ± 0.71 ^{abc}	129.00 ± 22.63 ^{bc}	96.50 ± 17.68 ^{ab}	129.50 ± 7.78 ^c	97.50 ± 2.12 ^{abc}
LDL-C/(mmol L ⁻¹)	0.49 ± 0.02 ^c	0.15 ± 0.04 ^a	0.36 ± 0.04 ^b	0.17 ± 0.02 ^a	0.26 ± 0.02 ^a	0.59 ± 0.08 ^d
HDL-C/(mmol L ⁻¹)	0.13 ± 0.01 ^{ab}	0.17 ± 0.03 ^c	0.16 ± 0.02 ^{bc}	0.14 ± 0.01 ^{abc}	0.13 ± 0.00 ^{abc}	0.11 ± 0.01 ^a

注: 总胆固醇 (CHO) 甘油三酯(TG)血糖(GLU)总蛋白 (TP)
碱性磷酸酶(ALP)低密度脂蛋白 (LDL-C) 高密度脂蛋白 (HDL-C)

表6-7: 饲料中添加PNE对杂交石斑鱼免疫指标的影响

Table 6

Effect of dietary *Panax notoginseng* extract on plasma immune parameters of hybrid grouper.

Diets (g kg ⁻¹)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	0	0.50	1.00	2.00	4.00	10.00
C3 (ug/ml)	47.18 ± 1.86 ^a	62.55 ± 1.07 ^b	74.08 ± 0.27 ^c	66.09 ± 1.48 ^b	72.39 ± 2.81 ^c	71.47 ± 4.40 ^c
C4 (ug/ml)	24.28 ± 1.46 ^a	57.91 ± 3.10 ^b	68.39 ± 5.19 ^c	71.31 ± 2.35 ^c	68.88 ± 1.46 ^c	52.79 ± 4.39 ^b
IgM (ng/ml)	1670.7 ± 12.3 ^a	1682.3 ± 35.1 ^a	1796.7 ± 104.4 ^{abc}	1722.9 ± 36.9 ^{ab}	1827.1 ± 61.4 ^{bc}	1866.3 ± 307 ^c

C3: component 3; C4: component 4; IgM: immunoglobulin M.

Values are means ± SD of three replications. Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 7

Effect of dietary *Panax notoginseng* extract on hepatic antioxidant ability of hybrid grouper.

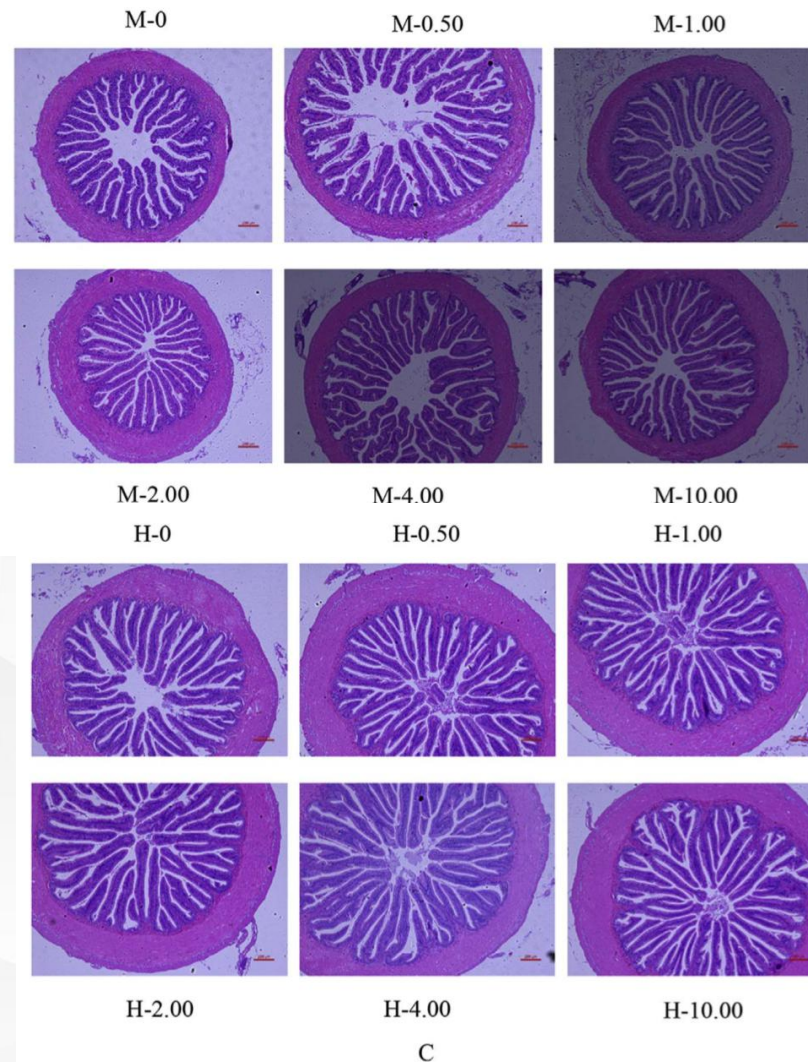
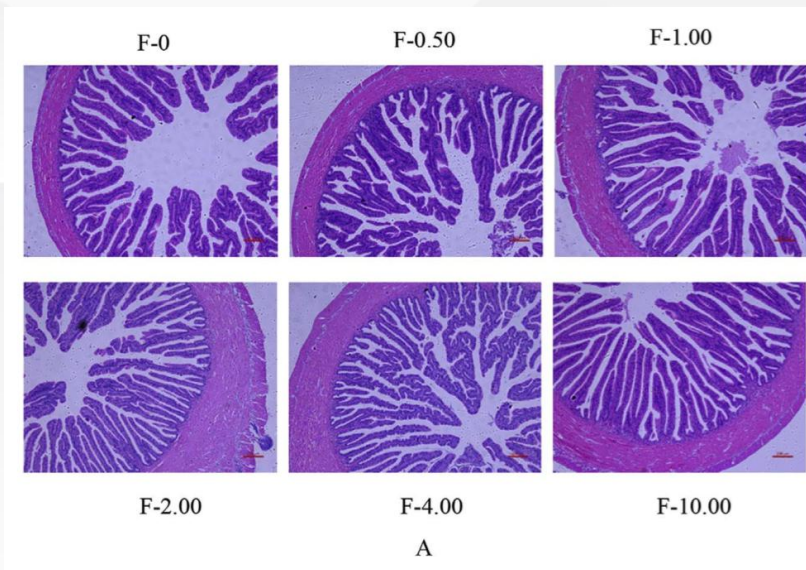
Diets (g kg ⁻¹)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	0	0.50	1.00	2.00	4.00	10.00
SOD (U/g)	1050.5 ± 39.9 ^a	1243.9 ± 65.9 ^b	1319.5 ± 50.1 ^b	1279.0 ± 107.6 ^b	1344.1 ± 132.1 ^b	1289.6 ± 68.8 ^b
CAT (U/g)	515.4 ± 12.5 ^a	523.5 ± 26.0 ^a	641.7 ± 28.2 ^{ab}	658.3 ± 14.1 ^b	554.5 ± 101.1 ^{ab}	522.0 ± 15.7 ^a
MDA (nmol/g)	112.8 ± 5.9 ^b	109.9 ± 9.4 ^b	100.3 ± 0.0 ^{ab}	104.3 ± 7.4 ^{ab}	94.2 ± 0.3 ^a	113.7 ± 3.2 ^b
T-AOC (U/g)	137.1 ± 1.4 ^a	152.3 ± 12.2 ^b	153.0 ± 1.4 ^b	167.2 ± 1.1 ^c	168.1 ± 0.7 ^c	153.2 ± 11.2 ^b

SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; T-AOC: total antioxidant capacity.

Values are means ± SD of three replications. Means in the same row with different superscripts are significantly different ($P < 0.05$).

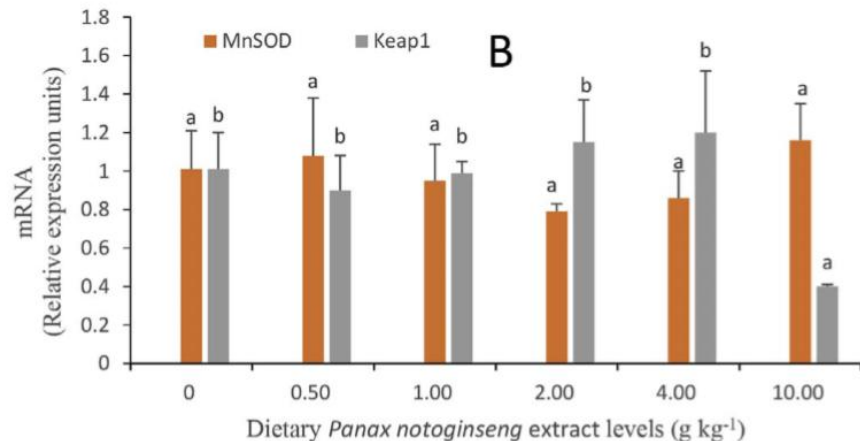
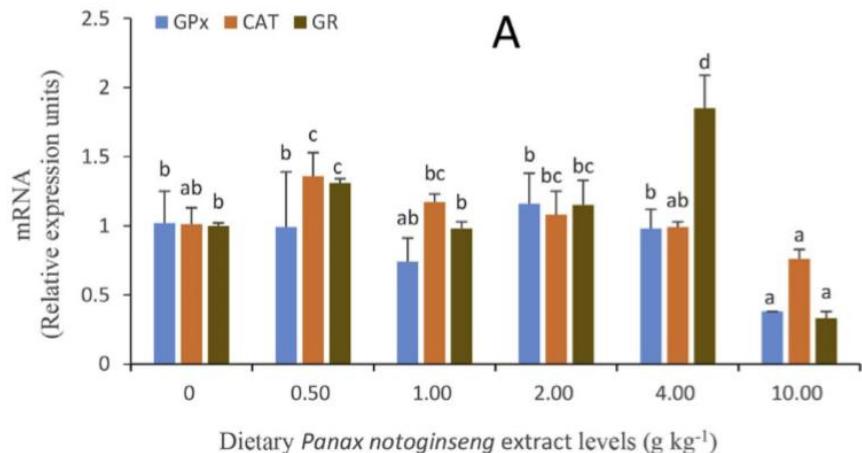
结果

图1: 前肠(A),中肠(B)和后肠(C)



结果

图2：饲料中添加PNE对杂交石斑鱼的头肾的相对表达



本研究发现，饲料中添加PNE的鱼的CAT和GR mRNA水平明显高于对照组($P < 0.05$)，但（除D6外）对Keap1 mRNA水平无显著影响($P > 0.05$)。

注：过氧化氢酶(CAT)、谷胱甘肽过氧化物酶(GPx)、谷胱甘肽还原酶(GR)、锰超氧化物歧化酶(MnSOD)和kelch - ECHassociated protein 1 (Keap1)

结果

图3: 饲料中添加PNE对杂交石斑鱼的头肾的相对表达

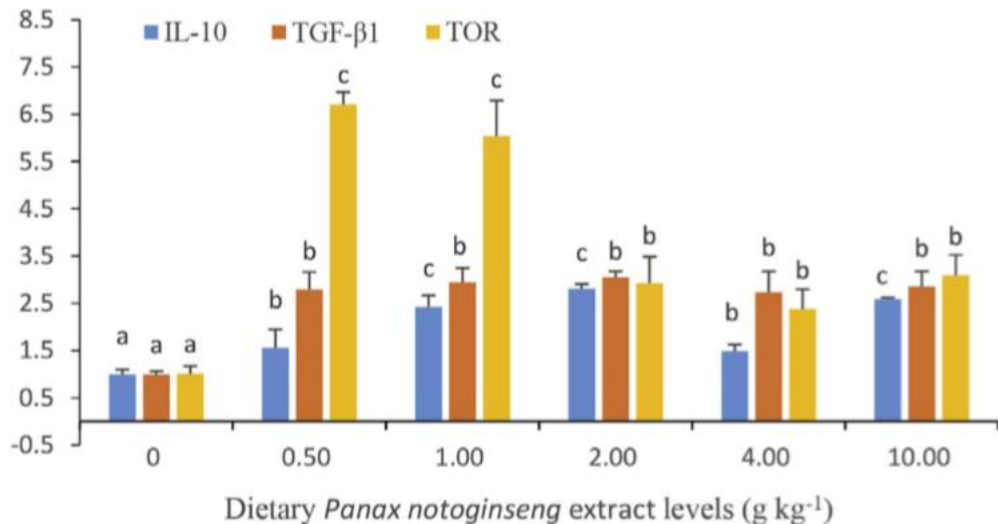


Fig. 3. Relative expression of interleukin 10 (IL-10), transforming growth factor β 1 (TGF- β 1) and target of rapamycin (TOR) in the head kidney of hybrid grouper fed diets supplemented with PNE. Values are means, error bars indicate S.D., and different letters above a bar denote the significant difference between treatments ($P < 0.05$).

与对照组相比, 饲料中添加PNE的鱼的 IL - 10, TGF- β 1和 TOR的表达明显增加。

注: 白介素10(IL - 10),转化生长因子- β (transforming growth factor- β ,TGF- β)雷帕霉素靶点(TOR)

结果

图4：饲料中添加PNE对杂交石斑鱼的头肾的相对表达

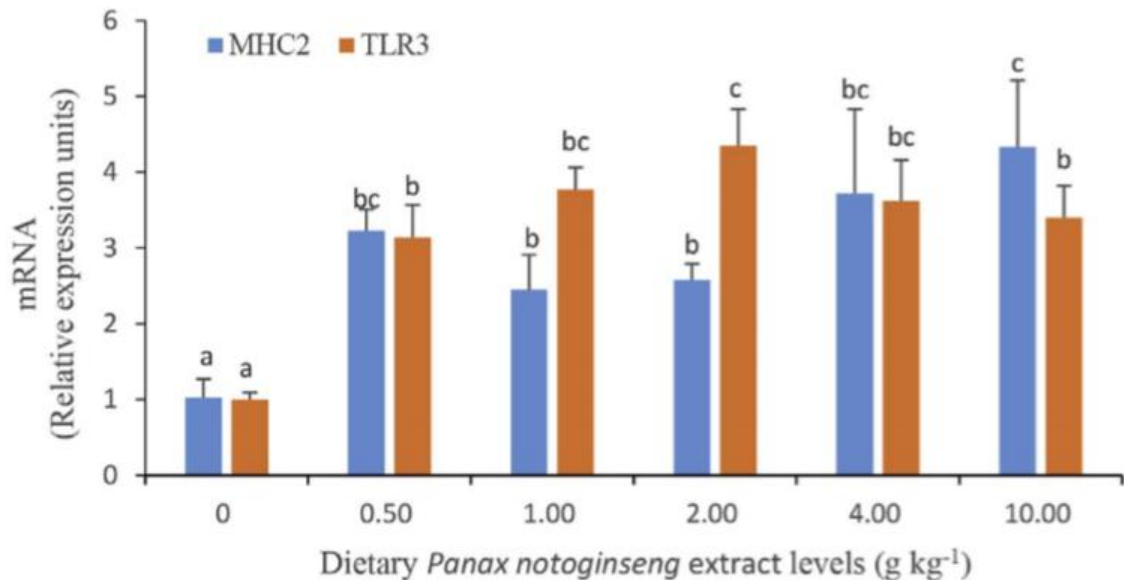


Fig. 4. Relative expression of major histocompatibility complex 2 (MHC2) and toll-like receptor 3 (TLR3) in the head kidney of hybrid grouper fed diets supplemented with PNE. Values are means, error bars indicate S.D., and different letters above a bar denote the significant difference between treatments ($P < 0.05$).

与对照组相比，饲料中添加PNE显著提高鱼头肾组织MHC2和TLR3 mRNA水平($P < 0.05$)。

注：主要组织相容性复合体 (major histocompatibility complex, MHC)Toll样受体3(Toll-like receptor 3, TLR3)

结果

图4：饲料中添加PNE对杂交石斑鱼的头肾的相对表达

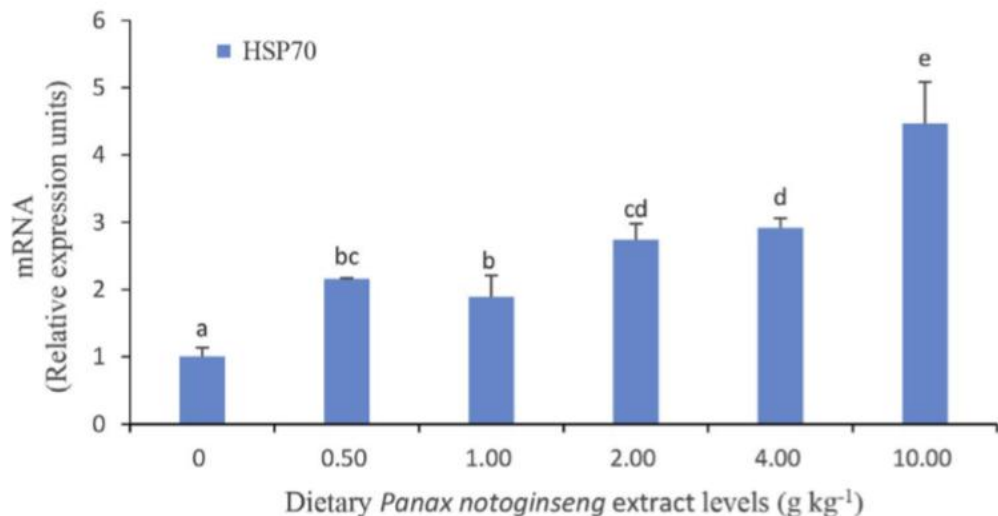


Fig. 5. Relative expression of heat shock protein 70 (HSP70) in the head kidney of hybrid grouper fed diets supplemented with PNE. Values are means, error bars indicate S.D., and different letters above a bar denote the significant difference between treatments ($P < 0.05$).

与对照组相比，饲料中添加PNE显著提高鱼头肾组织HSP70基因mRNA水平($P < 0.05$)。
注：HSP70是用于协同免疫作用的一种蛋白质

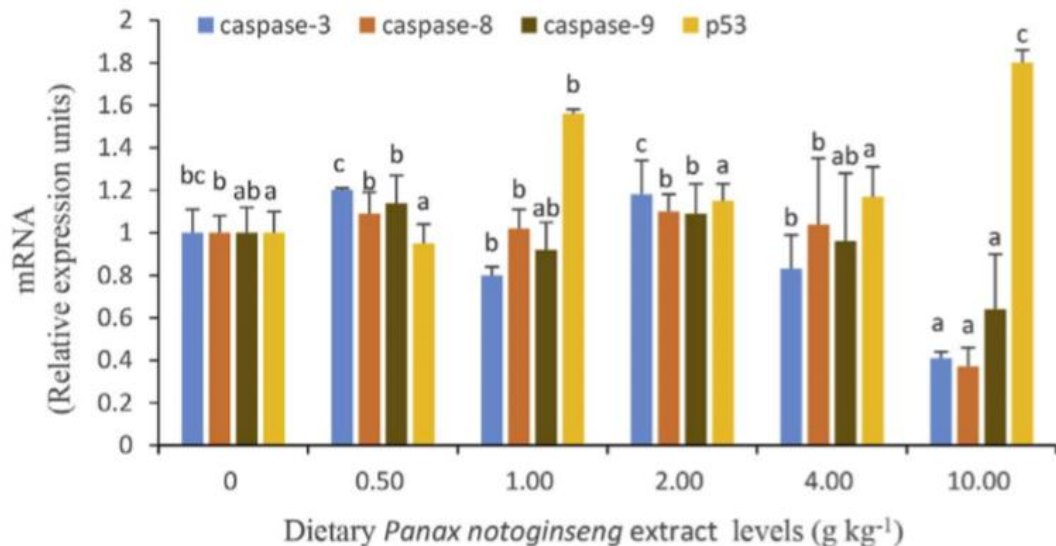


Fig. 6. Relative expression of caspase-3, caspase-8, caspase-9 and p53 in the head kidney of hybrid grouper fed diets supplemented with PNE. Values are means, error bars indicate S.D., and different letters above a bar denote the significant difference between treatments ($P < 0.05$).

与对照组相比, 饲料中添加PNE (除D6以外) 对头肾caspase-3、caspase-8和p53的表达无显著影响($P > 0.05$)。饲料中添加PNE对caspase-9表达无显著影响($P > 0.05$)。饲料中添加PNE (除D3、D6以外) 对头肾p53的表达无显著影响($P > 0.05$)。



结论与收获

01

合适的饲料添加剂，可提高生长性能，刺激免疫反应，保护肝、胃、肠的功能，是在低蛋白高脂饲料中成功替代鱼粉和鱼油的关键。在饲料中使用功能性添加剂是最有前途的策略之一，它不仅能提高鱼类的生长性能和饲料利用率，而且对鱼类的健康有益。

02

饲料中添加PNE对鱼类生长性能和饲料利用均有促进作用，降血脂，增强免疫力，提高肝脏的抗氧化能力，改善肠道形态，并导致饲料利用率提高，粘膜上皮的健康状况得到改善，且其阻断细菌感染的能力提高了。

03

饲料中添加PNE可上调杂交石斑鱼头肾中抗氧化基因的表达(CAT、GPx和GR)、免疫相关基因(MHC2和TLR3)和抗炎细胞因子(IL-10和TGF- β 1)的表达。考虑到生长性能、免疫反应和经济成本等因素,饲料中添加量PNE 0.5 g/公斤最佳。

04

收获：之前未关注过切片，免疫等相关指标的检测与分析，本文章对我的实验进展具有指导作用。



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THANKS

敬请各位老师同学批评指正
