行政院國家科學委員會專題研究計畫 成果報告

多磷酸態維生素C與石斑魚稚魚緊迫反應及疾病抵抗之關係

研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2313-B-041-003-
執 行 期 間 ：95年08月01日至96年07月31日
執 行 單 位 ：嘉南藥理科技大學生活應用與保健系

計 畫 主 持 人：林美芳

計畫參與人員：大專生：陳馨儀、湯悅琦

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96年10月31日

行政院國家科學委員會補助專題研究計□成果報告
□期中進度報告

（計畫名稱）

多磷酸態維生素 C 與石斑魚稚魚緊迫反應及疾病抵抗之關係

計畫類別：□ 個別型計畫　　□ 整合型計畫

計畫編號：NSC 95－2313－B－041－003－

執行期間：95 年 8 月 1 日至 96 年 7 月 31 日

計畫主持人：林美芳助理教授

共同主持人：

計畫參與人員： 陳馨儀

成果報告類型(依經費核定清單規定繳交)：□精簡報告　□完整報告

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢

□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：嘉南藥理科技大學生活應用與保健系

中　華　民　國　96 年 7 月 31 日

行政院國家科學委員會專題研究計畫成果報告

多磷酸態維生素 C 與石斑魚稚魚緊迫反應及疾病抵抗之關係

Dietary L-ascorbyl-2-polyphosphate on stress response and disease resistance in juvenile grouper, *Epinephelus malabaricus*

計畫編號：NSC 95－2313－B－041－003－

執行期限：95 年 08 月 01 日至 96 年 7 月 31 日

主持人：林美芳　嘉南藥理科技大學生活應用與保健系

e-mail : mayfang@mail.chna.edu.tw

計畫參與人員：陳馨儀　嘉南藥理科技大學生活應用與保健系

一、中文摘要

研究一 本研究為探討石斑魚餵食低量(1/2 適量)、適量、五倍適量及十倍適量之 C2PP(L-ascorbyl-2-polyphosphate) (分析值為 8, 14, 85 及 166 mg AA/kg diet)經促腎上腺皮質激素(adrenocorticotropic hormone, ACTH)刺激對其血漿可體松(cortisol)及組織維生素 C 含量之影響。實驗飼料總共 4 組，分別餵予石斑魚稚魚(平均初重：25.13 ± 2.29 g)，每組 10 尾魚，每組二重複。餵食實驗飼料四週魚體秤重後，將各餵食組之 20 尾石斑魚集合在一起，分成注射 ACTH 及生理食鹽水兩組，每組二重複，每組 5 尾石斑魚。於注射 ACTH 或生理食鹽水 30 分鐘後進行抽血，並取魚隻頭腎、後腎及肝臟組織測維生素 C 含量。無論是注射 ACTH 或生理食鹽水，石斑魚血漿 cortisol 濃度以餵予 166 mg AA/kg 顯著低於餵食 8 mg AA/kg diet 組。血漿葡萄糖濃度，無論是注射 ACTH 或生理食鹽水，以餵食 85 mg AA/kg 顯著低於餵食 8 mg AA/kg 各組。無論是注射 ACTH 或生理食鹽水，頭腎、後腎或肝臟維生素 C 濃度均隨飼料中 C2PP 時含量增加而上升。無論飼料中添加何種劑量之 C2PP，注射 ACTH 組，血漿 cortisol 及葡萄糖濃度均較注射生理食鹽水組顯著增高，且其頭腎維生素 C 濃度顯著低於注射生理食鹽水組。體外注射促腎上腺皮質激素會促進石斑魚腎間組織釋放緊迫激素 cortisol，且造成石斑魚頭腎維生素 C 含量下降，顯示石斑魚頭腎維生素 C 與緊迫激素 cortisol 生成具有相關性。研究二 本研究為探討餵食低量(1/2 適量)、適量、五倍適量及十倍適量之 C2PP (分析值分別為 9, 17, 88 及 173 mg AA/kg diet)對石斑魚低溫緊迫之影響。實驗飼料總共 4 組，分別餵予石斑魚稚魚(平均初重：5.40 ± 0.01 g)，每組 12 尾魚，每組三重複。餵食實驗飼料八週魚體秤重後，將各餵食組之 36 尾石斑魚集合在一起，分成非緊迫刺激(non-stress, NS)及緊迫刺激(stress, S)兩組，每組二重複，每組 9 尾石斑魚，NS 組注射弧菌(*Vibro carchariae*)感染，S 組則先以低溫緊迫刺激後，再進行弧菌感染試驗。連續紀錄七天各組魚隻累積之死亡率，並測定魚體之緊迫及免疫反應指標。石斑魚受緊迫刺激及弧菌感染之死亡率以餵食≦17 mg AA/kg，顯著高於≧88 mg AA/kg 各組。肝臟中維生素 C 濃度及血漿溶菌酶活性隨飼料 C2PP 含量增加而上升。超氧陰離子產率以餵予 173 mg AA/kg 顯著高於餵食≦17 mg AA/kg 各組。血漿 cortisol 含量以餵予 173 mg AA/kg 顯著低於餵予 9mg AA/kg 組。無論飼料中添加何種含量之 C2PP，緊迫刺激均會造成石斑魚血漿 cortisol 及葡萄糖濃度增加，溶菌酶活性及超氧陰離子產率下降。石斑魚餵食五倍適量或十倍適量之 C2PP 能降低弧菌感染死亡率及增進免疫反應。餵食十倍適量之 C2PP 能降低緊迫刺激對魚體之免疫抑制作用。

關鍵詞：石斑魚、多磷酸態維生素 C、促腎上腺皮質激素、免疫反應

Abstract

In Exp 1, Basal diet supplemented with either 50 mg (1/2 X adequate), 100 mg (adequate), 500 mg (5X adequate) or 1000 mg (10X adequate) C2PP (L-ascorbyl-2-polyphosphate, C2PP；25% vitamin C activity) per kg diet, analyzed values were 8, 14, 85 and 166 mg ascorbate (AA)/kg diet, respectively, for total of 4 experimental diets, each diet was fed to duplicate groups of grouper (mean initial weight: 25.13 ± 2.29 g, 10 fish per group) for four weeks. After final weighing, fish in each treatment pooled and divided into two sub-groups with duplicate per sub-group, each sub-group was subjected to intraperitioneal injection (i.p.) with adrenocorticotropic hormone (ACTH) or saline. Blood samples were taken from each sub-group 30 min after injection, pooled, and head kidney, trunk kidney and hepatic vitamin C concentrations were monitored. Regardless of injection with ACTH or saline, plasma cortisol concentration in fish fed the diet with 166 mg AA/kg diet was lower ($P<0.05$) than fish fed the diet with 8 mg AA/kg diet. Fish fed diets with 166 mg and 85 mg AA/kg diet had lower plasma glucose concentration than that fish fed the diet with 8 mg AA/kg diet. Head kidney, trunk kidney and hepatic vitamin C concentrations generally increased

as dietary vitamin C supplementation level increased. Regardless of vitamin C ingestion level, increased plasma cortisol and glucose concentrations and reduced head kidney vitamin C concentration were observed in fish injected with ATCH as compared to those fish injected with saline. These results revealed that ACTH treatment caused cortisol release and reduced head kidney vitamin C concentration in grouper, suggesting that dietary vitamin C ingestion level involves in stress response in grouper. In Exp. 2, Basal diet was supplemented with 1/2 X adequate (50 mg), adequate (100 mg), 5X adequate (500mg)and 10X adequate (1000 mg) L-ascorbyl-2-polyphosphate (analyzed values were 9, 17, 88 and 173 mg ascorbic acid equivalents kg^{-1} diet, respectively), for total of 4 experimental diets. Each diet was fed to triplicate groups of grouper (mean initial weight: 5.40 ± 0.01 g, 12 fish per group) in each aquarium for eight weeks. After final weighing, fish in each treatment pooled and divided into two groups with duplicate per group subjected to either non-stress or extreme temperature stress, then fish in both non-stress and stress groups were intraperitoneal injected (i.p.) with *Vibrio carchariae*. The cumulative mortality of fish fed diets with ≦17 mg AA/kg were higher than fish fed diets with ≧88 mg AA/kg after challenged with *V. carchariae*. Fish fed diet with 173 mg AA/kg had higher head kidney respiratory burst activity than that fish fed diets with ≦17 mg AA/kg. Plasma cortisol was lower in fish fed diet with 173 AA/kg than that in fish fed diet with 9 mg AA/kg Regardless of C2PP ingestion level, increased plasma cortisol and glucose concentrations and reduced lysozyme activity and head kidney respiratory burst activity were observed in fish with extreme temperature stress as compared to those fish with no stress. In summary, these results suggested that dietary 5X and 10X adequate C2PP supplementation enhanced the immune responses and disease resistance in grouper. Fish fed 10X adequate C2PP reduced the immunosuppression effect caused by the extreme temperature stress.

Keywords: Grouper; L-ascorbyl-2-polyphosphate; Adrenocorticotropic hormone; Immune response

二、緣由與目的

石斑魚仍屬於新興養殖項目，營養需求認知仍有限，以瑪拉巴石斑(*Epinephelus malabaricus*)為例，其已被探討之研究包括蛋白質需求量[1,2]、碳水化合物之利用性[3,4]、EPA/DHA 之利用性[5] 維生素 C[6,7,8]及維生素 E[9]需求量。

陸上動物的腎上腺皮質軸是緊迫反應產生激素之處，也具有高量的維生素 C。當動物受外界壓力刺激時，體內促腎上腺激素會釋放，並促進 cortisol 的生成，然而在另一方面腎上腺的維生素 C 會被消耗，顯示維生素 C 與腎皮質類固醇激素生成有關[10]。不同魚種其腎臟呈現分離或合一，兩者呈現分離者可分為頭腎(head kidney)及後腎(trunk kidney)二大部分，呈現合一者分為腎臟前部(anterior kidney)及腎臟後部(posterior kidney)。魚類腎臟為主要蓄積維生素 C 之處，腎臟相連者顯示腎臟前部蓄積的維生素 C 濃度比腎臟後部來的多，乃因腎臟前部被認為是腎上腺組織所在的位置[11]，所以腎臟前部被當作是評估魚類維生素 C 蓄積之指器官[12,13]。吳郭魚[14]或虹鱒[15]之體外研究顯示，ACTH 會作用於間腎組織而刺激 cortisol 釋放。注射外生性 ACTH 於鱸魚[16]、虹鱒[14]或高首鱚[17]，亦證實會造成血漿 cortisol 之上升。鮭鱒魚類經體外 ACTH 注射後，其血漿 cortisol 濃度上升外，魚體腎臟前部維生素 C 濃度則呈現下降[18]。魚類頭腎不但具有高量的維生素 C，亦是製造 cortisol 的組織[15, 19]，當石斑魚注射 ACTH 後，魚體腎臟維生素 C 與血漿 cortisol 生成是否具有相關性，是一值得探討的的課題。

緊迫反應會造成魚體血漿 cortisol 含量上升[20]，亦會造成魚體免疫抑制作用[20]及減少對疾病的抵抗力[21, 22]。大西洋鮭的研究中指出，水中鹽度及溫度變化造成魚體血漿 cortisol 濃度上升，當飼料中提供 1,000 mg C2MP/kg diet 可降低魚體血漿 cortisol 的生成，高劑量 C2MP 可以改善緊迫因子之影響[23]。Wedemeyer (1969) [18]以溫度變化(3℃及 17 ℃)刺激鮭鱒魚類，當降低溫度至 3 ℃或提升溫度至 17℃，造成腎臟維生素 C 降低。由上述研究結果顯示，魚類遭受緊迫作用時，造成血漿類固醇激素 cortisol 濃度上升，魚體血漿 cortisol 濃度與緊迫生理代謝有關，維生素 C 的提供是否能調節魚體緊迫生理代謝，而達到預防緊迫對動物免疫能力抑制的目的，此具有重要的意義。Li 及 Lovell (1985) [24]認為河鯰缺乏或餵食 300 mg AA/kg diet 時，感染愛德華氏菌(*Edwardsiella ictalari*)的死亡率上升、抗體產物明顯減少；餵食至 3,000 mg AA/kg diet 時則可降低死亡率，增進補體活性。Liu 等人(1989) [25]之河鯰研究中，0-4,000 mg AA/kg diet 對魚體免疫能力並無影響，但餵食至 1,000 mg AA/kg diet 可增加對 *E. ictalari* 之抵抗力。餵食 2,750 mg AA/kg diet 予大西洋鮭魚，可增進補體活性及降低 *Aeromonas salmonicida* 感染之死亡率，但頭腎細胞吞噬作用及抗體產物不受飼料中維生素 C 劑量之影響[26]。Waagbø 等人(1993) [27]餵食大西洋鮭達 4,000 mg C2MP/kg diet 時，可增加血清補體及溶菌酶活性，降低 *A. salmonicida* 感染之死亡率。維生素 C 顯示與緊迫反應有關，另一方面亦顯示飼料中添加高劑量時，可增加魚類抵抗疾病的能力，因此本研究將以穩定的多磷酸態維生素 C 探討其與緊迫反應及疾病抵抗的關係。

三、結果與討論

研究一、 多磷酸態維生素 C 與緊迫反應之關係－促腎上腺皮質激素對攝食不同劑量維生素 C 之石斑魚稚魚血漿可體松及組織維生素 C 含量之影響

石斑魚餵食不同含量 C2PP 四週後，其增重率以餵予 166 mg AA/kg 組顯著高於餵予 8 mg AA/kg，存活率於各組之間並無差異。無論注射 ACTH 或生理食鹽水，石斑魚血漿 cortisol 濃度均以餵予 166 mg AA/kg 組顯著低於餵予 8 mg AA/kg 組(table 1)。無論注射 ACTH 或生理食鹽水，血漿葡萄糖濃度，以餵予≧85 mg AA/kg 各組顯著低於餵予 8 mg AA/kg 組(table 1)。

Table 1. Plasma cortisol and plasma glucose concentration of grouper fed diets containing different amounts of C2PP for 4 weeks after intraperitoneal injection with adrenocorticotropic hormone (ACTH) or saline [1]

	C2PP[2]			
	8	14	85	166
Cortisol (ng/ml)				
ACTH	6.00 ± 0.21^{bx}	5.54 ± 0.22^{abx}	5.79 ± 0.03^{abx}	4.99 ± 0.64^{ax}
saline	3.86 ± 0.62^{by}	3.63 ± 0.64^{aby}	3.44 ± 0.62^{aby}	3.43 ± 1.21^{ay}
Glucose (mg/dl)				
ACTH	89.01 ± 10.10^{bx}	82.55 ± 2.53^{abx}	80.49 ± 6.60^{ax}	77.20 ± 6.22^{ax}
saline	78.02 ± 3.89^{by}	74.45 ± 2.33^{aby}	66.35 ± 22.34^{ay}	68.41 ± 1.94^{ay}

[1]Values are means ± SD (n=2), with 5 fish per group. ^{ab}Significant differences ($P<0.05$) among ascorbate levels within stress parameter. ^{xy}Significant differences ($P<0.05$) between nonstress or stress stimulated within ascorbate supplementation level.

[2]AA equivalent level.

無論注射 ACTH 或生理食鹽水，頭腎維生素 C 濃度，最高組為餵予 166 mg AA/kg 組，其次為餵予 85 mg AA/kg 組，最低組為餵予≦15 mg AA/kg 組(table 2)，後腎維生素 C 濃度，最高組為餵予≧85 mg AA/kg 各組，其次為餵予 14 mg AA/kg 組，最低組為餵予 8 mg AA/kg 組，肝臟中維生素 C 濃度，最高組為餵予 166 mg AA/kg 組，其次為餵予 85 mg AA/kg 組，最低組為餵予≦14 mg AA/kg 組(table 2)。無論添加劑量為何，注射 ACTH 之血漿 cortisol 及葡萄糖濃度均比注射生理食鹽水顯著增高。注射 ACTH 之頭腎維生素 C 濃度顯著低於注射生理食鹽水。無論注射 ACTH 或生理食鹽水，對後腎或肝臟維生素 C 濃度均無顯著影響。

ACTH 是一種壓力反應激素，吳郭魚[14]或虹鱒[15]之體外研究顯示，ACTH 會作用於間腎組織而刺激 cortisol 釋放。注射 ACTH 於鱸魚[16]或高首鱔[17]之研究，亦證實會造成血漿 cortisol 上升。本研究中注射 ACTH 於石斑魚，血漿 cortisol 濃度均比注射生理食鹽水高，顯示外生性之 ACTH 注射會作用於魚類壓力反應控制中樞下視丘-腦下腺-間腎組織反應軸(hypothalamus-pituatary-interrenal axis, HPI)，因而刺激腎皮質類固醇 cortisol 生成及血漿葡萄糖的上升。本實驗中顯示石斑魚餵食 C2PP 四週後，頭腎及後腎維生素 C 蓄積量高於肝臟維生素 C 蓄積量，頭腎及後腎維生素 C 蓄積量則為相似，顯示魚體腎臟是維生素 C 主要蓄積之處。

Table 2. Head kidney, trunk kidney and hepatic AA concentration of grouper fed diets containing different amounts of C2PP for 4 weeks after intraperitoneal injection with adrenocorticotropic hormone (ACTH) or saline [1]

	C2PP			
	8	14	85	166
Head kidney AA (μg/g tissue)				
ACTH	50.14 ± 1.06^{ax}	55.57 ± 6.62^{ax}	101.14 ± 13.17^{bx}	127.69 ± 7.12^{cx}
saline	67.92 ± 1.89^{ay}	66.89 ± 4.40^{ay}	111.69 ± 5.56^{by}	145.69 ± 5.18^{cy}
Trunk kidney AA (μg/g tissue)				
ACTH	67.72 ± 7.27^{a}	85.06 ± 10.01^{b}	100.34 ± 3.10^{c}	102.17 ± 20.39^{c}
saline	67.55 ± 11.54^{a}	86.06 ± 6.03^{b}	126.56 ± 7.54^{c}	126.62 ± 2.09^{c}
Hepatic AA (μg/g tissue)				
ACTH	36.80 ± 2.26^{a}	31.41 ± 8.12^{a}	78.94 ± 15.95^{b}	91.29 ± 16.20^{c}
saline	28.40 ±0.59^{a}	34.92 ± 13.97^{b}	69.07 ± 18.14^{c}	95.69 ± 20.74^{d}

[1]Values are means ± SD (n=2), with 5 fish per group. ^{abcd}Significant differences ($P<0.05$) among ascorbate levels within stress parameter. ^{xy}Significant differences ($P<0.05$) between nonstress or stress stimulated within ascorbate supplementation level.

[2]AA equivalent level

研究二、多磷酸態維生素 C 對石斑魚稚魚低溫緊迫反應及疾病抵抗之影響

石斑魚餵食不同含量 C2PP 八週後，各組之增重率及存活率。各組之間並無差異。無論是否給予緊迫刺激，石斑魚受弧菌感染死亡率均以餵予≧88 mg AA/kg 各組顯著低於餵食≦17 mg AA/kg 各組。肝臟中維生素 C 濃度及血漿溶菌酶活性，均隨飼料中 C2PP 含量增加而上升(table 3 及 table 4)。無論是否給予緊迫刺激，超氧陰離子產率以餵予 173 mg AA/kg 組顯著高於餵食≦17 mg AA/kg 之各組(table 4)。血漿 cortisol 含量，以餵予 161 mg AA/kg 組顯著低於餵予 9 mg AA/kg 組，血漿葡萄糖濃度在各組之間無顯著差異(table 5)。餵食 C2PP 組，無論添加劑量為何，緊迫刺激對石斑魚弧菌感染之死亡率及肝臟維生素 C 濃度亦無顯著影響，溶菌酶活性及超氧陰離子產率顯著降低，且血漿 cortisol 及葡萄醣濃度隨之上升。

無論低溫刺激與否，注射半致死劑量之 *V. carcharia* 感染石斑魚[28]，在餵食不同含量 C2PP 各組，死亡率為 0-33 %之間，其值均未達預期之半致死率。死亡的魚體中發現肝臟失色及明顯腸道腫脹充滿黃色液體等徵狀，此些徵狀與 Yii 等人(1997)[28]之研究相似，並可指出石斑魚隻死亡確實為 *V. carcharia* 感染所致。餵食五倍適量及十倍適量之 C2PP，死亡率顯著低於餵食低量及適量組，顯示至少五倍適量之 C2PP 能抵抗 *V. carcharia* 感染所造成之死亡率。

Table 3. Cumulative percent mortality and hepatic AA concentration of grouper fed diets containing different amounts of C2PP for 8 weeks after stimulated with extreme temperature and challenged with *Vibrio carchariae*[1]

	C2PP[2]			
	9	17	88	173
Mortality (%)				
nonstress	22.2 ± 0.0[b]	27.8 ± 7.9[b]	0 ± 0[a]	0 ± 0[a]
stress	27.8 ± 7.9[b]	27.8 ± 7.9[b]	5.6 ± 7.9[a]	5.6 ± 7.9[a]
Hepatic AA (μg/g tissue)				
nonstress	52.36 ± 20.63[a]	77.80 ± 18.67[b]	122.01 ± 39.08[c]	116.19 ± 2.80[c]
stress	48.79 ± 4.79[a]	78.91 ± 8.16[b]	112.30 ± 15.76[c]	120.20 ±4.55[c]

[1]Values are means ± SD (n=2), with 9 fish per group for mortality and with 5 fish for hepatic AA concentration. [abc] Significant differences ($P<0.05$) among ascorbate levels within stress parameter.

[2]AA equivalent level.

Durve 及 Lovell (1982) [29]指出至少提供河鯰之維生素 C 需求量的五倍，才能減少河鯰感染 *Edwardsiella tarda*ee 所造成的死亡率。Li 及 Lovell (1985) [24]指出 60 mg AA/kg 能使河鯰達到最大成長，但需提供其維生素 C 需求量的五倍或五十倍時才能防止 *E. ictaluri* 感染所造成的死亡率。虹鱒提供維生素 C 需求量的五倍至二十倍時能增加 *V. anguillarum* 感染之存活率(Navarre and Halver, 1989) [30]。Liu 等人(1989) [25]則認為提供 1000 mg。AA/kg 時，能增進河鯰對 *E. ictaluri* 之抵抗力。Sobhana 等人(2002) [31]亦認為提供 1000 mg C2PP/kg 予鯉科魚類，能改善 *A. hydrophila* 感染所造成之死亡率。WaagbØ 等人(1993) [27]則認為至少提供 4000 mg C2MP/kg 才能增加大西洋鮭魚感染 *A. salmonicida* 之存活率。餵食 C2PP，經低溫緊迫刺激組，死亡率均高於未刺激組。Li 等人 (1998) [21]指出以擁擠為緊迫因子刺激河鯰，而後再以 *E. ictaluri* 感染，發現魚隻經刺激者，死亡率比未刺激組高。

石斑魚肝臟維生素 C 濃度隨飼料中 C2PP 含量增加而上升，在未緊迫刺激或緊迫刺激組都有相似的結果($Y = 1.67X^2-0.24X -47.85$, $r^2 = 0.96$ (C2PP vs NS); $Y = 1.20 X^2-0.19X-49.38$, $r^2 = 0.93$ (C2PP vs S))，顯示提供 C2PP 能增進石斑魚肝臟維生素 C 蓄積濃度。緊迫刺激組肝臟維生素 C 濃度均稍低於未緊迫刺激組。Henrique 等人(1996) [32]發現金頭鯛受到淡水緊迫刺激時，肝臟維生素 C 濃度會降低，而 Li 等人(1998) [21]則指出以擁擠為緊迫因子刺激河鯰，對其肝臟維生素 C 含量無影響，此結果與本研究較為相似。本研究中經低溫緊迫刺激組，血漿 cortisol 濃度顯著上升，但提供至十倍適量之 C2PP 能降低魚體 cortisol 的生成。此結果與大西洋鮭的研究相似 (WaagbØ and Sandnes, 1996) [23]。石斑魚經低溫緊迫刺激血漿葡萄糖濃度顯著上升，提供不同含量或 C2PP 對血漿葡萄糖濃度無顯著影響。此結果與金頭鯛之研究結果不同，當金頭鯛缺乏維生素 C 提供時，低溶氧量刺激會使金頭鯛血糖顯著上升(Henrique et al., 1998) [33]。本研究的結果顯示以 zymosan 刺激石斑魚頭腎吞噬細胞時，餵食十倍適量之 C2PP 之頭腎吞噬細胞超氧陰離子產率顯著高於餵食低量之 C2PP。溶菌酶活性均隨飼料中 C2PP 含量增加而上升。大西洋鮭的研究指出，當受到 *A. salmonicida* 感染時，提供 4000 mg C2MP/kg 顯著增加頭腎細胞之溶菌酶活性，且具有較好之疾病抵抗能力[27]。低溫緊迫刺激組之溶菌酶活性及超氧陰離子產率顯著低於未刺激組，顯示低溫緊迫刺激顯著抑制石斑魚免疫反應。MÖck 及 Peters (1990) [34]之研究指出虹鱒受到污水、捕捉及運輸等緊迫因子刺激時，血清及頭腎溶菌酶活性明顯降低。

Table 4. Lysozyme activity and O_2^- production ratio of grouper fed diets containing different amounts of C2PP for 8 weeks after stimulated with extreme temperature and challenged with *Vibrio carchariae*[1]

	C2PP[2]			
	9	17	88	173
Lysozyme activity (unit/ml)				
nonstress	53.16 ± 13.97[ax]	127.23 ± 6.98[bx]	161.80 ± 13.97[cx]	265.50 ± 20.95[dx]
stress	58.09 ± 6.98[ay]	87.72 ± 20.95[by]	132.17 ± 13.97[cy]	161.80 ± 27.94[dy]
O_2^- production ratio				
nonstress	1.16 ± 0.12[ax]	1.46 ± 0.17[ax]	1.52 ± 0.05[abx]	1.81 ± 0.21[bx]
stress	1.05 ± 0.09[ay]	1.12 ± 0.02[ay]	1.25 ± 0.10[aby]	1.20 ± 0.09[by]

[1]Values are means ± SD (n=2), with 5 fish per group. [abcd]Significant differences ($P<0.05$) among ascorbate levels within stress parameter. [xy]Significant differences ($P<0.05$) between nonstress or stress stimulated within ascorbate supplementation level.

[2]AA equivalent level.

Table 5. Plasma cortisol and plasma glucose concentration of grouper fed diets containing different amounts of C2PP for 8 weeks after stimulated with extreme temperature and challenged with *Vibrio carchariae*[1]

	C2PP[2]			
	9	17	88	173
Cortisol (ng/ml)				
nonstress	3.75 ± 0.78[bx]	3.64 ± 0.65[abx]	3.55 ± 0.33[abx]	3.11 ± 0.43[ax]
stress	5.81 ± 0.86[by]	5.94 ± 0.46[aby]	5.01 ± 0.67[aby]	5.05 ± 1.07[ay]
Glucose (mg/dl)				
nonstress	76.10 ± 9.71[x]	78.16 ± 7.58[x]	75.96 ± 6.80[x]	80.36 ± 2.14[x]
stress	94.09 ± 5.25[y]	103.30 ± 5.05[y]	85.99 ± 5.05[y]	86.40 ± 4.08[y]

[1]Values are means ± SD (n=2), with 5 fish per group. [ab]Significant differences ($P<0.05$) among ascorbate levels within stress parameter. [xy]Significant differences ($P<0.05$) between nonstress or stress stimulated within ascorbate supplementation level.

[2]AA equivalent level.

四、計畫成果自評

達成本研究計畫之預期目標。

五、參考文獻

[1] Chen, H.Y., Tsai, J.C., 1994. Optimal dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. Aquaculture 119, 265-271.

[2] Shiau, S.Y., Lan, C.W., 1996. Optimum dietary protein level and protein to energy ratio for growth of grouper (*Epinephelus malabaricus*). Aquaculture 145, 259-266.

[3] Shiau, S.Y., Lin, Y.H., 2001. Carbohydrate

utilization and its protein-sparing effect of grouper, *Epinephelus malabaricus*. Animal Sci. 73, 299-304.

[4] Shiau, S.Y., Lin, Y.H., 2002. Utilization of glucose and starch by grouper *Epinephelus malabaricus* at 23℃. Fisheries Sci. 68, 989-993.

[5] Wu, F.C., Ting, Y.Y., Chen, H.Y., 2002. Docosahexaenoic acid is superior to eicosapentaenoic acid as the essential fatty acid for growth of grouper, *Epinephelus malabaricus*. J. Nutr. 132, 72-79.

[6] Lin, M.F., Shiau, S.Y., 2004. Requirements of vitamin C (L-ascorbyl-2-monophosphate-Mg and L-ascorbyl-2- monophosphate-Na) and its effects on immune responses of grouper, *Epinephelus malabaricus*. Aquacult. Nutr. 10, 327-333.

[7] Lin, M.F., Shiau, S.Y., 2005a. Dietary L-ascorbic acid affects growth, non-specific immune responses and disease resistance in juvenile grouper, *Epinephelus malabaricus*. Aquaculture 244, 215-221.

[8] Lin, M.F., Shiau, S.Y., 2005b. Requirements of vitamin C (L-ascorbyl-2-sulfate and L-ascorbyl-2- polyphosphate) and its effects on non specific immune responses of grouper, *Epinephelus malabaricus*. Aquacult. Nutr. 11, 183-189.

[9] Lin, Y. H., Shiau, S. Y., 2005c. Dietary vitamin E requirements of grouper, *Epinephelus malabaricus*, under two lipid levels, and their effects on immune responses. Aquaculture 248, 235-244.

[10] Kitabchi, A.E., 1967. Ascorbic acid in steroidogenesis. Nature 215, 1385-1386.

[11] Wilson, R.P., Poe, W.E., 1973. Impaired collagen formation in the antiscorbutic channel catfish. J. Nutr. 103, 1359-1364.

[12] Lovell, R.T., El Naggar, G.O., 1989. Vitamin C activity for L-ascorbic acid, L-ascorbyl-2-sulfate, and L-ascorbyl-2-phosphate Mg for channel catfish. In: Pro Third Int Symp on Feeding and Nutrition in Fish. Toba, Japan, Aug 28-Sept 1, pp 159-165.

[13] Moreau, R., Dabrowski, K., 1996. The primary localization of ascorbate and its synthesis in the kidneys of acipenserid (*Chondrostei*) and teleost (*Teleostei*) fishes. J. Comp. Physiol. 166 B, 178-183.

[14] Balm, P.H.M., Pepels, P., Helfrich, S., Hovens, M.L.M., Wendelaar Bonga, S.E., 1994. Adrenocorticotropic hormone in relation to in terrenal function during stress in tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 96, 347-360.

[15] Brodeur, J.C., Daniel, C., Ricard, A.C, Hontela, A., 1998. In vitro response to ACTH of the interregnal tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to cadmium. Aquatic Toxicolo. 42, 103-113..

[16] Girard, C, Brodeur, JC, Hontela, A., 1998. Responsiveness of the interregnal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo. Can. J. Fish. Aquat. Sci. 55, 438-450.

[17] Belanger, J.M., Son, J.H., Laugero, K.D., Moberg, G.P., Doroshov, S.I., Lankford, S.E., Cech, J.J., 2001. Effects of short-term management stress and ACTH injects on plasma cortisol levels in cultured white sturgeon, *Acipenser transmontanus*. Aquaculture 203, 165-176

[18] Wedemeyer, G., 1969. Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes. Comp. Biochem. Physiol. 29, 1247-1251.

[19] Izuru, K., 2002. Interrenal stress responsiveness of marbled sole, *Pleuronectes yokohamae*, inhabiting Tokyo Bay. Environ. Toxicol. 17, 1-6.

[20] Ortuño, J., Esteban, M.A., Meseguer, J., 2003. The effect of dietary intake of vitamin C and E on the stress response of gilthead seabeabream *Sparus aurata* L. Fish Shellfish Immunol. 14, 145-156.

[21] Li, M.H., Wise, D.J., Robinson, E.H., 1998. Effect of dietary vitamin C on weight gain, tissue ascorbate concentration, stress response, disease resistance of channel catfish *Ictalurus punctatus.* J. World Aquacult. Soc. 29, 1-8.

[22] Wise, D.J., Schwedler, T.E., Otis, D.L., 1993. Effects of stress on susceptibility of native channel catfish in immersion challenge with *Edwardsiella ictaluri*. J. Aquat. Anim. Health 5, 92-97.

[23] WaagbØ, R., Sandnes, K., 1996. Effect of dietary vitamin C on growth and parr-smolt transformation in Atlantic salmon, *Salmo salar* L. Aquacult. Nutr. 2, 65-69.

[24] Li, Y., Lovell, R.T., 1985. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115, 123-131.

[25] Liu, O.R., Plumb, J.A., Guerin, M., Lovell, R.T., 1989. Effect of megalevels of dietary vitamin C on the immune responses of channel catfish *Ictalurus punctatus* in ponds. Dis. Aquat. Org. 7, 191-194.

[26] Hardie, L.J., Fletcher, T.C., Secombes, C.J., 1991. The effect of dietary vitamin C on the immune response of the Atlantic salmon *Salmo salar* L. Aquaculture 95, 201-214.

[27] WaagbØ, R., Glette, J., Raa-Nilsen, E. Sandnes, K., 1993. Dietary vitamin C, immunity and disease resistance in Atlantic salmon *Salmo salar*. Fish Physiol. Biochem. 12, 61-73.

[28] Yii, K.C., Yang, T.I., Lee, K.K., 1997. Isolation and characterization of *Vibrio carchariae*, a causative agent of gastroenteritis in the groupers,

Epinephelus coioides. Curr. Microbial. 35, 109-115.
[29] Durve, V.S., Lovell, R.T., 1982. Vitamin C and disease resistance in channel catfish *Ictalurus punctatus*. Can. J. Fish. Aquat. Sci. 39, 948-951.
[30] Navarre, O., Halver, J.E., 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture 79, 207-221.
[31] Sobhana, K.S., Mohan, C.V., Shankar, K.M., 2002. Effect of dietary vitamin C on the disease susceptibility and inflammatory response of mrigal *Cirrhinus mrigala* (Hamilton) to experimental infection of *Aeromonas hydrophila*. Aquaculture 207, 225-238.
[32] Henrique, M.M.F., Morris, P.C., Davies, S.J., 1996. Vitamin C status and physiological response of the gilthead seabream, S*parus aurata* L., to stressors associated with aquaculture. Aquacult. Res. 27, 405-412.
[33] Henrique, M.M.F., Gomes, E.F., Gouillou-Coustans, M.F., Oliva-Feles, A., Davies, S.J., 1998. Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of seabream, *Sparus aurata* L. Aquaculture 161, 415-426.
[34] MÖck, A., Peters, G., 1990. Lysozyme activity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), stressed by handling, transport and water pollution. J. Fish Biol. 37, 873-885.