The effects and mechanisms of traditional chinese medicine and microalgae as supplement in fish food on the immunity.

Colby Lok Hay Lee, Ian Wing-Yin Mo, Fred Wang-Fat Lee, Emily Sze Wan Wong, Lam Macus Cheuk Ming, Tsui Yat Lai, Law Chui Lee
s1266795@live.hkmu.edu.hk School of Science and Technology, Hong Kong Metropolitan University, Hong Kong, China

1.INTRODUCTION

- Maintaining robust immune function in cultured fish is critical for aquaculture
- Many producers still rely on antibiotics to support fish health, but this practice carries risks such as antimicrobial resistance and residue concerns. While natural dietary supplements offer a safer and more sustainable alternative.
- In particular, Spirulina (Spir) and Astragalus Membranaceus (AM) have already been shown to exert positive effects on fish immunity when included as feed additives.



Spirulina: enhancing the antioxidant defense and immunostimulants

AM: improving immune cell activity

Fig 1. How combining two supplements within one fish food can provide a better immunity boost.

Aim: Examine the effect and mechanism on immunity in aquaculture fish under the influence of microalgae and Chinese medicine, and develop an optimum formula of the fish food with microalgae and Chinese medicine.

Objectives:

- Observe the effects of feed supplement on immunity
- Identify the immune responses and observe the mortality rate during the disease challenge.
- Based on the results of the above objectives, determine the best fish food formula

2.METHOD

Combintations of supplements in different dosages and control were created as below

Diet	AM (g/kg)	Spirulina (g/kg)	α-Cellulose (g/kg)
Control		0	10
AM control (dosage from literature review)	5	0	5
Spir control (dosage from literature review)	0	5	5
Diet A	5	2.5	2.5
Diet B	5	3.75	1.25
Diet C	5	5	0
Diet D	2.5	5	2.5
Diet E	3.75	5	1.25
Diet F	2.5	2.5	5
Diet G	3.75	3.75	2.5

Table 1. The dosage combination of experimental groups

- The test lasted 8 weeks
- Each diet treatments had triplicate
- Each replicate were made out of 5 Nile tilapia (*Oreochromis niloticus*)
- All fish were fed daily
- Water quality were monitored weekly

For the food supplments and basal diet:

- Basal diet was calculated to contain 3000 Kcal, 32.0% crude protein, 5.0% crude fat
- α-Cellulose was purchased from Rhawn (China), 1kg for 39.5 USD
- Spray dried Spirulina was purchased from Florida Aqua Farms (US), 2.5kg for 115 USD
- *Astragalus Membranaceus* Extract was purchased from NOW foods (US), 45g for 11.3 USD

The diets are prepared as followed:

1. Basal diet was crushed into dust form
2. Supplements were weighted and mixed in
3. Distilled water was added until the texture became paste like
4. Press the paste into thin layer and dry under 65 ℃ overnight

After the feeding period, sample were collected and the follow up tests were performed.

- Skin mucus were collected with filter paper
- Blood sample were collected with syringe from caudal vein
- Serum were obtained through centrifugation of blood

After sample collection, various immunity test were performed:

- Skin mucus& serum total IgM & lysozyme activity
- Serum protein level
- Full blood white blood cell (WBC) count
- Serum superoxide dismutase (SOD) & catalase (CAT) activity

After the sample collection, a disease challange was followed on the surviving subjects:

- *Aeromonas hydrophila* was injected into subjects with needles
- Mortality rate was observed for 2 weeks after injection

3.RESULT & DISCUSSION

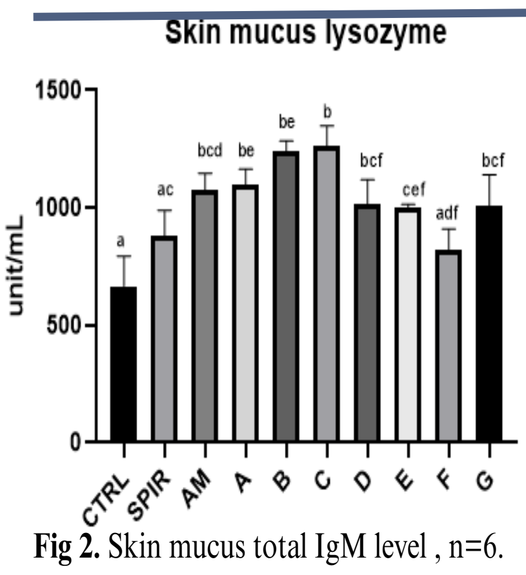


Fig 2. Skin mucus total IgM level , n=6. Groups with no repeating alphabets have significant difference ($p<0.05$).

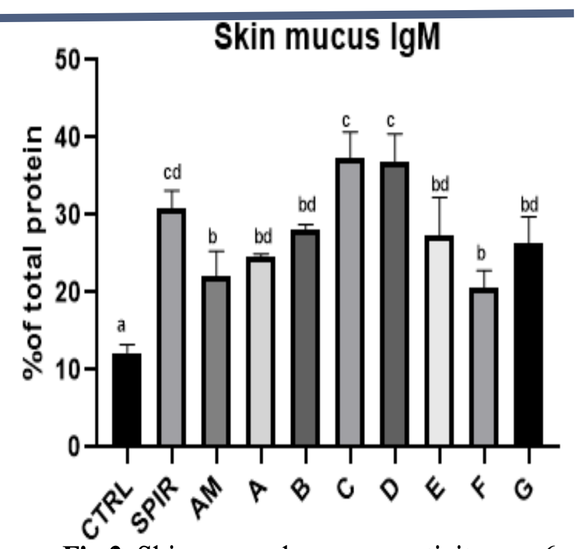


Fig 3. Skin mucus lysozyme activity , n=6. Groups with no repeating alphabets have significant difference ($p<0.05$).

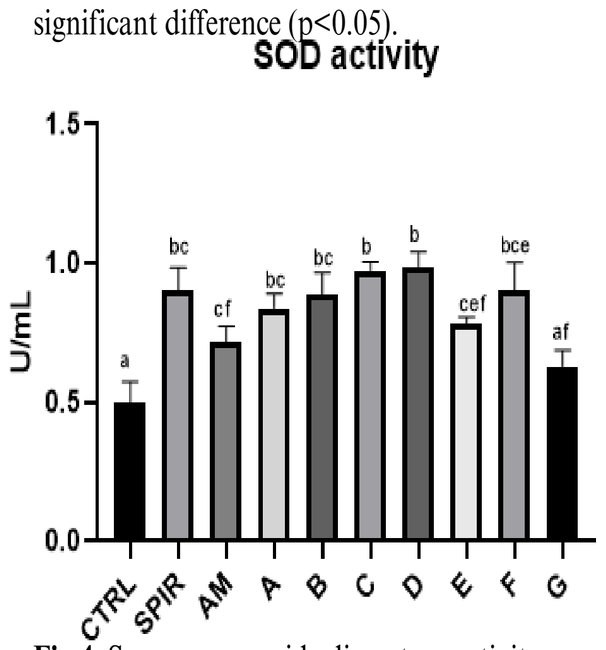


Fig 4. Serum superoxide dismutase activity , n=9. Groups with no repeating alphabets have significant difference ($p<0.05$).

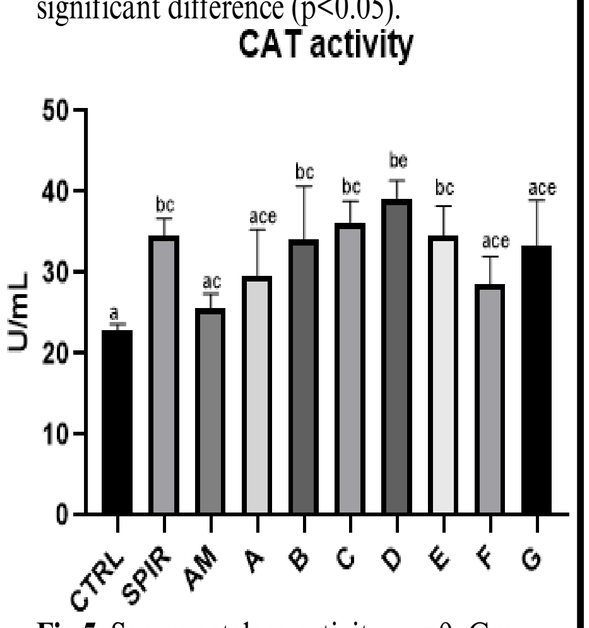


Fig 5. Serum catalase activity , n=9. Groups with no repeating alphabets have significant difference ($p<0.05$).

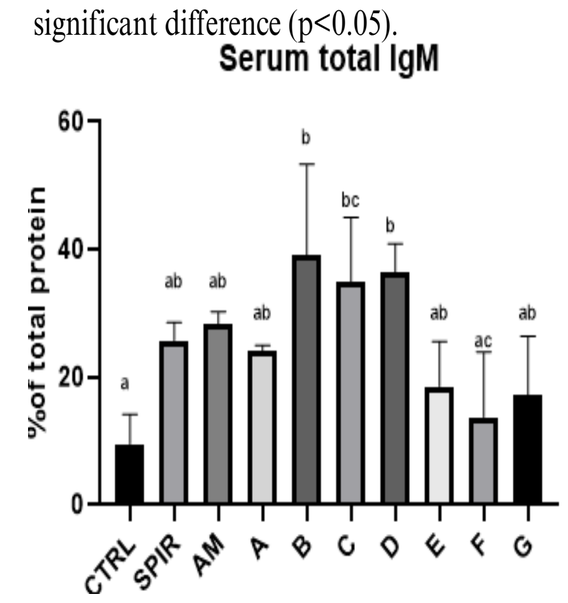


Fig 6. Serum total IgM level , n=9. Groups with no repeating alphabets have significant difference($p<0.05$).

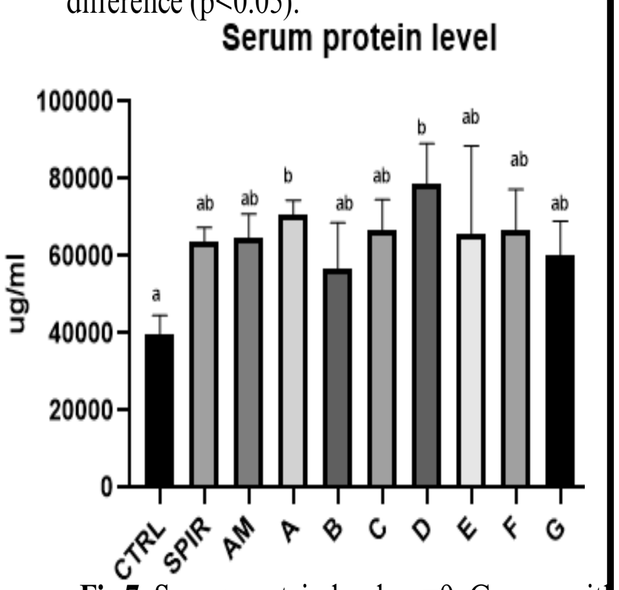


Fig 7. Serum protein level , n=9. Groups with no repeating alphabets have significant difference ($p<0.05$).

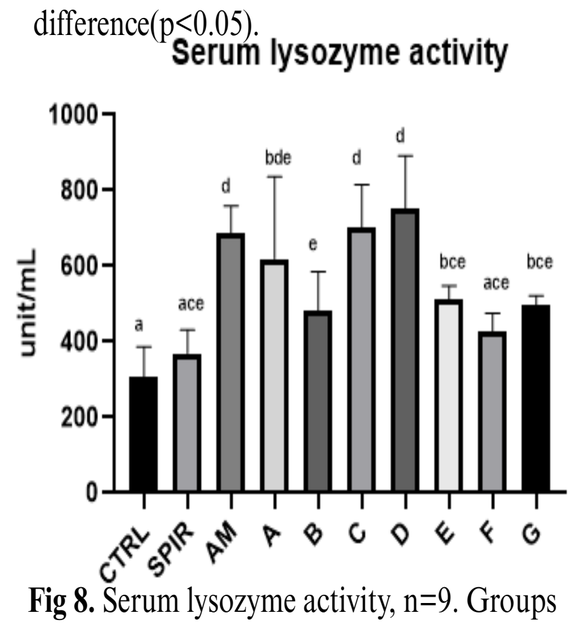


Fig 8. Serum lysozyme activity, n=9. Groups with no repeating alphabets have significant difference($p<0.05$).

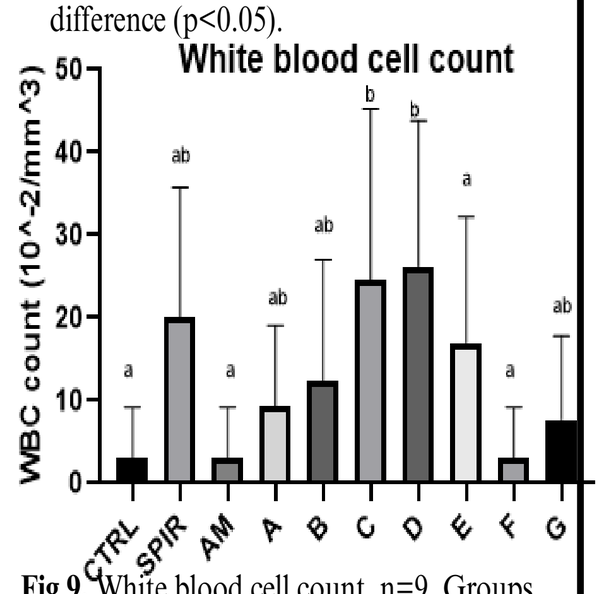


Fig 9. White blood cell count, n=9. Groups with no repeating alphabets have significant difference($p<0.05$).

Mortality rate during disease challange

Diet	Spir	A	B	C	D	E
Inital fish number	7	4	5	7	7	6
Number of death	4	2	2	3	2	3
Mortality rate(%)	57.1	50	50	42.9	28.5	50

Table 2. The mortality rate during the disease challenge

- Not every diet have enough fish for disease challenge

- Diets C,D perform significant better than other control and test groups
- Diet D was selected as the optimum diet, as it has the lowest production cost Comparing to diet C.(10kg production cost: 48.3 USD for diet C, 43.0 USD for diet D)

4.CONCLUSION

- By combining two supplements , addition and synergistic immunity boosting effects are noticeable
  - Addition effect: WBC count, AM control show no significant difference while diets C and D have significant increased WBC.
  - Synergistic effect: Serum total IgM, means increase in diets B,C,D compared with the optimum dosage control
- Using both supplements in high dosages show promising enhancement compared to single-supplement

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