EFFECTS OF DIETARY FISH MEAL REPLACEMENT WITH PLANT PROTEIN ON LEUCOCYTE CYTOKINE RESPONSES IN YELLOWTAIL *Seriola quinqueradiata*

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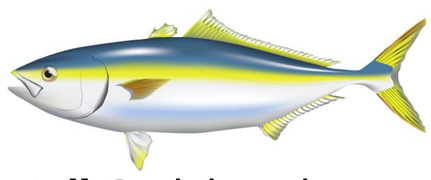
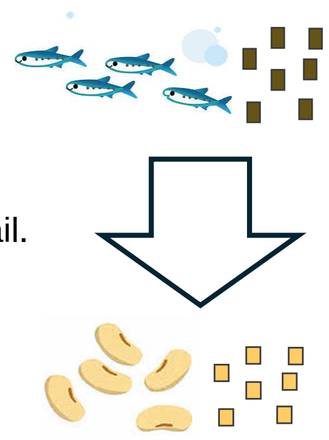
Background



Yellowtail *Seriola quinqueradiata*
- Carnivorous fish
- Most cultured fish in Japan

High fish meal (FM) content in aquacultural diet
Ex) FM is contains 50% in diet for around 2kg BW yellowtail.

For sustainable yellowtail culture…
Replacement of FM with plant proteins
Ex) Soybean meal (SBM), Corn gluten meal (CGM) and Soy protein concentrate (SPC)



Cytokine
- Important for teleost innate immunity
- Relates to cell-mediated immunity and humoral immunity
- Divided into 2 types : inflammatory or anti-inflammatory

Interleukin-1β (**IL-1β**) : Mediator of chronic inflammatory disease

Interferon-γ (**IFN-γ**) : Important for anti virus immunity

Tumor necrosis factor (**TNF-α**) : Involve in apoptosis

Interleukin-10 (**IL-10**) : negatively regulates cell-mediated immunity

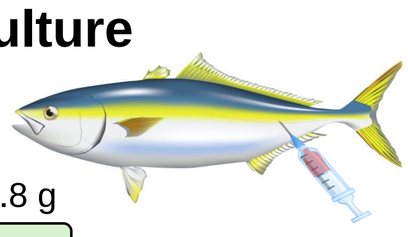
Low FM diet caused for differences in gene expression of the *il-1β* and *tnf-α* in European sea bass *Dicentrarchus labrax*

Toprrecillas et al., 2017

To evaluate the effect of dietary FM replacement with soybean meal (SBM) on the response of cytokine gene expression in yellowtail leucocytes

Materials, methods and results

① Leucocyte culture



Sampling
Blood — Yellowtail, Ave. BW 659.8 g

Separation of leucocyte

Seeding in 24 well plate

Leucocyte preculture

1.0×10^6 cells/well
Preculture : over night

Immune stimulation in leucocyte culture

With water-soluble fraction of feed ingredients
2 concentration,
2 sampling time

Measurement of cytokines
・*il-1β* ・*tnf-α* ・*ifn-γ* ・*il-10* With RT-qPCR

- FM
- SBM
- SPC
- CGM

Shake 2h
Leave 24h

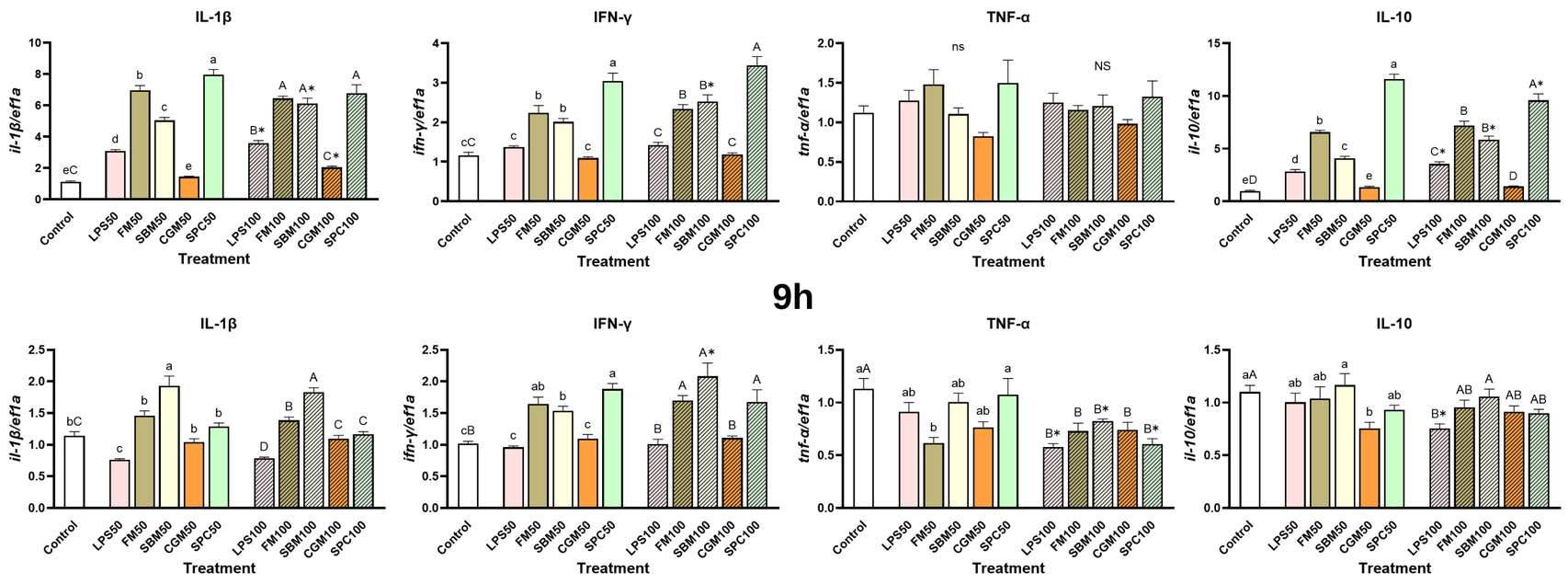
Water : Ingredient =1:6 (weight/volume)

Supernatant → Freeze-drying → Dissolve

Seeding : 1.0×10^6 cells/well
Stimulation : Medium (control), LPS* or Poly I:C*
3h and 9h

* Pathogen-associated molecular pattern molecules
LPS = lipopolysaccharide
Poly I:C =polyinosinic-polycytidylic acid

3h



9h

Fig.1 Relative gene expression of yellowtail leucocyte *il-1β*, *ifn-γ*, *tnf-α* and *il-10* after water-soluble fraction of feed ingredient stimulation.

Small letter: compare with control and 50μg treatment. Capital letter: compare with control and 100μg treatment.
*: Compare with 50μg and 100μg stimulation of same treatment.

✓ For ***il-1β***, ***ifn-γ***, and ***il-10*** were significantly **higher** in all water-soluble fraction treatments, except CGM after **3h** of stimulation.

✓ For ***il-1β***, the **SBM** treatment has significantly **higher** levels than the other treatments after **9h** of stimulation.

② Feeding trial and leucocyte stimulation

Initial body weight : 175.1 g

Density : 8 fish / 1.1 t tank

Diet : 4 types (FM replacement with SBM or Water washed SBM)

Feeding : 1 time / day (Satiation)

Period : 6 weeks

Temperature : 26.2~22.9℃ (Decreased)

Sampling : 6 week Blood

leucocyte isolation → Immune stimulation in leucocyte culture → Measurement of cytokines

Table1. Ingredient and composition of experimental diets for yellowtail

Ingredient (g/kg)	SBM0	SBM15	SBM30	WSBM
FM	585	485	385	385
Krill meal	50	50	50	50
CGM	60	60	60	60
SBM	0	150	300	0
Water washed SBM	0	0	0	225
Fish oil	85	90	95	97
Other	220	165	110	183
proximate composition				
Crude protein	48.2	50.7	51.5	49.3
Crude fat	13.7	14.1	14.2	13.8
Ash	24.3	18.3	13.6	18.5

Table2 Growth performance and feed utilization of yellowtail fed the experimental diets for 6 weeks

	Dietary groups			
	SBM0	SBM15	SBM30	WWSBM
Initial average body weight (g)	175.1±0.5	175.3±0.5	174.9±0.4	175.1±0.6
Final average body weight (g)	253.4±3.4 ^{A}	259.0±4.1 ^{A}	256.8±3.1 ^{A}	237.8±1.1 ^{B}
Weight gain (%)	44.7±2.4 ^{A}	47.8±2.7 ^{A}	44.8±0.9 ^{A}	35.8±1.0 ^{A}
Specific growth rate (%/day)	0.9±0.04 ^{AB}	0.9±0.04 ^{A}	0.9±0.03 ^{A}	0.7±0.02 ^{B}
Feed efficiency (%)	33.3±1.2 ^{AB}	36.7±1.7 ^{A}	36.6±0.3 ^{A}	30.1±0.8 ^{B}
Daily feed intake (% BW/day)	3.4±0.03 ^{A}	3.3±0.03 ^{A}	3.1±0.05 ^{C}	3.2±0.02 ^{BC}
Survival rate (%)	100.0±0.0	100.0±0.0	95.8±4.17	100.0±0.0

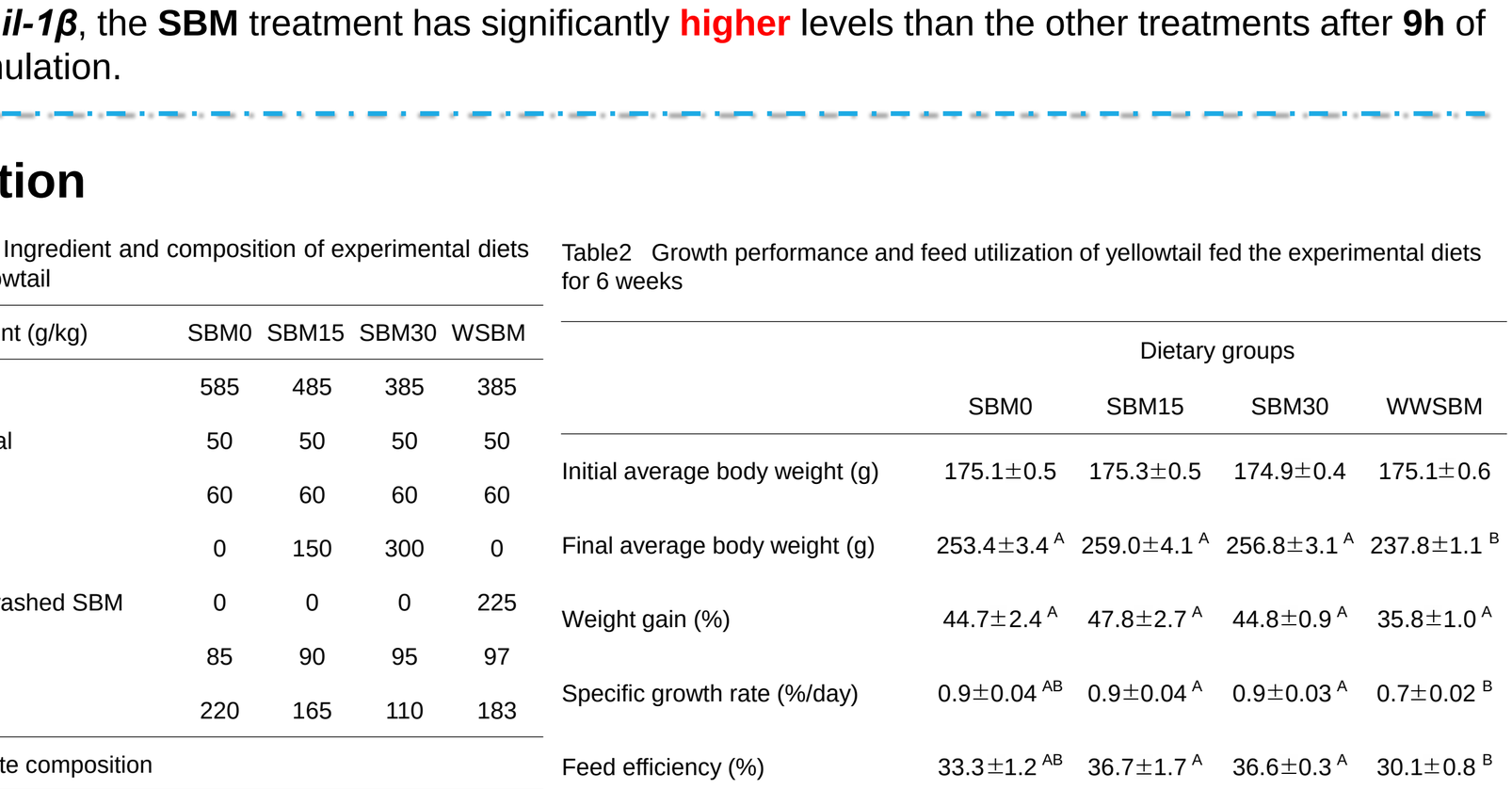
Table 3 Whole body composition (wet weight basis) of juvenile yellowtail fed the experimental diets for 6 weeks

	Initial	Dietary groups SBM0	SBM15	SBM30	WSBM
Whole body composition (%)					
Moisture	75.3^{A}	72.1±0.16^{B}	72.8±0.44^{B}	72.1±0.18^{B}	73.3±0.17^{B}
Crude protein	17.6	18.2±0.37	17.8±0.49	18.5±0.39	18.5±0.24
Crude fat	2.7^{C}	5.6±0.27^{A}	5.0±0.23^{B}	4.5±0.29^{AB}	4.5±0.11^{B}
Ash	3.0	3.0±0.16	3.4±0.19	3.4±0.18	3.0±0.17

Capital letter: Significantly difference compared with dietary groups. $p<0.05$.

3h

C : Control
L : LPS
P : Poly I:C



9h

Fig.2 Relative gene expression of *il-1β*, *ifn-γ*, *tnf-α* and *il-10* in cultured leucocyte of yellowtail after feeding trial.

Small letter: compare in same stimulant. *: Compare between Control and LPS or poly I:C stimulation groups.

✓ For ***il-1β*** and ***il-10***, LPS stimulation of the **WSBM** group was significantly **higher** than that of the other dietary groups after 3h of incubation.

✓ For ***ifn-γ***, the **SBM15** and **SBM30** groups with poly I:C stimulation were significantly **higher** than that of the other groups of 9h of incubation.

Conclusion

- The water-soluble fractions of FM, SBM, and SPC were found to have leucocyte-stimulating effects.
- Replacing dietary FM with SBM or WSBM in yellowtail might change the response of leucocytes to LPS and Poly I:C.

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