Effects of replacing fish meal with stalked sea squirt (*Styela clava*) tunic waste on growth performance, health status, and skin pigmentation in juvenile red sea bream (*Pagrus major*)

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Abstract

This study evaluated the effects of replacing fish meal (FM) with stalked sea squirt (*Styela clava*) tunic (TSC) on growth performance, health status, and skin pigmentation in juvenile red sea bream (*Pagrus major*). Five isonitrogenous and isolipidic diets were formulated, with TSC replacing FM at 0 % (TSC0), 5 % (TSC5), 10 % (TSC10), 15 % (TSC15), and 20 % (TSC20). After an 8-week feeding trial, TSC inclusion up to 10 % did not adversely affect growth, whereas TSC15 and TSC20 significantly reduced weight gain and feed efficiency. Immune response indicators, including lysozyme activity, immunoglobulin M, and interleukin-1, exhibited a linear decline with increasing dietary TSC levels ($P < 0.05$), suggesting potential immunosuppressive effects at higher inclusion rates. Skin color analysis revealed linear increases in redness (a*) and yellowness (b*) with higher dietary TSC levels. TSC15 significantly enhanced cranial skin a* and b* values compared to TSC0, although differences from TSC5, TSC10, and TSC20 were not significant. These results indicate that TSC can replace up to 10 % of FM in juvenile *P. major* diets without compromising growth or health, while higher replacement levels enhance skin pigmentation but may entail trade-offs in immune function and nutritional performance.

I. Introduction

- Red sea bream (*Pagrus major*) is a finfish that is highly sought for marine aquaculture in East Asia, including Japan and the Republic of Korea.
- Renowned for its high market value, *P. major* demands a protein-rich diet to support optimal growth, robust health, and vibrant skin pigmentation traits that significantly enhance its appeal in competitive markets (Kurnia et al., 2007).
- Fish meal (FM) has traditionally been the primary protein source in aquaculture feeds due to its balanced amino acid profile and high digestibility.
- However, the increasing cost, limited supply, and ecological concerns associated with FM have prompted the exploration of alternative protein sources (Tran et al., 2024; Xu et al., 2024).
- The skin color of *P. major* is a crucial quality criterion; yet this species tends to lose its natural pigmentation under cultured conditions, primarily due to carotenoid deficiency, particularly astaxanthin, in their artificial diets (Katayama et al., 1965).
- Synthetic pigments have become less desirable due to increasing consumer preference for natural products (Herrero et al., 2006), despite their demonstrated cost-effectiveness and environmental sustainability (Nguyen, 2013).
- This pattern has been observed since many countries have banned synthetic products due to concerns regarding their potential adverse effects on human health (Mohammed and Mohd, 2011).
- From a functional perspective, natural carotenoids are more valuable than synthetic alternatives due to differences in chemical structure that can impact biological functions, including antioxidant potential and shelf life (Ambati et al., 2014; Rao et al., 2013).
- While parts of *Styela clava* are edible, its inedible tunic has been largely overlooked, despite being rich in beneficial compounds.
- The stalked sea squirt tunic (TSC) also contains bioactive compounds, including antioxidants, pigments, and immune-boosting agents, which provide additional health benefits to fish (Lee et al., 2015).
- Therefore, this study investigated the effects of using TSC as an FM substitute in red sea bream diets. The main focus included growth performance, body composition, skin pigmentation, antioxidant activity, and immune response.

II. Materials and Methods

① TSC preparation

- Residual TSC, left after removing the edible portion, was collected from a private *S. clava* culture farm and processing facility (Jindong-myeon, Changwon-si, Korea).
- The collected tunics were washed with tap water, dried at 20 °C for 48 h, and then ground into a fine powder for feed formulation and analysis.

② Preparation of the experimental diets

- Jack mackerel meal, dehulled soybean meal and TSC were used the protein sources, and fish oil and soybean oil were used as the lipid source in the experimental diets (Table 1).
- Five isonitrogenous and isolipidic experimental diets were formulated to replace 0% (TSC0), 5% (TSC5), 10% (TSC10), 15% (TSC15), and 20% (TSC20) fish meal with TSC, respectively.



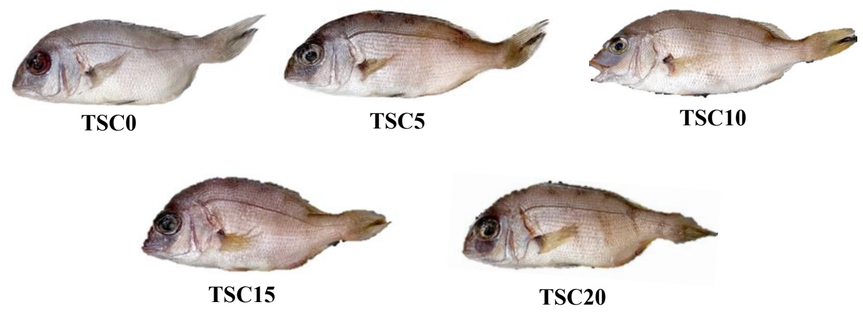
Table 1. Formulation and proximate composition of experimental diets (g/kg, dry matter basis)

	Experimental diets				
	TSC0	TSC5	TSC10	TSC15	TSC20
Jack mackerel meal	550	552.5	495	467.5	440
Dehulled soybean meal	150	150	150	150	150
Wheat flour	215	166.5	118	69.5	21
TSC	-	73	146	219	292
Fish oil	30	33	36	39	42
Soybean oil	30	30	30	30	30
Vitamin premix	10	10	10	10	10
Mineral premix	10	10	10	10	10
Choline	5	5	5	5	5
Proximate composition (g/kg)					
Dry matter	937	929	926	925	924
Crude protein	510	508	502	505	514
Crude lipid	116	113	108	112	130
Ash	109	121	125	146	155
Total carotenoids (ug/g)	4.6	27.3	61.7	83.2	104.8

③ Feeding trial



- Stocking density: 450 fish averaging 4.9 g were randomly stocked into 50 L plastic rectangular tanks (30 juvenile per tank)
- Water temperature: 20.6 ± 0.82°C (mean ± SD)
- Flow rate: 2.7 L/min/tank
- Feeding trial: 8 weeks



④ Chemical analysis

- Proximate composition: AOAC (1990)
- Digestive activity: amylase, lipase, and trypsin commercial kit (Abcam, UK)
- Antioxidant activity: SOD, CAT, and GSH commercial kit (Cayman Chemical, USA)
- Immune parameters: lysozyme (Lang et al., 2001), IgM and IL-1 commercial kit (MyBioSource, USA)
- Fish skin color measurement: Chroma Meter (CR400, Minolta Co., Ltd., Osaka, Japan), the colordistance R package and the Earth Mover's Distance

⑤ Statistical analysis

- R studio (version 2024.04.2+764)
- One-way ANOVA and Tukey's HSD test
- Orthogonal contrast analysis: significance was observed in linear, quadratic, or cubic trends
- Regression analysis: determine the best-fitting model
- Broken-line regression model: the optimal TSC substitution level based on WG data

III. Results

- The FBW, WG, and SGR of fish fed the TSC20 diet were significantly lower than those of fish fed the TSC0, TSC5, and TSC10 diet (Fig. 1, Fig. 2, Fig. 3).
- The FE and PER of fish fed the TSC0 diet were significantly higher than those of fish fed the TSC20 diet (Fig. 4, Fig. 5).
- Based on broken-line regression models of WG, the optimal substitution level of dietary FM with TSC was estimated at 9.66 % (Fig. 6).
- The lysozyme activity and IL-1 levels were significantly lower in fish fed the TSC20 diet than in those fed the TSC0 diet (Table 2).
- The IgM level was significantly lower in fish fed the TSC10 and TSC20 diets than in those fed the TSC0 diet (Table 2).
- Compared with the TSC0 treatment, the a* value of cranial skin in the TSC15 group was significantly higher (Table 3).
- The a* values of medial and caudal skin in fish fed the TSC15 and TSC20 diets were also significantly higher than those of fish fed the TSC0–TSC10 diets (Table 3).
- In the cranial, medial, and caudal regions, fish fed the TSC15 diet showed significantly higher b* values than those fed the TSC0 diet (Table 3).
- Juvenile red sea bream displayed similar pixel distributions across all treatment groups, with pixels classified into three bins (Table 4).
- The pair with the lowest distance score was the red sea bream from the TSC15 and TSC20 diets, followed by the TSC5 and TSC10 pair (Fig. 7).
- In contrast, the TSC0 group exhibited the greatest color distance from the TSC15 and TSC20 groups, indicating the most distinct color difference (Fig. 7).
- Overall, two major clusters were identified: one consisting of the TSC0 group, and the other comprising the TSC5, TSC10, TSC15, and TSC20 groups (Fig. 7).

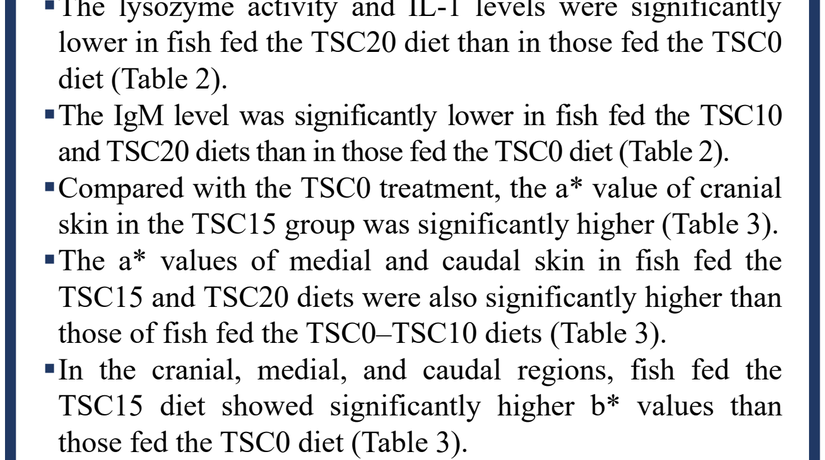


Fig.1. Effects of dietary replacement by TSC on final body weight (FBW, g) of juvenile red seabream.

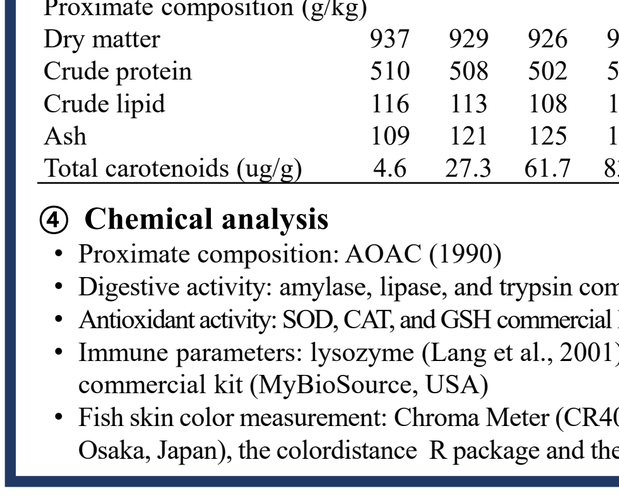


Fig.2. Effects of dietary replacement by TSC on weight gain (WG, g/fish) of juvenile red seabream.

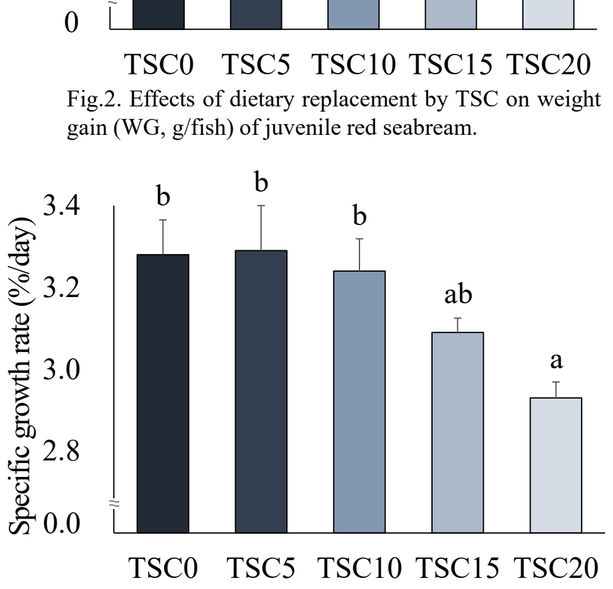


Fig.3. Effects of dietary replacement by TSC on specific growth rate (SGR, %/day) of juvenile red seabream.

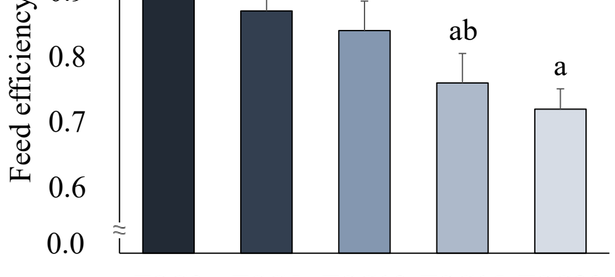


Fig.4. Effects of dietary replacement by TSC on feed efficiency (FE) of juvenile red seabream.

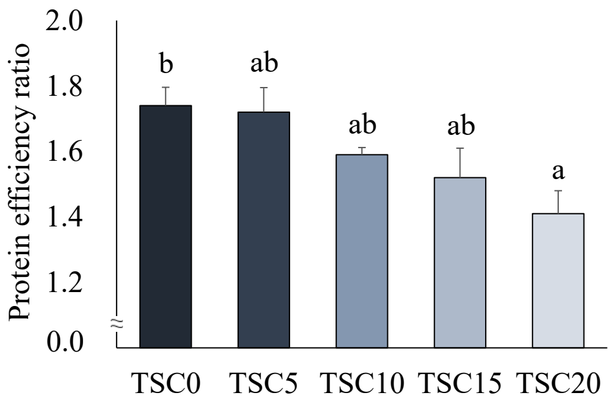


Fig.5. Effects of dietary replacement by TSC on protein efficiency ratio (PER) of juvenile red seabream.

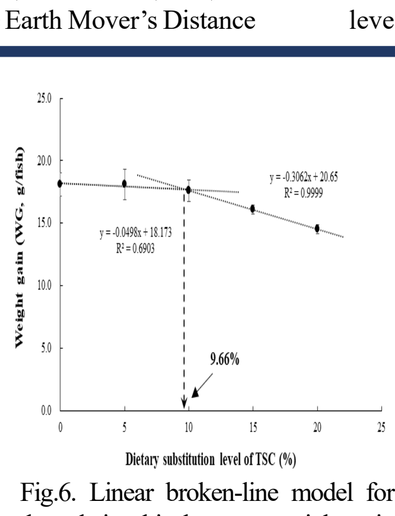


Fig.6. Linear broken-line model for the relationship between weight gain (WG) in red seabream and the replacement level of fish meal with a tunic of *Styela clava* (TSC)

Table 2. Effects of dietary replacement by TSC on immune response of juvenile red seabream

	Experimental diets				
	TSC0	TSC5	TSC10	TSC15	TSC20
Lysozyme (U/mL)	0.32 ± 0.039^{b}	0.30 ± 0.013^{ab}	0.29 ± 0.011^{ab}	0.24 ± 0.013^{ab}	0.21 ± 0.016^{a}
IgM (mg/mL)	467.8 ± 66.68^{b}	397.8 ± 32.92^{ab}	309.4 ± 24.54^{ab}	241.1 ± 56.73^{a}	203.3 ± 8.22^{a}
IL-1 (pg/mL)	268.0 ± 19.37^{b}	255.7 ± 7.49^{ab}	251.6 ± 4.82^{ab}	222.1 ± 8.74^{ab}	207.0 ± 4.24^{a}

Table 3. Effects of dietary replacement by TSC on skin color parameters [L* (lightness), a* (redness) and b* (yellowness) of juvenile red seabream

	Experimental diets				
	TSC0	TSC5	TSC10	TSC15	TSC20
L value measures lightness*					
Cranial	48.73 ± 2.550	49.19 ± 0.903	48.15 ± 2.518	48.73 ± 0.901	48.56 ± 2.011
Medial	52.56 ± 0.503	52.67 ± 0.817	53.56 ± 0.937	53.21 ± 0.899	53.19 ± 0.691
Caudal	59.86 ± 0.218	60.01 ± 0.873	60.48 ± 1.687	59.80 ± 1.210	59.31 ± 0.992
a* value measures lightness					
Cranial	0.89 ± 0.095^{a}	1.64 ± 0.082^{b}	1.74 ± 0.078^{b}	2.00 ± 0.060^{bc}	2.23 ± 0.148^{c}
Medial	0.62 ± 0.039^{a}	1.01 ± 0.111^{b}	1.11 ± 0.051^{b}	1.54 ± 0.090^{c}	1.67 ± 0.050^{c}
Caudal	0.94 ± 0.016^{a}	1.57 ± 0.041^{ab}	1.68 ± 0.077^{b}	2.44 ± 0.250^{c}	2.69 ± 0.164^{c}
b value measures lightness*					
Cranial	-1.90 ± 0.385^{a}	1.61 ± 0.344^{b}	2.43 ± 0.120^{bc}	3.06 ± 0.391^{cd}	4.45 ± 0.147^{d}
Medial	-4.25 ± 0.843^{a}	0.32 ± 0.058^{b}	0.54 ± 0.080^{b}	2.51 ± 0.405^{bc}	3.38 ± 0.532^{c}
Caudal	-2.08 ± 0.128^{a}	1.54 ± 0.446^{b}	2.79 ± 0.123^{bc}	4.45 ± 0.416^{cd}	5.77 ± 0.499^{d}

Table 4. RGB values and relative pixel percentages for the three main bins, identified by image analysis (n = 15) from juvenile red seabream

	Bin	R	G	B	Pixels (%)
TSC0	1	112	106	111	47.2
	2	94	86	90	31.4
	3	141	137	143	21.4
TSC5	1	124	103	96	48.7
	2	109	85	75	28.3
	3	148	129	126	23.0
TSC10	1	129	108	99	48.5
	2	122	99	90	28.4
	3	140	119	110	23.0
TSC15	1	124	98	87	47.9
	2	123	96	83	29.4
	3	127	101	90	22.7
TSC20	1	124	98	91	47.7
	2	124	99	89	31.4
	3	126	101	92	20.9

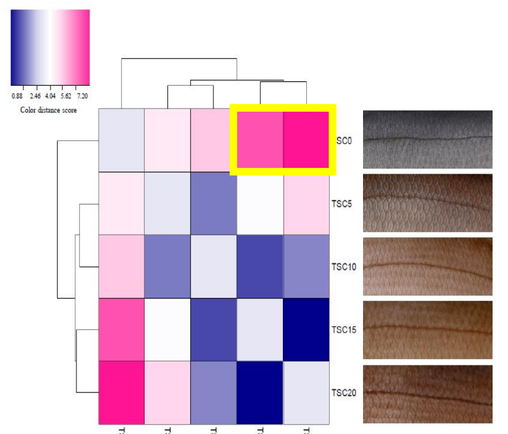


Fig.7. Distance matrix among dietary replacement levels on color distance scores and relative dendrogram. Central- epaxial portion of skin is shown for each dietary treatment.

IV. Conclusion

In conclusion, TSC can replace up to 10% of fish meal in juvenile red seabream diets without adverse effects on growth and feed utilization, while higher levels (≥ 15%) impair growth performance, nutrient retention, and immune function.