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STUDIES ON REDUCING OF PHOSPHORUS AND NITROGEN EXCRETION IN YELLOWTAIL (*Seriola quinqueradiata*) NUTRITION: I. EFFECTS OF DIETARY PROTEIN AND ENERGY LEVEL

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Abstract
The objective of the present study was to investigate the effect of increasing dietary energy at varying dietary protein levels on reducing phosphorus and nitrogen excretion in young yellowtail (*Seriola quinqueradiata*) in a 55-day growth trial by using 180g of fish. Three levels of dietary protein (46%, 50% and 54%) and two levels of dietary energy were tested in a complete randomized design with a 3 x 2 factorial arrangement. Dietary protein and energy levels did not cause any significant difference in final weight, weight gain, feed intake or survival. However, feed and protein efficiencies significantly ($P < 0.05$) improved with increasing dietary energy in each respective dietary protein levels. Phosphorus and nitrogen excretion rates were significantly ($P < 0.05$) reduced with increasing dietary energy at all dietary protein level. There was no interaction between dietary protein and energy level on the parameters measured. In conclusion, the diet containing 46% protein and 19.5% lipid with the total energy content of 5.2 kcal / g was recommended for yellowtail growing from 180 g to 460 g to obtain the lowest phosphorus and nitrogen excretion values without reduction of growth performances under the present experimental conditions.

Keywords: Phosphorus, nitrogen, excretion, dietary protein, lipid, yellowtail

Introduction
Phosphorus (P) is an essential mineral in fish nutrition, but it is also considered as a pollutant contributing algae and macrophyte growth in receiving waters (Roy & Lall, 2003; Bureau and

Cho, 1999). Fishmeal is the main P source in aquafeeds accounting for 30–50% by weight in most carnivorous fish feeds, however, its' P content is not well utilized by most of the aquatic animals and mostly turns into the waste (NRC, 1993; Lall, 1991). As the end product of protein metabolism, excreted nitrogen is an environmentally harmful substance and must be minimized through nutritional manipulations such as feeding fish with the diets having decreased protein-increased energy (Uyan et al., 2023). For particularly carnivorous fish species, lipid is considered as the main dietary energy source. The major benefit of this approach is to obtain a protein-sparing effect of lipid. Yellowtail production in Japan is the highest of any farmed fish species, and 57% of total Japanese fish culture production consists of yellowtail (Anonymous, 2024). Therefore, the development of effective nutritional strategies to maximize the utilization of P and minimize the P load in yellowtail aquaculture effluents requires an accurate estimation of the available P content in inorganic P sources. Yellowtail (Japanese yellowtail, *Seriola quinqueradiata*) is one of the most important aquaculture species in Japan, and the yellowtail production volume of Japanese marine aquacultures amounted to approximately 134 thousand tons in 2021 (Anonymous, 2024). In Mediterranean area, another similar species called as Mediterranean yellowtail (*Seriola dumerilii*) has also been assumed as one of the promising fish for Mediterranean aquaculture industry. Due to their importance, several nutritional studies are already available on both species (Uyan et al., 2007; Ren et al., 2008). However little information exists on P and N output status in yellowtail.

To reduce these two substance's output from aquaculture, efforts on decreasing dietary protein level with increasing energy without any retardation in growth performances could be contributed to lowering P and N excretion in yellowtail. Therefore, the present study was planned to understand how yellowtail tolerates the lipid-based increasing dietary energy under varying dietary protein level to reduce P and N output, and to monitor the growth performances at the present experimental conditions.

Materials and Methods

Test diets

The formulation of the experimental diets is shown in Table 1. A 3 x 2 (dietary protein level x dietary energy level) two-way factorial design was applied. Dietary protein levels (46%, 50% and 54%) were obtained using fish meal. The dietary gross energy level slightly increased with inclusion level of dietary lipid at each respective dietary protein level. Each protein treatment group was replicated with two dietary energy level as high lipid (H) and low lipid (L). Other energy sources are the same for all treatments.

The experimental diets were produced by a private feed company as extruded pellet (5 mm diameter). The experimental diets were stored at −30 °C until used.

Table 1. Ingredient composition and proximate analysis of the experimental diets

Ingredients	Diets					
	1	2	3	4	5	6
Fishmeal	45.0	45.0	51.5	51.5	58.0	58.0
Krill meal	5.0	5.0	5.0	5.0	5.0	5.0
Corn gluten meal	5.0	5.0	5.0	5.0	5.0	5.0
Defatted soybean meal	4.0	4.0	4.0	4.0	4.0	4.0
Pollock liver oil	10.0	13.8	6.6	10.4	3.2	7.0
α-Starch	3.0	3.0	3.0	3.0	3.0	3.0
Wheat flour	10.0	10.0	10.0	10.0	10.0	10.0

Vitamin mix[1]	4.0	4.0	4.0	4.0	4.0	4.0
Mineral mix[2]	4.0	4.0	4.0	4.0	4.0	4.0
Rice bran	10.0	6.2	6.9	3.1	3.8	0.0
Proximate composition (% in dry diet)						
Crude protein	46.3	46.1	50.1	49.8	54.2	53.2
Crude lipid	16.0	19.5	12.9	16.8	10.1	14.0
Crude fiber	3.6	3.0	3.3	2.5	2.8	2.3
Crude ash	13.2	12.8	13.8	13.7	14.5	14.1
Carbohydrate[3]	20.9	18.6	19.9	17.2	18.4	16.4
Gross energy (kcal/g)[4]	5.0	5.2	4.9	5.1	4.8	5.0
Protein/energy	9.3	8.9	10.2	9.8	11.3	10.6
Total phosphorus	2.1	2.0	2.2	2.1	2.3	2.2

[1]Vitamin mixture (g 100 g dry diet): β-caroten 12.84; Vitamin D_3 1.29; Menadione $NaHSO_4$ 6.11; DL-α-Tocopherol Acetate (E) 51.33; Thiamine-Nitrate (B_1) 7.70; Riboflavin (B_2) 25.65; Pyridoxine-HCl (B_6) 6.11; Cyanocobalamine (B_{12}) 0.01; d-Biotin 0.77; Inositol 513.22; Nicotinic acid 102.63; Ca Panthothenate 35.93; Folic acid 1.92; Choline Chloride 1049.24; Aminobenoic acid 51.10; Cellulose 256.60.

[2]Mineral mixture (g/100 g dry diet): NaCl 143.72; $MgSO_4 \cdot 7H_2O$ 506.70; $NaHPO_4 \cdot 2H_2O$ 322.54; KH_2PO_4 886.93; $Ca(H_2PO_4) \cdot 2H_2O$ 502.26; Fe citrate 109.84; Ca Lactate 1209.47; $Al(OH)_3$ 0.69; $ZnSO_4 \cdot 7H_2O$ 13.20; $CuSO_4$ 0.37; $MnSO_4 \cdot 7H_2O$ 2.96; $Ca(IO_3)_2$ 0.56; $CoSO_4 \cdot 7H_2O$ 3.69.

[3]Carbohydrate = 100 – (% protein + % lipid + % fiber + % ash)

[4]Gross energy is calculated based on protein, 5.65 kcal/g; lipid, 9.45 kcal/g; carbohydrate, 4.10 kcal/g.

Fish and experimental design

The experiment was conducted at Kagoshima Prefectural Fisheries Technology and Development Center (Tenpozan, Kagoshima, Japan). Yellowtail (*Seriola quinqueradiata*) (180.5 g) were used as test animal and randomly allocated to 12 FRP circular tanks (2 ton / tank) with duplicate groups consisting of 20 fish each. The fish were fed the respective test diets at apparent satiation level, two times a day (morning and evening). Every 20 days all fish were anesthetized (FA100, 150 ppm eugenol solution) and weighed in bulk. The feeding trial conducted for 55 days. All rearing tanks were provided with continuous aeration and maintained under natural light/dark regime (12 : 12 h). Filtered seawater was continuously provided at a flow rate of 12 l/min. All tanks were fed with seawater from a single filtration system. Water temperature ranged between 23.9 °C ~ 30.0 °C during the trial. Feed intake was recorded by subtracting the amount of uneaten diet from total amount of diet fed on a dry weight basis.

Chemical analysis

Crude protein was determined by the Kjeldahl method with a Tecator Kjeltec System (1007 Digestion system, 1002 Distilling unit and Titration unit). Total lipid was determined using the Bligh and Dyer (1959) method. Ash and moisture contents were analyzed by standard methods (AOAC, 1990). P concentrations in the samples were determined photometrically by the method of Lowry and Lopez (1946).

Statistical analysis

Data were subjected to one- or two-way analysis of variance (package super-ANOVA, Abacus Concepts, Berkeley, California, USA). In the absence of interactions, one-way ANOVA of single factors was performed. Students's t test was used as the mean separation procedure when significant ($P < 0.05$) differences were found in the ANOVA. Survival was transformed by arcsine square root before statistical analysis.

Results

The growth performances of yellowtail are presented in Table 2. Final weight, weight gain (WG), feed intake (FI) and survival rate were not significantly influenced by the dietary treatments, however improving tendency was observed with increasing energy content in each protein level. FE (Feed efficiency) was significantly improved with increasing energy content. PE (Protein efficiency) significantly decreased with increasing dietary protein in both energy levels. In both energy contents, the best PE values were obtained from the fish fed diet containing 46%. In the same dietary protein level, high energy diets produced significantly better results of PE. No significant interaction between dietary protein and energy level was identified on measured growth parameters.

The intake, accumulation and loading rates of P and N (kg / t WG) are presented in Table 3. Intake and excretion of P were significantly increased with increasing dietary protein while P retention significantly decreased in both energy levels. The lowest excretion and the highest retention values of P were obtained from the fish fed the diet containing 46% protein with high energy. The effect of energy level on intake, excretion and retention of P was relatively limited in each protein level, however decreasing trend with increasing energy content was appeared with or without significant difference.

The intake, accumulation and loading rates of N showed similar pattern as observed in P (Table 3). Excretion rates significantly increased with increasing dietary protein in both energy levels. Significantly lower N excretion rate was obtained from fish fed the diet containing 46% protein with high energy content. N excretion rate significantly decreased with increasing energy in each dietary protein level. No significant interaction between dietary protein and energy level was identified on any P and N related parameters.

Table 2. Growth performances and feed utilization in yellowtail fed the test diets over 55 days[a]

Dietary Protein (%)	Dietary Lipid (%)	FW[1]	WG[2]	FI[3]	FE[4]	PE[5]	Survival[6]
46	L	441.3 ± 10.9	141.3 ± 7.1	471.0 ± 12.5	0.55 ± 0.0	1.23 ± 0.0	100.0 ± 0.0
	H	461.0 ± 3.9	160.6 ± 10.8	467.3 ± 4.7	0.61 ± 0.0	1.37 ± 0.0	97.5 ± 2.5
50	L	441.0 ± 6.1	141.7 ± 3.2	473.8 ± 9.3	0.55 ± 0.0	1.12 ± 0.0	97.5 ± 2.5
	H	477.4 ± 22.3	166.0 ± 5.4	485.8 ± 21.7	0.61 ± 0.0	1.28 ± 0.0	97.5 ± 2.5
54	L	461.2 ± 25.4	166.0 ± 5.4	507.3 ± 17.0	0.55 ± 0.0	1.05 ± 0.0	100.0 ± 0.0
	H	472.1 ± 1.7	167.1 ± 10.7	501.4 ± 0.5	0.55 ± 0.0	1.15 ± 0.0	95.0 ± 0.0
Two-way ANOVA							
Protein level		0.604	0.174	0.084	0.084	***0.001***	0.729
Energy level		0.114	0.054	0.941	***0.011***	***0.001***	0.134
Interaction		0.698	0.347	0.765	0.106	0.598	0.423

[a]Values are means of duplicate groups ± S.E.
[1]Final weight (g)
[2](%): ((Final body weight (g) – Initial body weight (g)) / Initial body weight (g)) x 100
[3]Feed intake, g/fish
[4]FE: Wet weight gain (g) / dry feed intake (g)
[5]PE: Wet weight gain (g) / protein intake
[6](%)

Table 3. Phosphorus and nitrogen budget per unit body weight gain (kg P/t BW gain) and nutrient retention in yellowtail fed the test diets over 55 days[a]

Dietary		*Phosphorus (kg P/t BW gain)*				*Nitrogen (kg N/t BW gain)*			
Protein (%)	Lipid (%)	Intake[1]	Accum.[2]	Excretion[3]	Retention[4]	Intake[1]	Accum.[2]	Excretion[3]	Retention[4]
46	L	36.5 ± 0.7	5.8 ± 0.1	30.8 ± 0.7	15.8 ± 0.2	134.6 ± 2.6	32.6 ± 0.2	102.0 ± 2.4	24.2 ± 0.3
	H	34.6 ± 0.8	5.8 ± 0.1	28.9 ± 0.8	16.7 ± 0.3	122.1 ± 3.0	32.4 ± 0.0	89.7 ± 3.0	26.7 ± 0.7
50	L	38.5 ± 0.9	5.8 ± 0.0	32.7 ± 0.9	15.1 ± 0.4	146.1 ± 3.5	33.4 ± 0.4	112.7 ± 3.9	22.9 ± 0.8
	H	35.9 ± 0.5	5.8 ± 0.0	30.1 ± 0.5	16.3 ± 0.1	130.8 ± 1.9	32.5 ± 0.0	98.3 ± 1.9	24.9 ± 0.4
54	L	40.4 ± 1.3	5.7 ± 0.0	34.7 ± 1.3	14.2 ± 0.5	156.2 ± 5.0	33.5 ± 0.2	122.6 ± 4.8	21.5 ± 0.6
	H	39.1 ± 0.8	5.8 ± 0.1	33.2 ± 0.8	15.2 ± 0.2	147.5 ± 3.2	33.3 ± 0.3	114.2 ± 2.8	22.6 ± 0.3
Two-way ANOVA									
Protein level		***0.008***	0.548	***0.007***	***0.004***	***0.001***	0.122	***0.001***	***0.002***
Lipid Level		***0.034***	0.445	***0.025***	***0.003***	***0.004***	0.058	***0.005***	***0.006***
Interaction		0.744	0.548	0.788	0.823	0.629	0.343	0.662	0.499

[a]Values are means ± SE. (*P*>0.05).
[1]{(Feed intake (g/fish) X P or N concentration in diet (%) / 100) / (Mean body weight gain (g))} X 1000
[2]{(Final mean body weight (g) X Final whole body P or N concentration (%) / 100) – (Initial mean body weight (g) X Initial whole body P or N concentration (%) / 100)} / (Mean body weight gain (g)) X 1000
[3]P or N intake (g/kg body weight gain) – P or N accumulation (g/kg body weight gain)
[4]P or N accumulation (g/kg body weight gain) X 100) / P or N intake (g/kg body weight gain)

Discussion

Dietary protein level varying from 46 and 54% in the present study did not influence the WG of fish. However, an obvious improving tendency in WG at the any level of dietary protein towards to high-energy diet from the low energy was observed. Although no significant difference was identified between two energy levels, the significant level of 0.0536 (Table 2) must be considered as a sort of indication that might reflect the occurrence of possible significant difference between two energy levels if the experimental period was further extended. It would also be another sign presenting the higher energy level compared to mentioned one could have resulted higher WG.

Dietary energy level seems to be more important than the protein level in yellowtail nutrition. They could tolerate the high lipid as energy source, which have positive effect on protein utilization. This common phenomenon is called as protein-sparing effect in animal nutrition. Results indicated that yellowtail fed the diets containing higher levels of energy (lipid-based) had improved FE and PE (Table 2) compared to those fed the diets with lower lipid levels in any levels of dietary protein. These findings suggest that the high dietary lipid content exerted a protein-sparing effect. Such an effect has been well documented in rainbow trout and other species (Satoh et al. 2001; Weatherup et al., 1997; Steffens et al., 1999; Arzel et al., 1994; Kaushik & Medale, 1994; Kim & Kaushik, 1992). The improvement in protein utilization results from an increasing contribution of the non-protein energy sources (predominantly lipid but also some carbohydrates in some cases) to overall energy expenditure of the fish. Consequently, less ingested protein is catabolized for energy purposes. Based on the growth data, the diet containing 46% protein and 19.5% lipid with 5.2kcal/g energy could be recommended to obtain optimum growth compared to other experimental groups.

In the present study, PE significantly decreased with increasing dietary protein level. When the protein utilization of the fish fed to low protein diets was compared with those of fish fed the high protein diets, it appeared that the lower protein concentration in diets supported better PE (Chan et al., 2002). Thus, the high protein diets may have provided excess amounts of protein to the fish with the respect to the most efficient conversion of dietary protein into body protein. Hence, excess amount of protein is catabolized, and protein utilization decreases. Increasing N excretion and decreasing N retention with increasing dietary protein level at the same energy contents (Table 2) also supports this finding.

A nutritional strategy to reduce the waste load in fish is to produce high energy diets by lowering the protein level and subsequently increasing lipid levels. Hence, the increase in energy concentration improves feed conversion (Satoh et al., 2004). In the present study, increasing dietary lipid improved N retention (%) and thereby lowered its excretion (kg / t) (Table 1, Table 2). Another possible explanation of lowering N excretion with increasing energy content at each protein level might be lowering N intake. Although no significant difference was observed on feed intake, the amount of protein and lipid in digested feed must vary since the proximate composition of the diets is different. Therefore, at the same protein level, N intake of the fish consumed the diet with high lipid must be higher than those consumed the diet containing lower lipid. The reason of increasing N excretion with increasing dietary protein level at the same energy level is increasing N intake due to the increasing protein.

The P intake and excretion showed same tendency with N. Therefore, the mentioned explanation above is still valid for also in the case of P. Lowering P excretion with increasing energy at same protein level is due to decreasing P from FM since the amount of protein in digested feed decreased as explained above. Increasing dietary protein is responsible of increasing P excretion due to the increasing fishmeal, therefore increasing P, in the diets.

Earlier studies on environmentally friendly feeds for aquaculture have already emphasized that retention efficiencies of nutrients such as P and N are important for the evaluation of feed quality (Lall, 1991; Cho et al., 1994). In this regard, the results of present study demonstrated that the diet containing 46% protein and 19.5% lipid with the energy content of 5.2kcal/g for yellowtail nutrition from ~ 180 g to ~ 460 g could be recommended to obtain the lowest P and N excretion values without causing retardation on growth performances under the present experimental conditions.

Conclusion

Yellowtail farming is an increasingly widespread marine fish species not only in Japan but also in different parts of the world with suitable farming conditions. Therefore, environmental and financial feed optimization is of great importance. In this study, the lowest environmental impact was obtained with diet containing 46% protein and 19.5% lipid with the energy content of 5.2kcal/g for yellowtail nutrition from ~ 180 g to ~ 460 g.

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Informed consent
Not available

Data availability statement
The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest
There is no conflict of interests for publishing this study.

Contribution of authors
Orhan Uyan : Conceptualization, Data curation, Formal analysis, Writing draft
Shunsuke Koshio : Funding acquisition, Investigation, Methodology
Manabu Ishikawa : Project administration, Resources, Supervision, Validation
Saichiro Yokoyama : Resources, Supervision, Validation, Visualization
All authors have read and agreed to the published version of the manuscript.

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