

Effect of Japanese sandfish (*Arctoscopus japonicus*) meal in paste diet for *Anguilla japonica* in terms of growth, feed efficiency and digestibility

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Abstract

In this study, the effect of replacement of high-quality fish meal (HQFM) with Japanese sandfish (*Arctoscopus japonicus*) meal (SFM) and the utility of a viscous culture broth of *Leuconostoc mesenteroides* (VCL) as binding material in paste diet for Japanese eel (*Anguilla japonica*) was tested in terms of growth, feed efficiency and digestibility. Four experimental paste diets contained, fish meal and binding materials as, HQFM and starch (F0, control), SFM and VCL (F1), SFM and a mixture of starch and VCL (F2) and SFM and starch (F3). Each diet was formulated to be iso-nitrogenous and iso-lipidic. Each group of 10 juvenile eels with an average initial body weight of 20 ± 2.5 g were randomly distributed into 15 tanks in triplicates and fish were fed twice daily with one of the 4 experimental paste diets for 90 days. Eels fed with diet F2 exhibited good growth performance (final mean weight; 55.3 ± 0.67 g, WGR; $169 \pm 5\%$), being similar to eels (55.5 ± 0.26 g, $170 \pm 7\%$) fed a control diet ($p > 0.05$). In spite of iso-nitrogenous and iso-lipidic diets, the feed efficiency of F1 and F3 were inferior to other diets and the same trend was observed in growth performance also, probably due to reduced feed intake owing to poor water stability of the feeds. It should be considered that incorporation of SFM could lead to the discharge of undesirable matters such as phosphorus into the water environment, though it would contribute to lowering production costs. Therefore, further investigations are needed to improve the quality of SFM.

Introduction

Eel (*Anguilla* sp.), widely farmed worldwide, has great nutritive and commercial value. Japanese eel (*Anguilla japonica*) is an important cultured species in East Asia. Almost all the eels farmed are those reared from wild glass eels, and it was not until recent years that artificial seedling production and closing of full-lifecycle in captivity could be introduced (Tanaka, 2015; Castonguay and Durif, 2016; Nomura *et al.*, 2018). Eel is a carnivore fish demanding high protein diet and is fed on artificial diet prepared incorporating high-quality fish meal (HQFM) such as herring meal, anchovy meal and mackerel meal. With the growth of the aquaculture industry, the demand for fish meal is ever increasing

and the fluctuation in the price of fish meal exerts influence on fish production from farming.

Studies on alternative protein sources for fish feed mainly focused on plant proteins such as soybean meal and sunflower seed meal, as well as animal proteins such as meat meal, bone meal and feather meal (Ai *et al.*, 2006; Hernandez *et al.*, 2014; Delgado *et al.*, 2021). Proteins sources from plants are limited because of their deficiencies in essential amino acids/minerals and the presence of anti-nutritional factors (Tibbetts *et al.*, 2006; Olsen *et al.*, 2007; Zhang *et al.*, 2015), and those from animals, also are limited because of the potential risk of bovine spongiform encephalopathy and avian influenza (Yoshitomi *et al.*, 2006).



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Alternate protein from marine sources such as trash fish, fish byproducts, krill and crabs have attracted fish farmers' attention because of good protein quality and palatability (Uyan *et al.*, 2006; Alexandra *et al.*, 2011; Bunlipatanon *et al.*, 2014; Damusaru *et al.*, 2019).

Japanese sandfish (*Arctoscopus japonicus*) is distributed in the north-west Pacific, including north of the Korean East Sea, coastal regions of Hokkaido, Japan and Sakhalin, Russia. The species is reported to form an abundant fishery in Democratic Peoples' Republic (DPR) of Korea. Japanese sandfish contains less protein than that, but the amino acid composition is similar, except for lower contents of proline and glycine, to that of herring (Asano and Gudo, 1978). Also, essential amino acids form 43.5% of the total amino acids, indicating high biological value (Orlova and Kuranova, 1977). Owing to this, Japanese sandfish is one of the preferred species commonly used for fish sauce production (Klomklao *et al.*, 2009) and is worthy as a potential candidate for fish meal production. So far, there is no report on fish meal production and commercial availability based on Japanese sandfish. It is a locally available fishery resource, and its availability in fish meal production would help to reduce the cost of protein and feed for fish farming in DPR Korea.

Chiu and Pan (2002) reported that when HQFM is included up to about 70% in the eel diet and the dietary protein level is approximately 45%, it is sufficient to meet the protein requirement of eel. For the replacement of HQFM by sandfish meal, low protein levels should be compensated by increasing the fish meal content in the diet; this makes inevitably the other ingredients e. g. wheat flour and corn starch (pre-gel) to be reduced, and as a result, the energy balance of diet could be affected.

Also, the increase of fish meal content and decrease of substances for pre-gel in diet composition results in

increasing the dispersibility of diet particles in water. In this study, we proposed a way of using Japanese sandfish meal (SFM) as an alternative to HQFM, taking priority of formulation of eel diet using a viscous culture of *Leuconostoc mesenteroides* (VCL) as supplemental binding material. When *L. mesenteroides* grows in broth containing sucrose, the broth becomes highly viscous due to the concomitant formation of dextran, which is currently used in production of stabilisers and thickeners in food and cosmetic sector (Naessens *et al.*, 2005; UL Qader *et al.*, 2005). The present study aimed to investigate the feasibility of SFM as a protein source in eel diet and VCL as binding material for paste diet.

Materials and methods

Formulation and preparation of diets

Diets were formulated with ingredient composition as shown in Table 1. Four experimental paste diets containing fish meals and binding materials as HQFM and starch (F0, control); SFM and VCL (F1); SFM and a mixture of starch and VCL (F2) and SFM and starch (F3) were prepared. Each diet was formulated to be iso-nitrogenous and iso-lipidic. Sardine oil was included in the formulation as a lipid source; vitamin-mineral premixes as source of micronutrients; and starch was included as source of carbohydrate and to ensure water stability. Diets for digestibility assay were supplemented with 0.5% chromic oxide (Cr_2O_3) as an inert marker.

All diets were prepared in paste form. F1 and F2 diet formulations were prepared with VCL as binder. VCL was cultured under shaking for 20 h until a viscosity of 30 cP was reached. The viscous cultures were added to the premixes of F1 and F2 at a concentration of 40 and 22 ml 100 g⁻¹ premix, respectively, and then stirred uniformly to make a

Table 1. Ingredient composition of experimental diets (Dry matter %)

Ingredients	Cost per kg (USD)	Diets			
		F0	F1	F2	F3
SFM	0.75	-	77	77	77
HQFM	1.24	68	-	-	-
Soybean meal	0.56	5	5	5	5
α -starch	0.25	20	-	5	11
VCL*	0.11	-	11	6	-
Sardine oil	0.54	3	3	3	3
Vitamin premix ¹	3.25	2	2	2	2
Mineral premix ²	2.34	2	2	2	2

*1g Dry matter comprises dextran 0.55 g, fructose 0.34 g and the others 0.11g

¹ Vitamin premix: Vitamin A-6000 IU; Vitamin D-4000 IU; Vitamin E-250 IU; Vitamin K-30 mg kg⁻¹; Ascorbic acid, 200 mg kg⁻¹; Inositol-400 mg kg⁻¹; Thiamin-50 mg kg⁻¹; Biotin-1 mg kg⁻¹; Niacin-250 mg kg⁻¹; Pyridoxine-40 mg kg⁻¹; Vitamin B12-1 mg kg⁻¹

²Mineral premix (mg kg diet⁻¹): $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -20.0; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ -40.0; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ -50.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -50.0; KI-5.5; Na_2SeO_3 -1.0; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ -8.0; NaF-4.0

dough (diameter of approximately 100 mm). The other two diet formulations (F0 and F3) were formulated with starch as binder.

Water stability of paste diets

The experimental paste diets were prepared into paste forms of dough mass, placed respectively in beakers into which equal amounts of water (25°C, 2 l) were added. Those were allowed to stand with occasional gentle shaking for 10 s every 2 min. At every 5 min, samples of the paste diets were taken out of water and dried until the weights were constant. Water stability of diets was calculated as percentage of the final weight to initial weight.

Experimental feeding

Juvenile eels (*A. japonica*) with an average initial body weight of 20 ± 2.5 g were acclimatised for 2 weeks prior to initiation of the experiment. Each group of 10 juvenile eels was randomly distributed into 15 tanks in triplicates. Fish, were fed twice daily with one of the 4 experimental paste diets to visual satiety for 90 days. Water was supplied to each tank at a flow rate of 1 l min^{-1} and maintained at $25 \pm 0.2^\circ\text{C}$. Faecal matter was collected by siphoning, freeze-dried and stored at -20°C for analysis.

Biochemical analyses

The proximate composition of the diets and the fish body were determined as per AOAC (1995). Dry matter was calculated by gravimetric analysis after drying at 105°C . The Kjeldahl method determined crude protein content using an auto Kjeldahl System (8400 Auto analyzer, Sweden). Crude lipid was determined by ether extraction using a Soxhlet extractor. Ash content was gravimetrically determined by combustion in a furnace at 550°C . The amino acid composition was determined using a high-performance liquid chromatography (HPLC, Shimadzu, Japan). Energy content was calculated based on the standard physiological values, (4.5 Kcal per g of protein, 3.3 Kcal per g carbohydrate and 8 Kcal per g lipid). Each analysis was carried out in triplicates.

Analysis of growth performance, feed utilisation and digestibility

Growth performance was estimated as weight gain ratio (WGR) and specific growth rate (SGR):

$$\text{WGR (\%)} = 100 \times (W_f - W_i) / W_i$$

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times (\ln W_f - \ln W_i) / t$$

where W_i = Initial body weight, W_f = Final body weight and t = Experimental duration in days.

Feed utilisation was estimated as feed efficiency (FE) and protein efficiency ratio (PER):

Feed efficiency (FE) = Weight gain (g, Wet weight) / Feed consumed (g, Dry weight);

Protein efficiency ratio (PER) = Weight gain (g, Wet weight) / Protein intake (g, Dry weight).

Digestibility was expressed as apparent digestibility coefficient (ADC) for dry matter (DM), crude protein (CP), crude lipid (CL) and N-free extractive (NFE). Eels were fed diets supplemented with chromic oxide as an inert marker. The chromic oxide content in faeces were measured according to the method described by Furukawa and Tsukahara (1966).

The following formulae calculated the apparent digestibility coefficient (ADC):

$$\text{ADC}_{\text{DM}} = 100 - [100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in diet} / \% \text{ Cr}_2\text{O}_3 \text{ in feces})]$$

$$\text{ADC}_{\text{CP}} = 100 - [100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in diet} / \% \text{ Cr}_2\text{O}_3 \text{ in feces}) \times (\% \text{ Protein in feces} / \% \text{ Protein in diet})]$$

$$\text{ADC}_{\text{CL}} = 100 - [100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in diet} / \% \text{ Cr}_2\text{O}_3 \text{ in feces}) \times (\% \text{ Lipid in feces} / \% \text{ Lipid in diet})]$$

$$\text{ADC}_{\text{NFE}} = 100 - [100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in diet} / \% \text{ Cr}_2\text{O}_3 \text{ in feces}) \times (\% \text{ NFE in feces} / \% \text{ NFE in diet})]$$

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) and the significance of differences between means was tested using Duncan's multiple range tests using SPSS 10.0 Software. The significance was tested at a 95% confidence level and significant differences were considered when $p < 0.05$.

Results and discussion

In this study, four different paste diets (F0, F1, F2 and F3) were tested: F0 containing HQFM as a protein source was used as control. F1, F2 and F3 contained SFM as protein source, with differences in diet formulation. F1 and F3 contained VCL or starch as a binder, while F2 contained both VCL and starch. The proximate composition and amino acid profile of the experimental diets are given in Table 2 and 3 respectively. The relative comparison of water stability of paste diets showed that F2 was acceptable compared to F0. The water stability of F1 and F3 was poorer than that of the other two diets (Table 4).

Table 2. Proximate composition of experimental diets

Parameter	Diets			
	F0	F1	F2	F3
Moisture (%)	7.5	7.8	7.6	7.8
Crude protein (%)	45.5	45.4	45.4	45.4
Crude lipid (%)	12.9	13.0	12.9	12.85
Crude fiber (%)	0.22	0.15	0.21	0.2
Ash (%)	13.65	21.9	22.54	21.4
N-free extract (%)	20.17	11.67	11.3	12.3
Energy (MJ kg ⁻¹)	15.56	15.52	15.50	15.53
Digestible protein (DP)	38.6	38.4	38.4	38.4
Digestible energy (DE)	14.0	13.98	13.95	13.98
DP/DE (g MJ ⁻¹)	27.5	27.4	27.5	27.4

Table 3. Amino acid profile and proximate composition of SFM and HQFM

Amino acid profile (% protein)	SFM	HQFM
Alanine	5.68	5.72
Arginine	6.18	6.32
Aspartic acid	10.52	10.41
Cysteine	2.44	1.12
Glutamic acid	18.35	16.24
Glycine	3.48	5.86
Histidine	2.68	2.72
Isoleucine	4.86	4.95
Leucine	8.44	8.42
Lysine	8.08	8.24
Methionine	2.78	2.58
Phenylalanine	4.62	4.85
Proline	2.02	3.72
Serine	4.79	4.29
Threonine	4.72	4.85
Tyrosine	3.62	3.21
Valine	6.72	6.48
% Essential amino acid	44.46	44.56
Proximate composition (%dry matter)		
Crude protein	66.9	58.9
Crude lipid	14.5	11.4
Ash	15.2	26.2

Growth and feed utilisation of eels fed with various paste diets are shown in Tables 5 and 6 respectively. During the trial, WGR and SGR of eels fed with F0 and F2 were significantly higher than those of the other eels ($p < 0.05$) with $170 \pm 7\%$, $1.11 \pm 0.01\% \text{ day}^{-1}$ and $169 \pm 5\%$, $1.10 \pm 0.02\% \text{ day}^{-1}$, respectively. Eels fed with F2 were similar to those with F0 in all growth performance profiles. Survival of eel was not affected by diet. Feed efficiency analysis indicated that F2 was the best among all experimental diets inclusive of SFM with FE of 0.72 ± 0.02 and PER of 1.59 ± 0.05 , similar to F0 ($p > 0.05$). Protein efficiency ratio data showed that all the experimental diets containing SFM were inferior to diets containing HQFM, but that of F2 was slightly lower than that of F0. The feed efficiency profiles of F1 and F3 were inferior compared to that of F0 and F2.

Similar growth and feed efficiency response to F0 and F2 are probably due to the dietary protein quality level, and energy balance. The crude protein level and DP/DE of F2 and F0 were 45.5% , 27.5 g MJ^{-1} and 45.4% , 27.5 g MJ^{-1} respectively (Table 2), indicating similarity. At the same time, the essential amino acid level of sandfish meal was comparable to that of high-quality fish meal (Table 3). Our results are also supported by the report of Higuera *et al.* (1999), where eels fed diets containing meat meal or sunflower meal exhibited dietary utilisation and growth indices poorer than those fed diets containing fishmeal and essential amino acid supplementation significantly improved the performance of the diet containing sunflower meal.

Despite being iso-nitrogenous and iso-lipidic, the feed efficiencies of F1 and F3 were inferior to other diets and the same trend was observed in the growth performance of fish. In the case of F1, the absence of starch results in a deficiency of carbohydrates, though there are some saccharides such as sucrose and fructose in the *L. mesenteroides* culture, of which dextran is a non-digestible polysaccharide and unusable as an energy source. At such a low carbohydrate level, dietary lipids may be used not only as an energy source but as a precursor for gluconeogenesis (Suarez *et al.*, 1995). This may more or less limit the consumption of dietary lipids for energy sources only and consequently result in energy deficiency. Further, dietary protein may not be used for normal

Table 4. Water stability of experimental diets (%)

Time (min)	Diets*			
	F0	F1	F2	F3
5	99.5	99.5	99.5	99.5
10	99.3	98.6	99.3	98.6
15	97.4	92.4	97.5	94.5
20	91.1	84.1	91.1	87.2
25	84.4	74.4	82.6	75.8
30	80.4	68.5	80.4	70.5

*Diets were made in mass of dough with 100 mm of diameter

protein metabolic function, and some of that may be used as an inefficient energy source. Xu *et al.* (2015) explained this phenomenon, from the hypothesis that a proportion of protein would be deaminated and the carbon skeleton used as an energy source. The entire whole-body protein content ($13.2 \pm 0.42\%$) of eels fed with diet F1 was significantly lower than that (14.2 ± 0.25) of eels fed with diet F2 ($p < 0.05$). Like this, the metabolism of dietary protein is considerably relative to imbalance with energy in the diet, which is often defined as 'protein sparing effect' with non-protein energy sources (Vergara *et al.*, 1996; Kim and Lee, 2005; Ng *et al.*, 2008; Li *et al.*, 2010; Amin *et al.*, 2014).

On the other hand, the water stability of diet influences not only feed efficiency and growth performance but also water quality. As mentioned above, F1 and F3 had low water stability, which negatively affected feed intake (Table 6). Especially, F3 lost 30% of its weight after 30 min in water, resulting in reduced feed intakes. Reduced feed intake could be one of the reasons for a lower weight gain in the fish since lower

feed intake would reduce the amount of nutrients available for growth (Uyan *et al.*, 2006).

It should be noted that SFM used in the present study was not very acceptable, though the quality of protein included was fine. Higher levels of ash in diets containing SFM must be considered in terms of feed formulation for beneficial growth of fish and environmental impacts. There was no significant difference between the contents of whole-body ash of all eels (Table 7), which may, unfortunately, reflect that more undesirable matters such as phosphorus would be discharged into the environment. Further study for producing high-quality fish meal from sandfish is needed.

In treatments with F1 and F2, apparent digestibility coefficients for NFE were significantly lower than in those with F0 and F3 (Table 8), probably due to the presence of dextran in the *L. mesenteroides* culture. Despite its non-digestible property, dextran is widely used as a feed additive in poultry and livestock (Bozkurt *et al.*, 2008). It was

Table 5. Growth performance of eels fed paste diets containing SFM (means $\pm$ SD, $n=3$)

Diets	Mean initial weight (g)	Mean final weight (g)	WGR (%)	SGR (% day ⁻¹)	Survival (%)
F0*	20.5 $\pm$ 0.42	55.5 $\pm$ 0.26 ^a	170 $\pm$ 7 ^a	1.11 $\pm$ 0.01 ^a	97.2 $\pm$ 1.2 ^a
F1	20.2 $\pm$ 0.25	51.6 $\pm$ 0.55 ^b	155 $\pm$ 3 ^b	1.04 $\pm$ 0.02 ^b	97.2 $\pm$ 0.2 ^a
F2	20.5 $\pm$ 0.37	55.3 $\pm$ 0.67 ^a	169 $\pm$ 5 ^a	1.10 $\pm$ 0.02 ^a	97.5 $\pm$ 0.8 ^a
F3	20.3 $\pm$ 0.13	50.2 $\pm$ 0.46 ^b	147 $\pm$ 2 ^c	1.01 $\pm$ 0.03 ^b	97.3 $\pm$ 0.2 ^a

*F0 contains HQFM as protein source

Values within the same column with same superscripts are not significantly different ($p > 0.05$)

Table 6. Feed efficiencies of eels fed paste diets containing SFM (means $\pm$ SD, $n=3$)

Diets	Feed intake (g per fish)	Protein intake (g per fish)	FE	PER
F0*	48.3 $\pm$ 0.28 ^a	21.6 $\pm$ 0.26 ^a	0.73 $\pm$ 0.06 ^a	1.62 $\pm$ 0.05 ^a
F1	41.2 $\pm$ 0.54 ^b	18.7 $\pm$ 0.32 ^b	0.55 $\pm$ 0.01 ^b	1.26 $\pm$ 0.02 ^b
F2	48.4 $\pm$ 0.35 ^a	21.5 $\pm$ 0.14 ^a	0.72 $\pm$ 0.02 ^a	1.59 $\pm$ 0.05 ^a
F3	37.3 $\pm$ 0.26 ^c	16.9 $\pm$ 0.07 ^c	0.43 $\pm$ 0.05 ^c	1.19 $\pm$ 0.03 ^c

*F0 contains HQFM as protein source

Values within the same column with same superscripts are not significantly different ($p > 0.05$)

Table 7. Proximate composition of whole body in the experimental eels (means $\pm$ SD, $n=3$)

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Energy (kJ g ⁻¹)
Initial	68.4	15.8	11.6	3.5	7.0
F0*	67.8 $\pm$ 0.5 ^a	14.3 $\pm$ 0.34 ^a	12.5 $\pm$ 0.24 ^a	2.4 $\pm$ 0.67 ^a	7.8 $\pm$ 0.56 ^a
F1	67.7 $\pm$ 0.8 ^a	13.2 $\pm$ 0.42 ^b	12.7 $\pm$ 0.12 ^a	3.2 $\pm$ 0.12 ^a	7.3 $\pm$ 0.45 ^a
F2	64.3 $\pm$ 1.2 ^b	14.2 $\pm$ 0.25 ^a	12.8 $\pm$ 0.28 ^a	3.2 $\pm$ 0.25 ^a	7.2 $\pm$ 0.27 ^a
F3	65.8 $\pm$ 0.6 ^{ab}	13.9 $\pm$ 0.53 ^{ab}	13.1 $\pm$ 0.65 ^a	2.8 $\pm$ 0.12 ^a	7.3 $\pm$ 0.55 ^a

*F0 contains HQFM as protein source

Values within the same column with same superscripts are not significantly different ($p > 0.05$)

Table 8. Apparent digestibility coefficient for dry matter, protein, lipid and N-free extract (means $\pm$ SD, $n=3$)

Apparent digestibility coefficient	Diets			
	F0*	F1	F2	F3
ADC _{DM}	84.8 $\pm$ 0.75 ^a	84.1 $\pm$ 0.87 ^a	84.2 $\pm$ 0.54 ^a	80.2 $\pm$ 1.43 ^b
ADC _{CP}	92.3 $\pm$ 0.72 ^a	88.2 $\pm$ 0.66 ^b	90.5 $\pm$ 1.25 ^{ab}	85.4 $\pm$ 0.85 ^c
ADC _{CL}	87.7 $\pm$ 0.75 ^b	92.2 $\pm$ 1.07 ^a	91.5 $\pm$ 0.45 ^a	89.2 $\pm$ 1.12 ^{ab}
ADC _{NFE}	22.6 $\pm$ 0.75 ^a	19.8 $\pm$ 1.08 ^b	17.4 $\pm$ 1.12 ^b	21.8 $\pm$ 0.58 ^a

*F0 contains HQFM as protein source

Values within the same row with same superscripts are not significantly different ($p>0.05$)

already reported that dextran produced by lactic acid bacteria exhibited antiviral and immunomodulatory activity against fish viruses (Nacher-Vazquez *et al.*, 2015). Also, dextran-producing bacteria such as *Lactobacillus* and *Leuconostoc*, have a probiotic activity such as improvement of the intestinal microflora in fish (Vazquez *et al.*, 2005; Munoz-Atienza *et al.*, 2015). Therefore, it appears reasonable that VCL was selected as a supplementary binder in this study.

Replacement of HQFM by SFM in the Japanese eel diet was feasible by using VCL as a supplementary binding material. The effect of diet F2 containing SFM was as good as that of diet F0 containing HQFM, in terms of growth, feed efficiency and digestibility. This study would contribute to reducing the production cost in eel aquaculture by using cheaper fish meals (Table 9).

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Table 9. Cost of feed and feed cost per kg of fish produced using the different diets

Diet	Parameter	
	Cost per kg of feed produced (USD kg ⁻¹)	Feed cost per kg of fish produced (USD kg ⁻¹)
F0	0.934	1.825
F1	0.745	2.044
F2	0.751	1.223
F3	0.760	1.904

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