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Project Title: Replacement of Fish Meal and Fish Oil in Practical Diets for Japanese Sea Bass (*Lateolabrax japonicus*): III. Utilization of alternative fats in diets of Japanese sea bass.

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INDUSTRY SUMMARY

1. Introduction

Comparing with fish oil, territorial animal fats (lard, tallow, poultry fat) and plant oils (soy oil and corn oil) are with lower n-3 HUFA and lower cost. The least cost formulation for high-energy diets should be based on meeting the optimal essential fatty acids requirements by fish oil and/or soy oil, and use low cost fats as the clean energy sources.

China is the largest production of aquatic animals, and occupies more than 60% of aquatic outputs in the world. Although the cyprinids (Common carp, gible carp, grass carp etc.) are the main species for aquaculture, marine culture are being developed rapidly, which enhanced the development of high-lipid diet at the same time.

Japanese sea bass is an important marine-culture species in China and in recent years, has been cultured in fresh water in a large scale for it is a euryhaline fish. Lipid requirements for Japanese sea bass still were not reported. Most nutrition data are from the results of other related species (Navas et al., 1995; Asturiano et al. 2001). Previous research has shown that most terrestrial animal proteins (poultry-by-product, meat and bone meal and blood meal) can be very valuable ingredients for Japanese sea bass (Xue et al., not published data). It is also believed that animal fats could replace up to 50% of the fish oil into salmonid diets and reduce the diet cost (Bureau, personal communication).

2. Objectives

The objectives of the present study were to investigate the effects of replacement of fish oil by 3 territorial animal fats (lard, tallow and poultry fat), 2 plant oils (soy oil and corn oil) and a mix-fat (tallow, 60%; soy oil, 20%; fish oil, 20%) on growth and fatty acid profiles in muscle and liver of Japanese sea bass.

3. Industry summary

The present study showed that 50% of alternative fats replacement did not significantly affect fish growth performance, feed intake (palatability) and hepatosomatic index. Feed conversion rate and protein efficiency ratio in fish consuming the poultry fat diet were significantly lower

than those of soy oil and corn oil diets. Apparent digestibility of dry matter for pork lard was highest, and significantly higher than poultry fat diet. Significant differences in carcass moisture and liver lipid content were observed among the dietary treatments. The fatty acid composition of fillet largely reflected that of the diets, while that of liver were almost not affected. Except poultry fat group, other alternative fats using group got lower producing cost than fish oil group. The price of soy oil in the international market is going up after May 2003. It brings along the increasing price of other plant oil price. Relatively the costs of territorial animal fats were lower, but the safety using of territorial animal sources should be considered.

Replacement of Fish Meal and Fish Oil in Practical Diets for Japanese Sea Bass (*Lateolabrax japonicus*): III. Utilization of alternative fats in diets of Japanese sea bass.

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ABSTRACT

The studies were conducted to study the effects of replacement of fish oil by 3 territorial animal lipids (lard, tallow and poultry fat), plant oils (soy oil and corn oil) and a mix-fat (tallow, 60%; soy oil, 20%; fish oil, 20%) on growth and fatty acid profile in fillet and liver of Japanese sea bass. 7 basal diets were formulated, 10% of anchovy fish oil was used in control diet, 5% of alternative fats were used in other 6 diets. In the 8 weeks growth experiment, 28 tanks of fish were equally divided into 7 groups. Before and after the trial, fish of each tank were batch weighed and the samples of muscle and liver were collected to do the chemical analysis, for example moisture, crude protein, crude lipid, ash, and fatty acid composition.

Specific growth rate (SGR), feed intake and hepatosomatic index (HSI) fed the various experimental diets were not significantly different ($P>0.05$). FCE and PER of alternative groups were not significantly different from that of FO group, but the FCE and PER in fish consuming the PF diet were significantly lower than those of SO and CO diets. Alternative fat significantly affect the apparent digestibility of dry matter (ADC_d), but did not show significant effect on the apparent digestibility of protein (ADC_p) and apparent digestibility of lipid (ADC_l). ADCd of PL diet was highest, and significantly higher than PF diet. Significant differences in carcass moisture and liver lipid content were observed among the dietary treatments. The fatty acid composition of fillet largely reflected that of the diets, while that of liver were almost not affected.

Key word: Alternative fats, Japanese sea bass (*Lateolabrax japonicus*), fatty acids, growth

1. Introduction

In China, although the cyprinids (common carp, grass carp, gibel carp and black carp etc.) are still the main species for aquaculture, marine culture and some fresh water carnivorous species are being developed rapidly, which will enhance the development of high-lipid diet at the same time. Japanese sea bass (*Lateolabrax japonicus*), a widely cultured fish species, could be raised both in salty and fresh water because it is a euryhaline fish. However, although Japanese sea bass is one of the most important species for marine aquaculture in China, lipid requirements for Japanese sea bass still were not reported. Most nutrition requirements reports are from the results of European sea bass juveniles (*Dicentrarchus labrax*) and other related species (Navas et al., 1995; Geurden et

al., 1997; Company et al., 1999; Asturiano et al., 2001.). Along with the more culturing areas were developed, the market price of the fish was decreased rapidly. Thus, the relatively low value brings about which cannot be economically raised on expensive feed containing high levels of fish meal and fish oil. Previous research has shown that most territorial animal proteins (poultry-by-product, meat and bone meal and blood meal) can be very valuable ingredients for Japanese sea bass (Xue et al., not published data). Besides, the protein-sparing effect of dietary lipid in fish has been reported for both tropical and cold water fish species (DeSilva et al., 1991; Stowell and Gatlin, 1992; Chou and Shiau, 1996; Hemre and Sandnes, 1999; Gaylord and Gatlin, 2000). However, lipid resources of most studies were based on the fish oil, and most works have been carried out with salmonids and just a few reports of alternative oil utilization in fish feeds were reported (Arzel et al., 1994; Buzzi et al., 1996; Hemre and Sandnes, 1999). Marine fish oils and most vegetable oils, which contain a high proportion of polyunsaturated fatty acid (PUFA) are very susceptible to peroxidation. Hereby, the least cost formulation for high-energy diets should be based on meeting the optimal essential fatty acids requirements by fish oil and/or soy oil, and use the territorial animal fats, such as lard, poultry fat, tallow etc, which with high energy and low PUFA and low cost as the clean energy sources. The objectives of the present study were to investigate the effects of replacement of fish oil by 3 territorial animal fats (lard, tallow and poultry fat), 2 plant oils (soy oil and corn oil) and a mix-fat (tallow, 60%; soy oil, 20%; fish oil, 20%) on growth and fatty acid profiles in muscle and liver of Japanese sea bass.

2. Materials and methods

2.1 Experimental diets

According to the results of hybrid striped bass (*Morone chrysops* × *M.saxatilis*) showed that the fish fed 15% lipid achieved highest weight gain (Gaylord and Gatlin, 2000). 7 basal diets (47% crude protein, 15% crude lipid) were formulated. The alternative lipid sources tested in the experiment were fish oil as the control diet (FO), pork lard (PL), tallow (TL), poultry fat (PF), mix-fat (MF), soy oil (SO) and corn oil (CO) replaced 50% of fish oil of the control diet (Table 1). Each diet will be individually marked with Y_2O_3 at 100 mg/kg as inert marker for digestibility measurement. The diets will be made into dry pellets using an extruder (EXT50A, Φ1.5mm).

Fish oil was added in small amount (5% of uncoated extruded pellet weight) during the extrusion process, then the alternative lipids (5%) were coated to obtain seven isonitrogenous and isolipidic (47% protein and 14.5% total lipid) diets.

2.2 Fish, holding conditions and procedures

Japanese sea bass (*Lateolabrax japonicus*) were obtained from Beijing Fishery Institute, Beijing, and maintained in the laboratory for 2 weeks prior to the experiment. Initial body weight of juvenile Japanese sea bass were 5.87±0.02g. The fish were maintained in conical fibreglass tanks (water depth: 50 cm; volume: 100 l) in a recirculating system during the acclimation and experimental periods. Water temperature was 27-28℃, pH=7.5 and NH_4-N<0.5mg L^{-1}. Aeration was supplied to each tank 24h per day, and photoperiod was 12D:12L using six 40W fluorescent light. Each tank had a central outlet pipe connected to a net for collecting the uneaten diets and feaces.

During acclimation, the fish were fed to apparent satiation twice a day using a commercial diet (47% crude protein, 15% crude lipid, and supplied by Beijing Friendship Feed Co.). Four tanks were randomly assigned to each diet group in the 9 weeks growth experiment. At the start of the experiment, fish were not fed for 1d, then 20 fishes were batch weighed and stocked into each tank. During the feeding period, fish were fed the 7 experimental diets to satiation two times per day at 09:00 and 16:30, respectively. One h later, uneaten diet was removed, dried to constant weight at 70℃ and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying and reweighing. After 2 weeks of normal experiment, faeces were collected for each tank. To minimize nutrient leaching in the faeces, only intact faeces produced during 19:00-21:00 h each day were collected. All the animals of each tank were batch weighed at the end of 2nd, 4th, 6th and 9th week. Throughout the experiment, mortalities were recorded daily and dead fish were weighed. At the end of experiment, five fish were collected for the the carcass chemical analysis and other 10 fish from each tank were used for the fillet and liver fatty acid analysis.

2.3 Proximate composition and fatty acid analysis

The material for carcass analysis was prepared by grinding together 10 fresh fish for each tank.

Commercial pellets, experimental diets, carcasses and faeces were analysed for dry matter, protein, lipid, energy and ash, and the lipid content of fish fillets and livers were analysed using standard methods. Dry matter will be analyzed by drying the samples to constant weight at 105℃. Crude protein was determined by combustion using the Kjeldahl method (AOAC, 1997) and Crude protein content estimated by multiplying nitrogen by 6.25; Crude lipid by acid hydrolysis with a a Sotex System HT 1047 Hydorlyzing Unit (Tecator Application Note 92/87), followed by Soxhlet extraction using a Soxtex system 1043; gross energy in and adiabatic bomb calorimeter (Parr Instruments, Moline IL); ash was analysed by combustion in a muffle furnace at 550℃ for 16h. Concentrations of the inert markers, in the diets and faeces were determined by inductively coupled plasma atomic emission spectrometry (JY38S, Jobin Yvon, France). Duplicate analyses were conducted for each sample.

Fatty acid analysis was performed on each experimental diet and on fish fillets and livers samples for each tank. Total lipids were extracted and measured gravimetrically according to Folch et al. (1957) using dichloromethane. Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride methanol according to Santha and Ackman (1990) and were analysed in a HP gas chromatograph. The chromatograph was equipped with a HP-INNOWax Polythylene Glycol Capillary 30.0m ×320μm nomical (Model No. HP 19091N-213). Helium was used as carrier gas (1.4 mL/min) and the thermal gradient was 100 to 180°C at 8°C /min, 180 to 220°C at 4°C /min and a constant temperature of 220°C during 20min. Injector and flamme ionisation detector temperatures were 260 and 250°C respectively. Fatty acid methyl esters were identified by comparison with known standard mixtures (Sigma, 189-19) and quantified using a computer.

2.4 Statistical analysis

Data are reported as mean values ± standard error of mean (S.E.M). Homogeneity of variance was confirmed and comparison between means was by one-way ANOVA. Duncan's procedure is used for multiple comparisons. Differences were regarded as significant when $P < 0.05$. Data were subjected to liner regression and Pearson correlation where appropriated. All the statistical analyses were performed by STATISTICA 6.0.

3. Results

3.1 Growth and apparent digestibility coefficients (ADCs)

The growth parameters, feed utilization and apparent digestibility coefficients (ADCs) of Juvenile dry matter, protein and lipid were shown in Table 4. Except the feed conversion efficiency (FCE) and protein efficiency ratio (PER), the growth parameters, such as final body weight (FBW), specific growth rate (SGR), feed intake and hepatosomatic index (HSI) fed the experimental diets were not significantly different ($P>0.05$). FCE and PER of alternative groups were not significantly different from that of FO group, but the FCE and PER in fish consuming the PF diet was significantly lower than those of SO and CO diets. Survivals of all groups were 100%. Except PF group, fish culture cost of alternative fat groups were lower than the control group, and fish fed CO diet got lowest cost, it was significantly lower than that of PF diet ($P<0.05$). Besides, alternative fat significantly affect the apparent digestibility of dry matter (ADC_d), but did not show significant effect on the apparent digestibility of protein (ADC_p) and apparent digestibility of lipid (ADC_l). ADCd of PL diet was highest, and significantly higher than PF diet.

3.2 Chemical composition

The results of carcass proximate composition and lipid contents of fillets and livers were showed in Table. 5. Fish carcass moisture of PL group was significantly higher than SO group, and did not show significant different from other groups. Fish carcass protein of PL and PF group were significantly higher than SO group. Animal fats enhanced the liver lipid accumulation, fish fed PF diet got highest liver lipid and significantly higher than that of FO, SO and CO groups, liver lipid of control group was lowest. Alternative fats replacement did not significantly affect the carcass lipid, carcass ash and fillet lipid contents ($P>0.05$).

The fatty acid composition of the experimental diets (Table 2) showed some differences in both individual fatty acids and major fatty acid classes. The diet containing lowest amount of Saturated fatty acid (SFA) (27.2%) was the SO diet. The content of palmitic acid (16:0) was highest in the SFA structure for each diet, and that of diet C and animal fat using (PL, TL and PF) diets were significantly higher than vegetable oil using diet (SO and CO). The amount of monounsaturated fatty acids (MUFA), particularly 18:1n-9, was highest in animal fat using diet, then vegetable oil using diet, and lowest in the control diet. The control diet, containing only fish oil (FO), had the highest amount of polyunsaturated n-3 fatty acid, while n-6 fatty acids were

higher in SO and CO diets. The n-3/n-6 ratio varied from 2.4 for C to 0.6 for SO and CO diets.

The fatty acids found in high amounts in fillets (>5.0%), irrespective of the dietary treatment, were C16:0, C18:0, C18:1n-9, C18:2n-6, eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA, 22: 6n-3) (Table 5). Fillets of fish reared on the animal fat diets (FO, PL, TL and PF) showed higher sum of SFA than vegetable oil diets (SO and CO) ($P<0.05$), and that of MF was in median. Sum of MUFAs were significantly higher for fish on FO, SO and CO diets than that on territorial animal fats or mix-fat replacing diets ($P<0.05$). Linoleic acid value (C18:2n-6, LA) and sum of n-6 fatty acid in the fillets was highest for SO and CO groups ($21.3\pm 1.8\%$, $19.5\pm0.9\%$ and 22.8 ± 1.5, 21.2 ± 0.9, respectively) and lowest for FO group ($9.0\pm0.7\%$) ($P<0.05$). Arachidonic acid value (C20:4n-6, ARA) in all the fillets were similar ($P>0.05$). Although eicosapentaenoic acid (C20:5n-3,EPA) in the fillet of FO group was lower, but higher in the other groups' than in the diets, EPA value in the fillet of FO was significantly higher than that of PL ($P<0.05$). Docosahexaenoic acid (C22:6n-3, DHA) in the fillets of all groups were higher than in the diets, DHA value in the FO group was significantly higher than other groups ($P<0.05$), except in the MF group. The n-3/n-6 ratio was higher in the muscle than in the diets irrespective of the dietary treatment, and maintaining an average level in the muscle of 1.9 ± 0.6 respect the average diet value (1.2 ± 0.6). Moreover, the n-3/n-6 ratio was highest for fish on FO diet, then for TL and MF diets, thirdly for PL and PF diets, and lowest for SO and CO diet ($P<0.05$). The C18:1n/n-3 ratio in the muscle of FO group was significantly lower than animal fat replacing groups (PL, TL, PF and MF)($P<0.05$).

Fatty acids present in the fillet were also identified in livers (Tables 6). Livers of fish reared on FO diets showed higher sum of SFA than on PF and MF diets ($P<0.05$), mainly because of the difference among C16:0. Sum of MUFAs were not significantly different in the groups ($P>0.05$), while the liver C18:1n-9(7) content was lowest ($P<0.05$). Linoleic acid value (C18:2n-6, LA) and sum of n-6 fatty acid in the livers was highest for SO group ($10.9\pm1.7\%$ and $12.0\pm2.0\%$, respectively) and lowest for FO group ($3.9\pm1.0\%$ and 5.1 ± 1.3) ($P<0.05$). C18:3n-6 and arachidonic acid value (C20:4n-6, ARA) in all the fillets were similar ($P>0.05$). Only linolenic acid (C18:3n-3, LNA) in the n-3 series showed difference ($P<0.05$), while sum of n-3 of livers were not significantly different among groups ($P>0.05$). The n-3/n-6 ratio in livers of fish fed FO and SO was lower, while higher for other groups than in the diets. Hereby, the n-3/n-6 ratios for

the fish fed terrestrial animal fats replacing diets were not significantly different from the control group (FO), but significantly higher than for vegetable oils using diets (SO and CO) ($P<0.05$). The C18:1n/n-3 ratio in the livers were not significantly different among groups ($P>0.05$).

The sum of SFA, MUFA, PUFA, the n-3/n-6 ratio, and the C18:1n-3/n-3 ratio in fillets showed a positive linear correlation ($r>0.76$) between the diets (Table 7), while the data in livers were not significantly correlated with the dietary contribution.

4. Discussion

Regarding the optimum dietary lipid levels of Japanese sea bass, no definite results were reported until now. Other related species, Peres and Oliva-Teles (1999) found no growth differences in European sea bass (*Dicentrarchus labrax*) fed diets ranging from 12 to 24% lipids, while with 30% lipids a growth depression occurred. The results of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) showed that the fish fed 15% lipid achieved highest weight gain (Gaylord and Gatlin, 2000). In the present study, juvenile Japanese sea bass responded well to all the experimental diets (47% of crude protein and 15% of crude lipid contents). 100% survival of all groups indicated that all the tested alternative lipid sources did not have any adverse affect on the health of the fish. In spite of the differences in dietary fatty acids composition, the experiment did not lead to significant difference in most growth parameters, such as SGR, feed intake and HSI. Although FCE and PER values of PF diets were significantly lower than that of SO and CO diets, they were still enough high for satisfaction. The differences of FCE among groups may be induced by the lower apparent digestibility coefficients of dry matter and lower PER. The partial replacing of fish oil (50%) with alternative lipid sources could be a good tool in the reduction of the production costs. Similar reports of pork lard, poultry fat, soybean oil and canola oil etc. for salmonaids and marine fish were reported. For example, Hertrampf and Piedad-Pascual (2000) reported that suitability of PL and PF could be used as a lipid source for rainbow trout; Feeding canola oil or soybean oil to Atlantic salmon (*Salmo salar*), Chinook salmon (*Oncorhyncus kisutch*) and grey mullet (*Mugil cephalus*) did not negatively affect growth, feed conversion or survival (Greene and Selivonchick, 1990; Argyropoulou et al., 1992; Bell et al., 2001).

In spite of the constancy of the dietary lipid and similar lipid content of carcass and fillet, the

significant difference on fish liver lipid showed that PL using would induce higher fat depots on liver.

16:0 and 18:1n-9 were main non-essential fatty acids in fish muscle and liver. Higher 18:1n-9 will indicate the fatty acid deficiency, and 18:1n-9:n-3 ratio can be a critera to evaluate the fatty acid requirement (Takeuchi et al., 1990; Sargent et al., 2002). In the present study, the 18:1n-9:n-3 ratios in liver were not significantly different ($P>0.05$). Plant oil replacing did not significantly affect the 18:1n-9:n-3 ratio of fish fillet, even the ratio of territorial animal fats group were significantly higher than FO group, all of them were lower than 1.0. It indicated that 5% of anchovy fish oil had met the essential fatty acid requirements of Japanese sea bass.

Fish, like all other vertebrates, require three long chain polyunsaturated fatty acids (PUFA) for their normal growth and development: AA, EPA and DHA, which involved in maintaining cell membrane structure and function (Sargent et al., 1999). Findings reported by Thrush et al. (1993) and Bell et al. (1996) pointed strongly to the the importance of AA, and suggested tuna orbital oil, which has a DHA : EPA ratio of 8.6 and an EPA : AA ratio of 2.2 as a more suitable dietary oil for European sea bass larvae broodstock than northern hemisphere fish oil. As fish value for human consumption is related to its high fillet HUFA levels, special care must be taken to guarantee a high quality of the final product. As the results of present study showed that fatty acid structure of fish fillets other than liver has close correlation with which of diets, and could be easily modified by diet manipulation. EPA in the fillet of FO group was lower, but higher in the other groups' than in the diets, and the increasing of muscle DHA and AA in alternative fats groups higher than that of FO. It indicates that Japanese sea bass will selectively absorb these fatty acids when the PUFA could meet the requirements. In the present study, for juvenile Japanese sea bass, all diets induced satisfying growth performance. However, although plant oils and terrestrial animal fats can be used up to a certain level to replace fish oil (Hardy et al., 1987; Bell et al., 2001; Turchini et al., 2003), adequate amounts of fish oil must be incorporated in the diets to cover the EFA requirements, as fish oils are the only dietary source of n-3 HUFA (Sargent et al., 1989). 1% of HUFA will be suitable for most of marine fish (Sargent et al, 2002), and in all experimental diets n-3 HUFA were higher than this level. The results of present study concluded that when EFA of diets was enough for requirement, alternative fats using would not have significantly negative effect on Japanese sea bass. Similar result was reported by Craig and Gatlin (1995) on juvenile red

drum (*Sciaenops ocellatas*).

Considering the disease induce by animal sources protein and oil, such as BSE, bird flu, and dioxin, how to safely use the animal protein and fats in animal feed should be studied. Cannibalism should be avoided. As far as Japanese sea bass, comparing studies on the fatty acid requirements for which cultured in seawater or fresh water need to be conducted.

5. Conclusion

The 50% of alternative lipid replacement (10% of fish oil used in control diet) could be used effectively for oil coating extruded diets for Japanese sea bass, no effect on SGR and feed intake (palatability). These alternative lipid sources are more cost-effective and marine resources friendly. Fish fed mix-fat, soy oil and corn oil replacing diets got higher PUFA content in fish muscle ($P<0.05$) and liver ($P>0.05$), more difficult to stabilize. The fatty acid structure of fillet could be modified and had close correlation to the fatty acid structure of diets.

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Table 1. Formulation and analysed composition of the experimental diets

Ingredients	FO	PL[1]	TL[1]	PF[1]	MF[1]	SO	CO
Fish meal (6.0RMB/kg, Peru)	30	30	30	30	30	30	30
Soybean meal (2.0RMB/kg, China)	20	20	20	20	20	20	20
Wheat gluten (7.0RMB/kg, China)	15	15	15	15	15	15	15
Wheat flour (1.3RMB/kg, China)	14	14	14	14	14	14	14
Wheat middlings (0.9RMB/kg, China)	7	7	7	7	7	7	7
Vitamin and mineral premix[2]	4	4	4	4	4	4	4
Anchovy fish oil[1]	10	5	5	5	5	5	5
Alternative fats		5	5	5	5	5	5
Analysed chemical composition							
Crude protein (% dry matter)	47.26	47.47	47.04	47.47	47.50	47.25	47.02
Crude lipid (% dry matter)	14.53	14.55	14.51	14.59	14.48	14.42	14.48
Ash (% dry matter)	10.69	10.73	10.63	10.74	10.72	10.63	10.61
Gross energy (MJ/kg)	19.66	19.84	19.79	19.82	19.69	19.87	19.80
EPA	1.5	0.8	0.8	0.8	0.8	0.8	0.8
DHA	2.2	1.4	1.3	1.3	1.3	1.3	1.3
Feed Cost (RMB/kg feed)	4.52	4.36	4.36	4.37	4.36	4.42	4.42

[1] Fish oil was supplied by Weihai Colong Fish Meal Co. Ltd; Lord, tallow and poultry fat will be supplied by National Renderers Association, 500g/kg Vitamin E was added as antioxidant. Price of anchovy fish oil, lard, tallow, poultry fat, mix-fat, soy oil and corn oil were 7500RMB/ton, 4300RMB/ton, 4300RMB$/ton, 4500RMB/ton, 4300RMB/ton, 5500RMB/ton and 5500RMB/ton in China before May, 2003.

[2] Vitamin premix (mg/kg diet, 7.0RMB/kg): thiamine-HCl, 10.0; riboflavin, 12.0; niacin, 50; pyridoxine-HCl, 10.0; cyanocobalamine (1%), 4; pantothenic acid, 30; biotin, 1.0; inositol, 400; folic acid, 3.0; choline chloride, 1500.0; L-ascorbyl-2-monophosphate-Mg, 300.0; Vitamin A, 20.0; Vitamin D, 4.0; Vitamin E, 150.0; Vitamin K, 7.0; BHT, 10.0; α -cellulose, 7489; Mineral premix (g/kg diet): $Ca(H_2PO_4)_2 \cdot H_2O$, 10.00; $MgSO_4 \cdot 7H_2O$,3.00; $NaHCO_3$,2.00; $FeSO_4 \cdot 7H_2O$, 0.60; $ZnSO_4 \cdot 7H_2O$, 0.35; $MnSO_4 \cdot 7H_2O$, 0.18; KI, 0.01; Na_2SeO_3, 0.01; $CoCl \cdot 6H_2O$(1%), 0.05; $CuSO_4 \cdot 5H_2O$, 0.01; zeolite, 13.79.

Table 2. Fatty acid composition (% total lipid) of the experimental diets

Fatty acid	FO	PL	TL	PF	MF	SO	CO
C14:0	6.0	4.3	5.0	4.1	4.3	3.8	3.9
C16:0	20.8	23.5	24.0	23.4	21.9	18.4	18.6
C18:0	4.3	8.3	9.4	5.1	6.3	4.4	4.1
C20:0	0.8	0.5	0.6	0.6	0.6	0.7	0.7
C22:0	0.2	0.0	0.2	0.0	0.2	0.3	0.3
C24:0	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Σ Saturates	32.2	36.8	39.3	33.3	33.6	27.8	27.9
C14:1	0.9	0.5	0.9	0.7	0.7	0.5	0.6
C16:1*n*-7	5.6	4.3	5.0	6.2	4.9	3.6	3.7
C18:1*n*-9	11.3	21.4	20.3	22.0	23.2	15.1	17.7
C20:1*n*-9	2.4	1.6	1.5	1.5	1.7	1.7	1.7
C22:1*n*-11	3.3	1.7	1.8	1.7	1.8	1.7	1.8
C24:1*n*-9	0.8	0.4	0.4	0.4	0.4	0.4	0.4
Σ Monoenes	23.4	29.4	29.1	31.8	32.2	22.5	25.3
C18:2*n*-6	9.1	13.7	10.7	14.8	12.9	28.3	26.4
C18:3*n*-6	2.2	1.4	1.4	1.4	1.4	1.3	1.5
C20:2*n*-6	0.3	0.2	0.2	0.2	0.2	0.1	0.1
C20:3*n*-6	0.2	0.1	0.1	0.0	0.0	0.0	0.0
C20:4*n*-6	0.8	0.6	0.5	0.3	0.5	0.5	0.4
Σ *n*-6	12.6	15.9	12.9	16.7	15.0	30.2	28.5
C18:3*n*-3	1.8	1.6	1.4	1.6	2.0	3.4	1.6
C20:5*n*-3	11.4	5.6	5.9	5.6	6.0	5.6	5.8
C22:5*n*-3	1.3	0.7	0.7	0.7	0.7	0.7	0.7
C22:6*n*-3	16.3	9.5	9.8	9.6	9.9	9.4	9.7
Σ *n*-3	30.8	17.4	17.8	17.5	18.6	19.1	17.8
Σ PUFA	43.4	33.2	30.7	34.2	33.6	49.2	46.2
Σ *n*-3/Σ *n*-6	2.4	1.1	1.4	1.0	1.2	0.6	0.6
DHA/EPA	1.43	1.7	1.7	1.7	1.7	1.7	1.7
EPA/AA	14.3	15.8	19.6	32	19.8	18.8	19.4
C18:1*n*-9/Σ *n*-3	0.4	1.2	1.1	1.3	1.3	0.8	1.0

Table 3. Growth performance, feed utilization and apparent digestibility coefficients (ADCs) of Juvenile Japanese sea bass. Data are mean ± S.E.M: means with different superscripts are significantly different ($P<0.05$)

Parameters	FO	PL	TL	PF	MF	SO	CO
IBW(g)	5.87±0.02	5.87±0.03	5.87±0.01	5.87±0.02	5.87±0.04	5.87±0.01	5.87±0.03
FBW (g)[1]	30.63±1.31	28.49±2.63	28.89±2.39	31.35±1.35	30.29±2.96	30.58±1.94	30.39±2.59
SGR (%/d)[3]	2.84±0.08	2.72±0.16	2.74±0.15	2.89±0.07	2.82±0.16	2.84±0.11	2.83±0.15
Feed intake[2]	7.77±0.54	6.97±0.85	7.06±0.59	7.42±0.54	7.29±0.87	7.40±0.38	7.31±0.47
FCE[4]	93.66±1.49^{ab}	95.65±3.24^{ab}	93.67±0.57 ^{ab}	90.46±6.91 ^{a}	94.67±0.44^{ab}	97.78±2.38^{b}	96.93±2.22^{b}
PER[5]	2.15±0.03^{ab}	2.16±0.05^{ab}	2.16±0.01^{ab}	2.05±0.15^{a}	2.14±0.02^{ab}	2.19±0.05^{b}	2.19±0.05^{b}
HSI[6]	1.33±0.03	1.44±0.10	1.44±0.06	1.44±0.07	1.38±0.12	1.38±0.09	1.40±0.10
ADC_d[7]	80.61±2.27^{ab}	81.77±0.30^{b}	79.47±1.20^{ab}	78.91±1.40^{a}	80.69±1.14^{ab}	79.85±1.26^{ab}	79.27±0.33^{ab}
ADC_p[7]	94.97±0.97	95.06±0.31	95.50±0.18	95.08±0.40	95.17±0.09	95.02±0.21	94.68±0.28
ADC_l[7]	93.94±3.49	94.01±2.73	94.37±1.89	93.44±3.08	93.35±0.25	95.84±1.91	95.02±1.71
Cost (RMB￥/kg)	4.83±0.04^{a}	4.57±0.06^{ab}	4.65±0.02^{ab}	4.85±0.22^{a}	4.62±0.01^{ab}	4.52±0.06^{b}	4.56±0.06^{ab}

[1] FBW, final body weight (g). He threw out

[2] Feed intake, expressed as % initial weight/day

[3] SGR, specific growth rate (% day) = 100×[ln(FBW)-ln(IBW)]/feeding days, where IBW is initial body weight.

[4] FCE, feed conversion efficiency (%) = 100 × wet weight gain/feed consumption.

[5] PER, protein efficiency ratio = body weight gain (g)/protein intake (g)

[6] HSI, Hepatosomatic index (%) = 100 × (weight of liver / total fish weight)

[7] ADCd, apparent digestibility coefficient of dry matter (%) = 100 × (1-Di/Fi); ADCp, apparent digestibility coefficient of protein (%) = 100 × (1-(Di×Fj)/(Fi×Dj); where Di=concentration of Y2O3 in diet; Dj=crude protein content in diet; Fi=concentration of Y2O3 in faeces; Fj=crude protein content in faeces; ADCl, apparent digestibility coefficient of lipid (%) = 100 × (1-(Di×Fj)/(Fi×Dj); where Di=concentration of Y2O3 in diet; Dj=crude lipid content in diet; Fi=concentration of Y2O3 in faeces; Fj= crude lipid content in faeces.

Table 4 Proximate composition of fish carcasses and lipid content of fillets and livers fed the different dietary treatments (in % of dry weight basis, mean ± S. E. M).

Diets	Carcass moisture	Carcass protein	Carcass lipid	Carcass ash	Fillet lipid	Liver lipid
FO	69.35 ± 1.60^{ab}	49.00 ± 2.32^{ab}	27.69 ± 3.95	12.38 ± 1.25	6.39 ± 0.85	17.79 ± 1.80^{a}
PL	69.75 ± 1.56^{a}	52.09 ± 2.53^{a}	28.09 ± 1.81	12.10 ± 0.26	5.77 ± 0.59	20.86 ± 3.59^{abc}
TL	69.06 ± 0.79^{ab}	50.15 ± 1.56^{ab}	29.91 ± 1.20	11.82 ± 0.80	5.21 ± 1.06	21.82 ± 2.11^{bc}
PF	69.50 ± 0.33^{ab}	50.45 ± 0.87^{a}	28.29 ± 1.07	12.60 ± 0.55	6.15 ± 0.91	23.93 ± 0.23^{c}
MF	69.38 ± 0.70^{ab}	49.95 ± 1.36^{ab}	29.25 ± 2.41	12.09 ± 0.95	5.80 ± 0.70	21.08 ± 1.03^{abc}
SO	67.71 ± 0.64^{b}	47.00 ± 0.12^{b}	31.43 ± 1.23	11.24 ± 0.51	6.34 ± 0.69	18.01 ± 2.35^{ab}
CO	69.44 ± 0.36^{ab}	48.70 ± 2.17^{ab}	28.50 ± 1.01	12.28 ± 0.31	5.31 ± 0.74	19.25 ± 1.13^{ab}

* Means with different superscripts are significantly different ($P<0.05$)

Table 5. Fatty acid composition of fillet total lipid of juvenile Japanese sea bass fed experimental diets (mean ± S. E. M).

Fatty acid	FO	PL	TL	PF	MF	SO	CO
C14:0	3.3±0.0^{a}	2.4±0.3^{b}	2.5±0.3^{b}	2.6±0.3^{b}	2.7±0.2^{b}	2.4±0.3^{b}	2.3±0.3^{b}
C16:0	21.8±0.6^{a}	21.9±1.2^{a}	21.7±1.0^{a}	21.3±0.6^{a}	20.6±0.0^{ab}	19.2±0.4^{c}	19.6±0.5^{bc}
C18:0	5.6±0.6^{a}	6.6±0.5^{b}	6.9±0.4^{b}	5.4±0.3^{a}	5.8±0.2^{a}	5.5±0.5^{a}	5.6±0.4^{a}
C20:0	0.4±0.1	0.2±0.1	tr	0.2±0.1	0.1±0.1	tr	0.2±0.1
ΣSFA	31.1±1.1^{a}	31.0±1.4^{a}	31.1±1.4^{a}	29.5±0.7^{ab}	29.2±0.3^{bc}	27.2±0.4^{d}	27.7±0.1^{cd}
C14:1	0.8±0.1^{a}	0.6±0.0^{b}	0.7±0.0^{ac}	0.6±0.0^{bc}	0.7±0.0^{ac}	0.7±0.0^{bc}	0.6±0.0^{bc}
C16:1*n*-7	5.3±0.3^{ab}	4.2±0.3^{ac}	4.9±0.6^{abc}	6.0±0.6^{b}	5.0±0.5^{abc}	3.8±0.3^{c}	3.9±0.3^{c}
C18:1*n*-9(7)	15.0±0.2^{a}	22.5±0.6^{b}	21.4±1.0^{b}	22.2±1.3^{b}	22.3±1.0^{b}	16.9±0.6^{c}	18.0±0.4^{c}
C20:1*n*-9	1.6±0.2^{a}	1.2±0.0^{b}	1.0±0.0^{b}	1.1±0.2^{b}	1.2±0.1^{b}	1.2±0.2^{b}	1.1±0.1^{b}
C22:1*n*-11	0.9±0.4^{a}	0.1±0.1^{ab}	0.1±0.1^{a}	0.6±0.3^{ab}	0.3±0.1^{ab}	0.1±0.1^{a}	TR
C24:1*n*-9	0.6±0.0	0.5±0.0	0.5±0.1	0.5±0.1	0.5±0.0	0.6±0.1	0.6±0.0
ΣMUFA	24.2±1.1^{a}	29.1±0.7^{b}	28.7±1.7^{b}	31.0±2.2^{b}	30.0±1.9^{b}	23.2±1.2^{a}	24.2±0.6^{a}
C18:2*n*-6	9.0±0.7^{a}	13.1±1.0^{b}	11.2±1.9^{ab}	12.9±0.6^{b}	11.2±0.3^{ab}	21.3±1.8^{c}	19.5±0.9^{c}
C18:3*n*-6	1.1±0.2^{a}	0.5±0.4^{b}	0.7±0.1^{ab}	0.9±0.2^{ab}	0.7±0.3^{ab}	0.7±0.2^{ab}	0.7±0.1^{ab}
C20:4*n*-6	1.2±0.3	1.3±0.1	1.2±0.1	1.2±0.2	1.2±0.2	0.9±0.4	1.1±0.2
Σ*n*-6	11.2±0.9^{a}	14.9±0.6^{b}	13.1±1.9^{ab}	15±0.6^{b}	13.1±0.4^{ab}	22.8±1.5c	21.2±0.9^{c}
C18:3*n*-3	1.3±0.0^{a}	1.3±0.1^{a}	1.2±0.5^{a}	1.3±0.0^{a}	1.4±0.1^{a}	2.1±0.2^{b}	1.1±0.1^{a}
C20:5*n*-3	8.5±0.3^{a}	6.3±0.3^{b}	6.9±0.4^{ab}	6.3±0.4^{ab}	6.9±0.4^{ab}	6.5±0.3^{ab}	6.7±0.3^{ab}
C22:5*n*-3	1.6±0.1^{a}	1.5±0.1^{ab}	1.4±0.0^{ab}	1.3±0.1^{b}	1.4±0.1^{ab}	1.4±0.2^{ab}	1.3±0.1^{b}
C22:6*n*-3	22.0±0.3^{a}	17.5±0.7^{b}	17.6±1.0^{b}	15.6±2.1^{b}	17.9±2.2^{ab}	16.9±1.9^{b}	17.8±1.4^{b}
Σ*n*-3	33.5±0.6^{a}	24.9±2.4^{b}	27.1±1.4^{b}	24.5±2.5^{b}	27.7±2.5^{b}	26.8±2.1^{b}	26.8±1.6^{b}
ΣPUFA	44.7±1.1^{a}	39.8±1.9^{b}	40.2±2.7^{b}	39.5±2.2^{b}	40.8±2.2^{b}	49.6±0.8^{c}	48.1±0.8^{c}
Σ*n*-3/Σ*n*-6	3.0±0.3^{a}	1.7±0.2^{b}	2.1±0.3^{c}	1.6±0.2^{b}	2.1±0.2^{c}	1.2±0.2^{d}	1.3±0.1^{d}
C18:1*n*-9/Σn-3	0.5±0.0^{a}	0.9±0.1^{b}	0.8±0.1^{bc}	0.9±0.1^{b}	0.8±0.1^{bc}	0.6±0.1^{ac}	0.7±0.1^{abc}

*Means with different superscripts are significantly different (P<0.05)

Table 6. Fatty acid composition of liver total lipid of juvenile Japanese sea bass fed experimental diets (Mean ± S. E. M)

Fatty acid	FO	PL	TL	PF	MF	SO	CO
C14:0	3.1±0.5^{a}	2.5±0.1^{b}	2.5±0.2^{b}	2.3±0.2^{b}	2.3±0.2^{b}	2.4±0.0^{b}	2.6±0.3^{b}
C16:0	30.0±0.4^{a}	26.9±0.4^{bc}	26.9±1.5^{bc}	25.8±1.5^{b}	25.8±1.2^{b}	26.7±1.4^{bc}	28.5±0.4^{ab}
C18:0	7.0±0.5	6.7±1.0	7.1±0.4	6.0±0.3	6.3±0.4	7.4±1.2	7.1±0.5
C20:0	0.5±0.1^{a}	0.3±0.0^{b}	0.2±0.0^{b}	0.3±0.1^{b}	0.3±0.0^{b}	0.4±0.1^{ab}	0.3±0.0^{ab}
ΣSFA	40.4±0.3^{a}	36.6±1.0^{ab}	36.8±2.0^{ab}	34.5±2.0^{b}	34.9±0.9^{b}	37.1±2.6^{ab}	38.8±0.3^{ab}
C14:1	0.6±0.2	0.5±0.0	0.3±0.2	0.5±0.0	0.4±0.1	0.4±0.1	0.5±0.1
C16:1*n*-7	11.4±1.0	9.5±1.4	10.6±0.6	10.6±0.8	9.6±1.2	9.0±0.8	10.6±1.4
C18:1*n*-9(7)	29.4±3.4^{a}	34.4±0.9^{ab}	35.3±0.5^{b}	33.6±2.4^{ab}	35.2±1.6^{ab}	31.1±3.0^{ab}	31.6±1.0^{ab}
C20:1*n*-9	1.8±0.3^{a}	1.6±0.1^{ab}	1.3±0.2^{ab}	1.4±0.1^{ab}	1.6±0.3^{ab}	1.5±0.1^{ab}	1.2±0.2^{b}
C22:1*n*-11	0.9±0.2^{a}	0.2±0.1^{b}	0.3±0.1^{b}	0.3±0.1^{b}	0.4±0.1^{b}	0.4±0.1^{b}	0.4±0.2^{b}
C24:1*n*-9	1.0±0.3	0.8±0.2	0.6±0.1	0.8±0.1	0.7±0.2	0.8±0.1	0.6±0.1
ΣMUFA	45.0±3.7	47.0±1.6	48.4±0.8	47.1±3.2	47.8±2.5	43.2±3.2	44.9±1.5
C18:2*n*-6	3.9±1.0^{a}	6.3±0.8^{ab}	4.1±0.6^{a}	6.2±0.6^{ab}	5.5±1.2^{ab}	10.9±1.7^{c}	8.2±2.8^{bc}
C18:3*n*-6	0.4±0.1	0.2±0.0	0.2±0.1	0.3±0.1	0.1±0.1	0.3±0.1	0.1±0.2
C20:4*n*-6	0.8±0.3	0.8±0.1	0.6±0.1	0.9±0.3	0.8±0.1	0.6±0.2	0.5±0.1
Σ*n*-6	5.1±1.3^{a}	7.4±0.8^{ab}	5.9±2.0^{a}	7.5±0.8^{ab}	6.6±1.1^{a}	12.0±2.0^{b}	8.9±2.7^{ab}
C18:3*n*-3	0.6±0.1^{ab}	0.5±0.1^{ab}	0.4±0.1^{a}	0.5±0.1^{ab}	0.6±0.1^{ab}	0.8±0.1^{b}	0.4±0.1^{a}
C20:5*n*-3	2.2±0.6	2.0±0.1	2.0±0.2	2.3±0.9	2.3±0.6	1.6±0.8	1.7±0.3
C22:5*n*-3	0.6±0.1	0.5±0.0	0.5±0.0	0.6±0.2	0.6±0.1	0.4±0.2	0.4±0.0
C22:6*n*-3	6.3±2.0	6.0±0.3	6.0±0.5	7.6±4.0	7.2±3.0	5.0±2.5	4.9±0.9
Σ*n*-3	9.5±2.9	9.0±0.3	9.0±0.6	11.0±4.7	10.7±3.6	7.8±3.6	7.4±1.2
ΣPUFA	14.6±4.0	16.4±0.6	14.8±2.0	18.5±5.2	17.3±3.0	19.7±5.5	16.4±1.7
Σ*n*-3/Σ*n*-6	1.9±0.3^{a}	1.2±0.2^{abc}	1.6±0.5^{ac}	1.5±0.5^{abc}	1.7±0.8^{ac}	0.6±0.2^{b}	0.9±0.4^{bc}
C18:1*n*/Σn-3	3.4±1.5	3.8±0.2	4.0±0.2	3.5±1.5	3.6±1.1	4.6±2.1	4.3±0.7

* Means with different superscripts are significantly different (P<0.05)

Table 7. Statistical interrelationship amongst fatty acid factors in the diets(X) and Japanese sea bass fillets or livers (Y)

	Fish fillets			Fish livers		
	Liner regression	R^2	P	Liner regression	R^2	P
SFA	Y=18.61+0.33X	0.58	<0.05	Y=42.03-0.15X	0.07	>0.05
MUFA	Y=5.11+0.80X	0.82	<0.05	Y=34.57+0.42X	0.57	<0.05
PUFA	Y=21.82+0.55X	0.83	<0.05	Y=13.80+0.08X	0.03	>0.05
n-3/n-6	Y=0.67+0.99X	0.86	<0.05	Y=0.62+0.61X	0.38	<0.05
C18:1n-3/n-3	Y=0.26+0.48X	0.76	<0.05	Y=4.11-0.23X	0.00	>0.05