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Substitution of Fishmeal in a Diet for the Carnivorous Marine Fish *Pagrus auratus* (Bloch and Schneider) from Southeastern Australia

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Abstract

The growth of Australian snapper (*Pagrus auratus*)(Bloch and Schneider) fed diets based on different sources of protein was compared. Two commercial diets for other carnivorous species and two experimental diets for snapper were compared. Fishmeal was the predominant source of protein for one experimental diet (60% content), while the other contained only 10% fishmeal with the remainder of the protein sourced from soybean meal and poultry offal meal. Replacement of fishmeal gave a lower growth rate and higher (poorer) apparent food conversion ratio (FCR, weight of feed given/ live weight gain) compared to the fishmeal diet. The growth rate and FCR obtained with the soybean meal and poultry offal meal diet was similar to that of the two commercial diets tested. Fatty acid analysis of the two experimental diets indicated that concentrations of essential fatty acids exceeded those reported to reduce growth of snapper. Concentration of lysine in the experimental diet associated with reduced growth was lower than that in the diet based on fishmeal.

Introduction

Farming of carnivorous marine fish in temperate Australia is likely to be based on an indigenous species for which a suitable low-cost diet can be produced. One of the species with promise for establishment of such an industry is the snapper, *Pagrus auratus* (Bloch and Schneider). Snapper has been farmed successfully in Japan for about 20 years (Foscarini 1988; Fukusho 1991), where it is known as red sea bream. In 1990, the Japanese production of snapper by aquaculture was 40,000 t (Davy 1990). Preliminary trials in New South Wales (NSW) indicate that snapper should also be suitable for farming in temperate Australia (Bell *et al.* 1991).

Fishmeal is the protein source of choice for fish feeds (Lovell 1989), although increasing demand, reduced supply and increasing cost (Barlow 1989) have contributed to a major research effort to replace fishmeal with alternative

sources of protein for a number of fish species (Mohsen and Lovell 1990; Olvera-Navoa *et al.* 1990). In Australia, the production of large quantities of fishmeal is not feasible because baitfish are scarce. Therefore, a pre-requisite for large scale development of marine fish farming is the identification of a local supply of high quality protein to replace fishmeal.

This paper describes an experiment that compares four diets for carnivorous fish. Two were formulated for snapper; one based on fishmeal (60% content) and the other with 10% fishmeal and the rest replaced with soybean meal and poultry offal meal. The other two were commercially available diets for other carnivorous species.

Materials and methods

Juvenile snapper were collected from Port Hacking, NSW (34° 5'S, 151° 8'E), by seine net. The fork lengths and weights of these fish ranged from 70-90 mm and 5-20 g, respectively. The juvenile snapper were acclimated in 2000-l fiberglass tanks with flow-through (0.3 l seconds^{-1}) aerated seawater at a stocking density of approximately 2 kg m^{-3}. Faeces and other particulate matter that settled on the bottom of the tank were removed daily by siphoning.

Four dry pellet diets were compared; two were formulated by us and two were available commercially. The two experimental diets formulated for snapper (Diets 1 and 2) fulfilled the requirements for Japanese red sea bream given by Yone (1975). Diet 1 was based predominantly on fishmeal as the source of protein and, in Diet 2, all but 10% of the fishmeal was replaced by soybean meal (heat treated, hexane extracted) and poultry offal meal (Table 1). No diets for snapper were commercially available in Australia. The two experimental snapper diets were evaluated by comparing them with two commercially available diets for other carnivorous species. The nominal protein content of both these diets was about 50%, similar to the experimental diets. However,

Table 1. Diet formulation of experimental snapper diets.

Ingredients	Diet 1	Diet 2
Fishmeal[1]	0.0	10.1
Soybean meal	-	22.4
Poultry offal meal	3.8	50.5
Blood meal	-	3.0
Lupins	7.0	-
Sorghum	15.1	-
Wheat	8.6	8.5
Fish oil	3.0	3.0
Vitamin/mineral premix[2]	2.5	2.5

[1]72% protein fishmeal was used

[2]Included the following per kilogram of diet:
Retinol 2.4 mg; cholecalciferol 25 mg; a-tocopherol acetate 125 mg; mendione sodium bisulfite 16.5 mg; thiamine.HCl 10 mg; riboflavin 25.5 mg; nicotinamide 200 mg; Ca-pantothenate 54.5 mg; pyridoxine.HCl 15 mg; cyanobalamin 20 mg; folic acid 4 mg; biotin 1 mg; ascorbic acid 450 mg; myo-inositol 600 mg; choline.Cl 1.5 g; $CaCO_3$ 7.5 g; MnSO 300 mg; $ZnSO_4 7H_2O$ 700 mg; $FeSO_4.7H_2O$ 500 mg; $CuSO_4$ 60 mg; NaCl 7.5 g, and; KIO_3 2 mg.

proximate analysis showed that one of the diets had a protein content of only 35% (Table 2). Fifty kilograms each of Diets 1 and 2 were manufactured. The ingredients for these diets were mixed using a 100-kg horizontal mixer and diets were pelleted using a Henry Simon (Cheshire, England) Californian pellet press (without steam conditioning). Pellet diameter was approximately 2 mm for all diets. The starting daily ration of 1.3% of the mean total wet weight of fish per tank was based on a feeding table for gilthead seabream (G. Lemarie, pers. comm.). The ration per tank was increased twice during the trial to account for growth of fish. The fish were fed twice daily 6 days per week.

Immediately prior to setting up the experiment, all fish were placed in a 2000-l tank. Groups of 10-20 fish were caught at random and lightly anesthetized with 50-70 mg l^{-1} benzocaine (ethyl p-aminobenzoate). Individual fish were then taken at random, weighed, measured and distributed among 32 tanks by systematic interspersion. This process continued until there were eight fish per tank. Each tank held 200 l of aerated seawater at ambient temperature, giving an initial stocking density of approximately 2 kg m^{-3}.

The laboratory facility was comprised of two rows of tanks at two different heights (eight tanks per row) along opposing walls. The four diets were randomly allocated to two tanks in each row so we could test for any effect of side and/or height on growth.

At the start of the experiment, there was no significant difference in the mean weight and fork length (FL) of fish per tank among the four diet groups

Table 2. Proximate composition (%) of experimental diets and of juvenile snapper (*Pagrus auratus*) sampled before the experiment commenced and when the experiment was terminated after 12 weeks[1].

Sample	Dry matter	Organic matter	Crude protein[2]	Fat	Fiber	Gross Energy[3] (MJ kg^{-1})
Diets[4]						
1	87.94	91.94	51.19	11.99	6.23	21.76[5]
2	88.45	91.18	53.75	14.03	6.54	22.25
3	92.08	88.37	35.13	18.99	16.56	21.68
4	94.17	81.26	51.06	12.61	6.58	20.06
Fish						
Initial	97.97	82.74	55.56	26.94		23.79
Fed diet 1	97.14	82.93	58.00	24.54		23.45
Fed diet 2	98.02	84.13	53.69	30.56		24.72
Fed diet 3	97.50	87.44	46.25	40.76		27.09
Fed diet 4	95.75	80.20	59.13	18.64		21.74

[1]All results (except dry matter) are expressed on a dry basis and are the mean of duplicate samples. Two fish (from separate replicates) were freeze dried and combined. Methods used follow those of Faichney and White (1983).

[2]Protein = Nx6.25

[3]Calculated based on 23.6 MJ kg-1 for protein, 39.5 MJ kg^{-1} for lipid and 17.2 MJ kg^{-1} for carbohydrates (NRC 1993).

[4]Diet 1, experimental snapper diet based on fishmeal.
Diet 2, experimental snapper diet based soybean meal and poultry offal meal.
Diets 3 and 4, commercial diets for other carnivorous species.

[5]Digestible energy of Diet 1 was 14.3 MJ kg^{-1} when calculated in a separate study using faeces collected by settlement (Quartararo et al., unpublished data).

($F_{3,28}$=1.18, P<0.33; $F_{3,28}$=0.82, P<0.49, respectively). The means (± SD) of both the mean weight and FL per tank at the start of the experiment were 71±4 g and 142±2 mm (n=32), respectively. The coefficient of variation (CV) for weight in each tank averaged 19% and ranged from 10 to 31%.

Fish that died during the experiment were replaced immediately by fish of a similar size whose pelvic fins were clipped back to the body. Replacements were easily identified at the end of the experiment. The experiment lasted 86 d.

Water quality was maintained by a continuous supply of filtered seawater at 0.03 $l \cdot seconds^{-1}$, and by siphoning the bottom of the tanks every second day. Water quality was assessed weekly by measuring dissolved oxygen using the modified Winkler's Method, calculating the unionized ammonia (NH_3) from the measured total ammonia concentration, pH, salinity and temperature and measuring nitrite concentrations (Major *et al.* 1972; Dal Pont *et al.* 1974; APHA 1985). Dissolved oxygen always exceeded 5 $mg \cdot l^{-1}$ and salinity was between 34 and 35 ppt. NH_3 and nitrite never exceeded 0.05 $mg \cdot l^{-1}$ and 0.03 $mg \cdot l^{-1}$, respectively.

Water temperatures were measured in each tank on at least four days during each week of the experiment. The weekly mean of the daily modal temperatures increased from 15.9 to 22.5°C during the experiment (Fig. 1). The natural photoperiod was augmented by fluorescent lights which were switched on automatically in sequence half an hour after sunrise and switched off half an hour before sunset.

Proximate analysis of diets and fish was done using the methods outlined by Faichney and White (1983). Lipids were extracted from whole fish by the method of Folch *et al.* (1957). Neutral and polar lipid fractions were separated by chromatography and lipid classes were separated by thin layer chromatography as described by Anderson and Arthington (1992). Amino acid profiles were determined using Waters Pico-Tag (Waters Chromatography Division, Millipore Pty Ltd, Lane Cove, NSW) with a Waters HPLC. Samples were acid hydrolyzed prior to amino acid analysis. As this technique degrades the sulphur amino acids, separate samples were oxidized using performic acid to determine methionine and cystine. Tryptophan was lost during acid hydrolysis and is not reported.

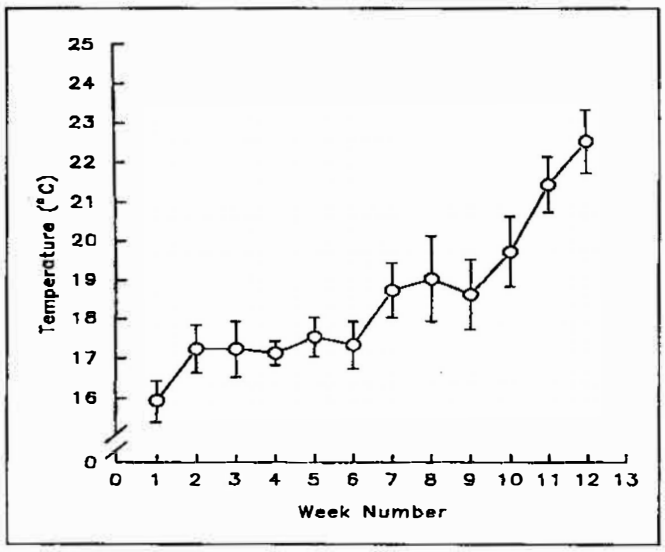

Fig. 1. Weekly mean of daily modal temperatures during the 12 weeks of the experiment. Error bars give SD.

We used mean weight, mean FL, mean FL gain, mean weight gain, relative weight gain (mean weight gain/mean initial weight), apparent food conversion ratio (FCR=weight of feed offered/wet weight gain) and the protein efficiency ratio (PER=wet weight gain/dry weight of crude protein offered) of the fish in each tank to test the null hypothesis that there was no effect of diet on growth. In tanks where some of the fish had died, the mean was based on the remaining original fish. The number of such fish in all tanks was always greater than four. We used a 3-way orthogonal ANOVA to test the effects of diet, side of the room and height of the tank on fish growth. All three factors were treated as fixed effects and the total number of degrees of freedom was 31. All variables had homogeneous variances by Cochran's Test. Significant differences among means were identified using the Student-Newman-Keuls (SNK) procedure.

Results

Neither the side of the room on which the tank was located nor the height of the tank above the ground had a significant effect on any of the variables used to measure growth. After pooling non-significant terms (see Underwood 1981), there was a significant effect of diet on the growth of snapper. This was true for all variables used to measure growth (Table 3). In all cases, growth of fish fed the experimental diet with the most fishmeal (Diet 1) was significantly greater than fish fed the other three diets (Table 4, SNK tests). There was no significant difference in growth of fish fed the latter three diets for any of the variables (Table 4, SNK tests). Although growth rates for all treatments were lower than for nutritional experiments with this species reported elsewhere (Yone 1975; Foscarini 1988), temperatures during the present study were relatively low and fluctuated according to ambient conditions. However, growth rates were similar to those reported for fish cultured commercially in sea cages (for fish over a similar size range) in both Japan and Australia (Foscarini 1988; Quartararo 1996). The apparent FCR was lower for fish fed Diet 1 (mean 1.6; SD 0.2; n=8 replicate tanks) than for the other three diets (mean 2.2; SD 1.0; n=24 replicate tanks).

Table 3. F statistics obtained from ANOVA for the variables used to assess the effects of diet, side of the room and tank height on the growth of snapper (*Pagrus auratus*).

Source	Final FL	FL gain	Final Wt	Weight gain	Relative Wt gain[-1]
Diet (d)	7.15[2]	7.76[2]	5.50[2]	5.45[2]	4.62[2]
Side (s)	0.86	4.00[2]	0.89	3.26[2]	4.13[2]
Height (h)	1.04	0.20	3.51[2]	0.70	0.07
d*h	0.67	0.44	0.66	0.59	0.74
d*s	0.74	0.38	0.20	0.13	0.25
s*h	1.42[2]	2.37[2]	1.24	1.84[2]	1.43[2]
d*s*h	0.28	0.21	0.17	0.66	0.09

[1]Weight gain/initial weight.
[2]F statistic calculated after nonsignificant terms with P>0.25 were pooled with the residual.

Table 4. Growth, feed conversion and survival of snapper (*Pagrus auratus*) fed the four diets[1].

Diet	1	2	3	4
Fork Length (mm)				
initial	142±3a	141±2a	143±2a	142±3a
final	168±3a	162±3b	164±4b	161±4b
gain	26±3a	21±3b	21±4b	19±4b
Weight (g)				
initial	71±4a	69±3a	72±3a	71±4a
final	118±6a	104±6b	110±8b	106±10b
gain	47±5a	35±7b	37±6b	35±10b
Relative weight gain[2]	0.66±0.08a	0.51±0.12b	0.52±0.08b	0.49±0.15b
FCR[3]	1.6±0.2	2.2±0.6	2.0±0.3	2.5±1.7
PER (%)[4]	15.8±1.6	11.0±2.4	8.4±2.8	11.6±3.2
Survival (%)	83±19	81±7	88±13	80±18

[1]Values are the means±SD of eight replicate tank means. Means within a row having a common superscript were not significant (P>0.05).
[2]Relative weight gain = weight gain/initial weight.
[3]Apparent food conversion ratio = weight of feed offered/wet weight gain.
[4]Apparent protein efficiency ratio = wet weight gain/dry weight of crude protein offered.

The estimated protein and fat contents for fish carcasses sampled at the termination of the experiment reflected the contents of the diets. The highest carcass fat content was measured in fish fed Diet 3, which had the highest fat content of the four diets (Table 2). The protein content of fish carcass before the experiment was 55.6%, similar to that of three of the diets (1, 2 and 4) and the content of the fish carcass after the experiment (Table 2). Fish fed the diet with the lowest protein content (35%, Diet 3) had a protein content of 46% at the end of the experiment.

The fatty acid profiles of the experimental diets reflected the fatty acid profiles of the ingredients. Diet 1 had higher concentrations of the linolenic series fatty acids and highly unsaturated fatty acids (HUFAs) than the diet based on soybean meal and poultry offal meal (Diet 2; Table 5). The fatty acid profile of fish fed Diet 1 was closer to that of fish sampled before the experiment commenced than the profiles of fish fed the other diets (Table 6). However, as limited numbers of fish were available for analysis of fat (two fish from separate replicate tanks), care should be taken when interpreting body composition results.

Discussion

Growth of snapper on a diet containing 60% fishmeal (Diet 1) was significantly greater than on a diet containing only 10% fishmeal with the remainder of the protein sourced from soybean meal and poultry offal meal (22% and 51%, respectively, in Diet 2). Substituting fishmeal with less expensive protein sources reduced growth rates in other species (Cowey *et al.* 1971; Davis and Stickney 1978; Dabrowski and Kozak 1979; Fowler 1980; Jackson *et al.* 1982; Viola *et al.* 1982; Hilton and Slinger 1986; Shiau *et al.* 1987; Fowler 1991; Webster *et al.* 1992; Reigh and Ellis 1992), although Reinitz (1980)

Table 5. Fatty acid composition (% of total fatty acids found) of two diets formulated for snapper (*Pagrus auratus*) and of juvenile snapper sampled before the experiment commenced, and when the experiment was terminated after 12 weeks.

Fatty acid[1]	Diet[2]		Fish		
	1	2	Initial	Fed Diet 1	Fed Diet
14:0	7.43	3.58	4.47	4.28	3.48
14:1	0.22	0.53	0.52	0.72	0.55
16:0	23.80	23.09	15.65	17.47	18.06
16:1 (n-7)	10.34	8.39	8.87	8.47	8.03
16:2 (n-6)	0.31	0.76	0.72	1.26	0.91
18:0	2.90	6.15	5.20	5.28	5.28
18:1 (n-9)	18.51	34.23	21.03	21.06	27.17
18:2 (n-6)	8.43	12.11	8.93	9.24	10.43
20:0	0.00	0.00	0.01	0.01	.14
18:3 (n-3) + 20:1 (n-9)	2.14	2.22	4.24	5.59	4.60
20:2 (n-6)	2.29	0.69	2.29	1.72	1.21
20:3 (n-6)	0.00	0.12	0.02	0.06	0.92
20:4 (n-6)	5.92	1.24	3.35	4.10	2.75
20:4 (n-3)	0.10	0.00	1.10	0.83	0.75
20:5 (n-3)	11.33		7.84	6.65	5.32
22:4 (n-6)	3.38	3.57	1.24	1.29	0.84
22:4 (n-3)	1.01	0.05	1.08	1.01	0.68
22:5 (n-3)	1.34	0.86	2.59	1.80	1.74
22:6 (n-3)	14.74	11.43	12.94	12.10	9.18

[1]Two fish were combined and analyzed for initial fish. For fish fed Diets 1 and 2, values are means for three and four fish, respectively, from different replicate tanks.
[2]Diet 1 experimental diet for snapper based on fishmeal;
Diet 2 experimental diet for snapper based on soybean meal and poultry offal meal.

Table 6. Amino acid composition (g/16 g N) of diets[1].

Amino acid	Diet[2]			
	1	2	3	4
Asparagine	8.06	7.70	7.23	7.05
Glutamine	13.35	12.22	12.86	12.31
Serine	4.51	5.87	4.05	3.95
Glycine	5.50	6.92	6.49	8.59
Histidine	2.02	2.04	2.85	2.28
Arginine	4.42	4.88	4.67	4.84
Threonine	3.75	3.52	3.21	3.02
Alanine	5.94	5.07	5.72	6.27
Proline	4.82	6.86	5.59	6.57
Valine	4.71	4.50	4.33	4.25
Methionine	2.60	1.37	2.01	1.37
Cystine	1.05	2.44	0.87	0.69
Isoleucine	4.17	3.62	3.65	3.49
Leucine	7.20	6.67	6.43	6.20
Phenylalanine	3.78	3.72	3.68	3.54
Tyrosine	2.71	2.54	2.56	2.43
Lysine	6.82	4.65	6.21	6.07

[1]Results are expressed on an as is basis (moisture contents are given in Table 1) and are the mean of duplicate samples.
[2]See Table 2 for a description of diets.

found no reduction in growth rate of rainbow trout (*Oncorhynchus mykiss*) when soybean meal was used to replace 75% of the herring meal.

Possible explanations for the lower growth of fish fed Diet 2 include deficiencies in essential energy or nutrients and presence of antinutritional factors or factors which reduce feed intake. Gross energy and crude protein were higher in Diet 2 than Diet 1, although concentrations of methionine, leucine, isoleucine and lysine were all lower in Diet 2 than in Diet 1. If these were below minimum requirements, they may have restricted growth. Cystine can spare requirements for methionine (Wilson 1989) and although methionine was lower in Diet 2 than Diet 1, total cystine plus methionine was similar for both diets (3.7 and 3.8 for Diets 1 and 2, respectively) and this total is similar or above requirements for most species (NRC 1993). Concentrations of leucine and isoleucine in both diets were above requirements (NRC 1993). Conversely, the concentration of lysine in Diet 2 was 4.65 16 g^{-1} N (dry basis) compared with 6.07 16 g^{-1} N or above in the other diets. This concentration is below published requirements for warm water marine carnivorous finfish (NRC 1993; Halver 1989) and may have contributed to reduced growth. Davies and Morris (1997) found that supplementing a soybean-based diet for rainbow trout (soybean meal replaced 66% of the fishmeal) with methionine and lysine significantly improved growth compared with an unsupplemented diet. However, even the supplemented diet was inferior to the fishmeal-based control. Digestibility of poultry offal meal and soybean meal were lower than for fishmeal (herring meal) for rainbow trout and chinook salmon (NRC 1993) and gilthead seabream (Nengas *et al.* 1995) and this may have reduced growth of fish fed Diet 2. Supplementing diets with crystalline amino acids is not always effective (Lovell 1989; Murai 1992).

Marine fish have a definite requirement for the n-3 series fatty acids, and specific requirements for n-3 highly unsaturated fatty acids (HUFAs) have also been demonstrated (Halver 1989; Tucker 1992; NRC 1993). Snapper have been reported to require 2% n-3 HUFAs (Fuji and Yone 1976). Although fish oil was added to both diets, concentration of n-3 series fatty acids and HUFAs was lower in Diet 2 than Diet 1. This was also reflected in the concentration of fatty acids in fish fed the two diets.

Although many antinutritional factors are inactivated by the heat treatment involved with producing soybean meal, the product still contains a variety of compounds which may make it unpalatable and cause growth reductions (Tacon *et al.* 1983). Several authors have reported that soybeans are unpalatable (Lovell 1989; Reigh and Ellis 1992) and this may reduce or delay feed intake. Olli and Krogdahl (1995) concluded that the alcohol-soluble components of soybeans comprise antinutrients which reduced fat digestibility in Atlantic salmon. Recently, Baeverfjord and Krogdahl (1996) described the development of a non-infectious sub-acute enteritis in Atlantic salmon fed soybean meal. They found the first morphological changes after 2 d on a soybean meal diet, fully developed condition after 3 weeks and, following a change of diet, apparent complete recovery after a further 3 weeks. Such a condition has not been previously described for snapper but warrants investigation if increased concentrations of soybean meal in diets for this species are considered.

The growth of fish fed Diet 2 was similar to that of fish fed either of the two commercial diets, despite the nutritional composition of at least two of these three diets being very different. Crude protein content of Diet 3 was only 35%, compared with over 50% for all other diets, and fat content was about 19%, compared with between 12-14% for the other diets. The similarity of growth of fish fed Diets 2, 3 and 4 may reflect a protein sparing mechanism of lipid in Diet 3. Vergara *et al.* (1996) found evidence of a protein-sparing effect of dietary lipid for *Sparus auratus* and that an increase in dietary lipid produced an increase in body lipid deposition. In our study, fish fed Diet 3 had the highest lipid composition (40.8% dry basis) compared with 18.1-30.1% for fish fed the other diets.

This research has provided a useful practical formulation for a fishmeal-based snapper diet. Substituting all but 10% of the fishmeal with poultry offal meal and soybean meal reduced growth of snapper, possibly because of reduced lysine concentrations or antinutritional factors in one or both of the substitute ingredients. Evidence of a protein-sparing effect in lipids was found, although this resulted in marked elevation of body fat. In a future trial, we plan to examine a range of diets in which the substitution of fishmeal falls between the extremes examined here.

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