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EFFECT OF PROTEIN LEVEL AND VARYING PROTEIN – LIPID CONCENTRATIONS ON GROWTH CHARACTERISTICS OF JUVENILE SPOTTED GRUNTER *POMADASYS COMMERSONNII* (HAEMULIDAE)

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A 60-day feeding trial was conducted to evaluate the effects of dietary crude protein level on growth, feed conversion and protein efficiency ratio of juvenile (3–6 g) spotted grunter *Pomadasys commersonnii.* In the experiment, six semi-purified diets containing casein and fishmeal as protein sources, and with crude protein levels ranging from 35 to 60%, were fed to three replicate groups of fish per treatment in a recirculating system at the optimum temperature for growth. The relationships between dietary crude protein level and growth parameters were analysed by broken-line regression models. Results suggest that 48–50% dietary protein is needed for optimum growth and feed conversion for *P. commersonnii*. In a second experiment, three protein levels (35, 45 and 55%) at three different lipid concentrations (6, 8 and 12%) were used to formulate nine semi-purified diets that were fed for 60 days to triplicate groups of fish per treatment. Results suggest that a diet of at least 45% protein with a 12% lipid inclusion level is required for the best specific growth rate (5.96% per day) and feed conversion ratio (1.72) at this specific stage in the growth phase of *P. commersonnii*.

Key words: growth, lipid, *Pomadasys commersonnii*; protein

The commercial viability of any intensively cultured fish species depends on market demand and cost of production. The largest fraction of the production cost lies in feed, with protein comprising the most expensive component. It is therefore crucial that optimal protein levels are known to ensure best growth and survival at the lowest cost (Serrano *et al*. 1992, Chen and Tsai 1994). Fish, like other animals, do not have a true protein requirement, but they do require a well-balanced mixture of essential and non-essential amino acids. It is, however, the protein in the diet that provides the amino acids. Inadequate protein in the diet will result in a reduction or cessation of growth and a loss of weight owing to the withdrawal of protein from less vital tissues to maintain the functions of the more essential ones (Wilson 1989).

The optimal dietary protein requirement of a species is influenced by a number of factors. Among others, these include the protein source, the protein to energy (P:E) ratio in the diet (Sargent *et al*. 1989), the size and age of the fish and the ambient temperature, and the quantity of food ingested is determined by the energetic requirements of a fish (Lee and Putnam 1973). Increasing dietary protein in fish diets generally results in an increase in growth rate. However, protein inclusion levels in excess of the optimum requirement results in increased rates of deamination and catabolism of protein and amino acids (Cai *et al*. 1996) and may not further enhance growth. At high inclusion levels, protein is used for somatic growth and to satisfy maintenance energy requirements. The use of protein for the latter is neither necessary nor desirable. It has been shown for numerous species that partial replacement of a fraction of the optimum protein requirement with relatively inexpensive high-energy sources such as carbohydrates and/or lipids may result in a significant degree of "protein sparing" with no reduction in somatic growth rate (Austreng 1979, Nematipour *et al*. 1992). However, the degree to which protein can be spared can only be determined once an "optimal" dietary protein inclusion level has been established (Fuller 1988, Tibaldi *et al*. 1996).

Because of its high value, taste and texture, the spotted grunter *Pomadasys commersonnii* has been identified as a candidate species for marine aquaculture in South Africa. Under natural conditions these fish attain a size of approximately 200–300 g within the first 16 months (Wallace and Schleyer 1979) and under cage or tank culture conditions reach 500 g in the same time (Hecht and Mperdempes 2001).

The present study was designed to examine the effect of dietary crude protein levels on growth, feed conversion ratio (*FCR*) and protein efficiency ratio (*PER*) of *P. commersonnii.* Further it aimed to define the optimal dietary protein:energy (P:E) ratio in order to facilitate maximum growth under culture conditions and to determine the extent to which protein can be spared by lipid inclusion. This was achieved by as-

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Table I: Ingredients and nutrient composition (dry weight basis) of diets to evaluate the influence of dietary protein level on growth of *P. commersonnii*

Ingredient	Protein level (%)					
	35	40	45	50	55	60
Casein[1]	20	25	30	35	43	50
Fish meal[2]	24	24	25	25	22	20
Dextrin[3]	30	25	20	15	10	5
α-Cellulose[1]	2.5	2.2	1.5	1.5	1.5	1.5
Sodium alginate[4]	2.5	2.5	2.5	2.5	2.5	2.5
Sodium hexamata-phosphate[5]	1	1	1	1	1	1
Cod liver oil	8	8	8	8	8	8
Vitamin mixture[6]	4	4	4	4	4	4
Mineral mixture[7]	8	8	8	8	8	8
Determined nutrient analysis						
Crude protein (%)	36.67	40.45	46.22	50.86	57.83	61.84
Gross energy (kJ g^{-1})	17.79	17.82	18.35	18.16	18.93	18.33
Ash (%)	14.39	14.59	14.27	15.62	14.10	14.44

[1] Sigma Chemicals (St Louis, MO, USA)
[2] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Denmark)
[3] Sigma Chemicals (St Louis, MO, USA)
[4] Sigma Chemicals (St Louis, MO, USA)
[5] Sigma Chemicals (St Louis, MO, USA)
[6] Per kg: Vitamin A 500 000 IU; Vitamin D_3 400 000 IU; Vitamin E 10 000 IU; Vitamin K_3 1 g; Vitamin B_1 0.25 g; Vitamin B_2 1.5 g; Vitamin B_6 0.5 g; Vitamin C 2.5 g; Folic acid 0.09 g; Biotin 0.025 g; Niacin 2.5 g; Inositol 2.5 g; Calpan 2.5 g.
[7] Per kg: Potassium 74 g; Vermiculite RSU 516 g; Salt 14 g; Ammonium chloride 0.05 g; Choline chloride 31 g; Cobalt 0.31 g; Copper 0.15 g; Iron 1.5 g; Iodine 0.05 g; Manganese 0.22 g; Magnesium 41 g; Zinc 1 g

sessing the effect of three different P:E ratios at three protein levels on growth, *FCR* and *PER*.

MATERIAL AND METHODS

Experimental diets, feed preparation and feeding

Six semi-purified diets (Table I) were formulated with protein levels ranging from 35 to 60% to evaluate the influence of dietary protein levels on growth, *FCR* and *PER* of *P. commersonnii*. Diets were kept isocaloric at 18.23 ± 0.31 mg kJ^{-1}, with the supplementation of dextrin.

To evaluate the influence of P:E ratio on growth, *FCR* and *PER*, nine diets (Table II) were formulated to contain either 35, 45 or 55% protein and 6, 8 or 12% lipid. Marine fish oil and soybean oil served as the lipid sources. Because the digestible energy values for the individual dietary ingredients have not been determined for *P. commersonnii*, digestible energy was calculated, based on the estimated values most commonly used in artificial diet preparation for carnivorous marine fish, i.e. 16.7, 16.7 and 37.7 kJ g^{-1} for carbohydrate, protein and lipid, respectively.

The protein sources used for all experimental diets were casein and low temperature Danish fishmeal. The dry ingredients were thoroughly mixed and oil was added gradually, whereafter distilled water was slowly added until a stiff dough was formed. The dough was mechanically kneaded in a commercial food mixer for 10 minutes and then extruded through a 2-mm die and sliced into small pellets (4 mm long).

The experimental groups consisted of eight fish per tank with three replicates per treatment. The fish were fed to satiation three times a day for 60 days, which has been found optimal for this species (Deacon and Hecht 1996). Food for each tank was kept in individual screw-top containers at -20°C, which were weighed before and after each feed to determine the mass of food consumed per day.

Experimental animals and system

All fish used in this study were juveniles, estimated to be younger than 6 months, caught in the estuary of the Great Fish River on the south-east coast of South Africa (33°30´S, 27°07´E) with a 15-mm mesh seine net. Fish were acclimated to tank conditions for a period of two weeks prior to the initiation of feeding trials. During the first week of acclimation, the fish were fed pellets consisting of a 2:1 ratio of minced sardine *Sardinops sagax* and low temperature Danish fishmeal to induce acceptance of pellets. During the second week the fish were switched to their respective semi-purified diets to ensure complete acceptance of the moist pellet diet prior to starting the experiment. The average weight and total length (*TL*) of the fish for the protein experiment were 3.38 ± 0.48 g and 55 ± 3 mm *TL*, whereas those for the P:E experiment were 6.42 ± 0.72 g and 66 4 ± 3.2 mm *TL*.

A 4 000-ℓ recirculating system (excluding experimental tank volume), incorporating both trickle and submerged biological filters was used for this trial. Water in the system was replaced at a rate of 20% per week. A total of 40-ℓ glass aquaria (maintained at a volume of 36 ℓ) were arranged in three tiers, with three replicates for each treatment being allocated to tanks. Water temperature was controlled at a constant 24 ± 1.5°C for the duration of the experiment by means of a thermo-controlled 4 kW immersion heater. This temperature is the preferred optimum (Deacon and Hecht 1996). Photoperiod was maintained at

Table II: Ingredients and nutrient composition (dry weight basis) of diets to evaluate the influence of dietary protein levels at different dietary lipid concentrations on growth of *P. commersonnii*

Ingredient and nutrient composition	Value at different levels of portein and lipid								
	35% protein			45% protein			55% protein		
	6% lipid	8% lipid	12% lipid	6% lipid	8% lipid	12% lipid	6% lipid	8% lipid	12% lipid
Casein[1]	11	11	11	15	15	15	25	25	25
Fishmeal[1]	35	35	35	44	44	44	45	45	45
Pregelatinized maize starch[2]	26	34	37	16	24	27	6	14	17
α-Cellulose[1]	20.2	10.1	3.2	18.4	8.3	1.3	17.4	7.3	0.4
Fish oil[3]	1.2	2.6	5.2	0.4	1.8	4.5	0.4	1.8	4.4
Soy bean oil	0.6	1.3	2.6	0.2	0.9	2.2	0.2	0.9	2.2
Vitamin mixture[1]	2	2	2	2	2	2	2	2	2
Mineral mixture[1] 4	4	4	4	4	4	4	4	4	
Determined nutrient analysis									
Crude protein (%)	35.7	35.5	35.5	44.8	45.3	45.3	54.7	55.4	55.3
Gross energy (kJ g^{-1})	8.0	8.2	8.5	8.2	8.3	8.5	8.5	8.6	8.8
Ash (%)	9.4	9.4	10.3	11.7	11.8	10.3	10.4	10.6	11.0
Calculated values									
DE (kJ g^{-1})	12.7	14.8	16.8	12.8	14.9	16.9	12.9	15.0	17.0
P:E (mg kJ^{-1})	28.1	23.9	21.1	35.3	30.3	26.7	42.3	36.8	32.5

[1] As in Table I
[2] African Products (Pty), Ltd (Johannesburg, South Africa)
[3] Marinol-R, Marine Oil Refiners (Pty), Ltd (Cape Town, South Africa)

14L:10D (Deacon and Hecht 1996) at a light intensity of 1500 lux (equivalent to 1.95×10^{-3} μE s^{-1} cm^{-2}). The flow rate to the tanks was 0.6 ℓ min^{-1}, resulting in one water exchange per hour. Aeration was supplied via an airstone in each tank, coupled to a low-pressure air blower. Oxygen saturation and salinity were maintained at a constant 97 ± 1% and 33 ± 1 respectively. Nitrite and total ammonia levels were tested weekly and remained below 0.1 and 0.02 mg ℓ^{-1} respectively.

Growth parameters

At the beginning of the trial period, and every 10 days thereafter, fish were weighed. This was achieved by anaesthetizing the fish in a bath of 0.02% 2-phenoxyethanol, then gently blotted using a paper towel to remove excess water, and individually weighed (using a Mettler 3000 Electronic Scale). The use of 2-phenoxyethanol has no effect on fish growth (Deacon *et al*. 1997).

The parameters used to evaluate the growth and food assimilation of the fish were:

$$\text{Specific growth rate } (SGR \text{ in } \%) = \frac{\ell n(w_{t2}) - \ell n(w_{t1})}{t_2 - t_1} \times 100 ,$$

where w_2 and w_1 are the weights at time (t) 1 and 2 and $t_2 - t_1$ is the time (in days) between w_2 and w_1.

$$\text{Food conversion ratio } (FCR) = \frac{\text{Food intake (dry weight in g)}}{\text{Body weight gain (wet weight in g)}}$$

$$\text{Protein efficiency ratio } (PER) = \frac{\text{Body weight gain (wet weight in g)}}{\text{Protein intake (dry weight in g)}} .$$

Analysis

Ashing was performed at 550°C for 7 h, crude protein ($N \times 6.25$) was determined using the micro-Kjeldahl technique, and the gross energy content of diets was determined through direct combustion in a CP400 Calorimeter Systems adiabatic calorimeter.

Data on total body weight gain, *SGR*, *FCR* and *PER* were analysed using one-way analysis of variance. Tukey's *D*-test was used to compare means between treatments at $p = 0.05$ (Snedecor and Cochran 1990).

Protein requirements were estimated from total body weight gain, *SGR*, *FCR* and *PER* using the two-slope, broken-line analysis technique (Robbins *et al*. 1979).

RESULTS

Total body weight gain, *SGR*, *FCR* and *PER* of fish fed the experimental diets are presented in Table III.

Table III: Influence of different dietary protein levels on total body weight gain, specific growth rate (*SGR*), feed conversion ratio (*FCR*) and protein efficiency ratio (*PER*) of juvenile *P. commersonnii* over 60 days (means ± *SD*, *n* = 3)

Protein level (%)	Weight gain (g)	*SGR*	*FCR*	*PER*
35	$5.67^{a} \pm 0.51$	$2.02^{a} \pm 0.15$	1.89 ± 0.21	$1.80^{ab} \pm 0.09$
40	$7.68^{b} \pm 2.87$	$2.31^{b} \pm 0.11$	1.96 ± 0.25	$1.17^{a} \pm 0.12$
45	$6.13^{b} \pm 2.30$	$2.33^{b} \pm 0.14$	1.85 ± 0.12	$0.94^{b} \pm 0.10$
50	$7.15^{b} \pm 1.67$	$2.42^{b} \pm 0.09$	1.67 ± 0.11	$0.88^{b} \pm 0.11$
55	$5.64^{a} \pm 0.80$	$2.09^{a} \pm 0.13$	1.85 ± 0.17	$0.80^{bc} \pm 0.15$
60	$5.07^{a} \pm 0.45$	$1.81^{a} \pm 0.15$	1.87 ± 0.24	$0.59^{c} \pm 0.14$

[a, b, c] Values in the same column with different superscripts are statistically different at $p < 0.05$

Dietary protein content significantly affected ($p < 0.05$) total body weight gain, *SGR* and *PER*, but had no influence on *FCR* ($p > 0.05$). Whereas an optimum *SGR* was reached at 50% dietary protein, there was a decreasing trend in *PER* with increasing dietary protein levels.

On the basis of total body weight gain, *SGR*, *FCR* and *PER*, the broken-line model predicts an optimum dietary crude protein requirement of 48.56–50.00% for juvenile *P. commersonnii* (Table IV).

Whereas *SGR* increased ($p < 0.05$) with increasing lipid concentrations at a dietary protein level of 35%, no differences ($p > 0.05$) were found among lipid concentrations at 55% dietary protein (Table V). The highest ($p < 0.05$) *SGR* (5.96% day^{-1}) was at a dietary protein level of 45% and a lipid concentration of 12%. On the basis of performance (*SGR*, *FCR* and *PER*) the optimal P:E ratio (26.7 mg kJ^{-1}) was derived from a diet containing 45% protein and 12% lipid. Except for *SGR*, increasing lipid concentration at 35 and 45% dietary protein did not have a positive influence on parameters within protein levels, indicating the absence of the "protein sparing effect" (Steffens 1989).

DISCUSSION

The present study showed that the optimum dietary protein level (48–50%) required by juvenile *P. commersonnii* is comparable to results presented for carnivorous species such as Atlantic salmon *Salmo salar* and striped bass *Morone saxatilus* (40–55%; Millikin 1983), rainbow trout *Oncorhynchus mykiss* (51%; Austreng and Refstie 1979), red drum *Sciaenops ocellatus* (45%; Serrano *et al*. 1992) and grouper *Epinephelus malabaricus* (47.8%, Chen and Tsai 1994). *P. commersonnii* has been described as an opportunistic benthivore (Van der Westhuizen and Marais 1977). Best growth and *FCR* was achieved with a 50% dietary protein diet, indicating the most efficient utilization of the food. This empirical estimate of the optimum dietary protein content for juvenile grunter (<70 mm *TL*) is similar to the predicted value of 58% from an analysis of the proximal composition of the mysid *Mesopodopsis slabberi*, the preferred prey of the fish at this size (Irish 1997). *PER* decreased with increasing dietary protein levels, indicating that the maintenance requirement of dietary protein for juvenile *P. commersonnii* probably lies between 35 and 40%. Fish are physiologically capable of utilizing a maximum amount of protein for growth, after which the excess protein will either be converted to glycogen for energy or will be deaminated and excreted (Wilson 1989).

Optimal growth of the juvenile grunter was achieved at a P:E ratio of 26.7 mg kJ^{-1} (i.e. the 45% protein:12% lipid diet). The use of dietary lipid levels as a means of reducing the protein requirement has been well documented, and protein sparing has been noted for

Table IV: Comparison of the dietary crude protein requirements of *P. commersonnii* determined by ttwo-slope, broken-line analysis

Statistical model	Weight gain	*SGR*	*FCR*	*PER*
Broken-line analysis	50.00 ± 9.95	48.71 ± 1.49	50.00 ± 7.80	48.56 ± 11.44
Goodness of fit				
R^2	0.5449	0.9527	0.6108	0.9066
Absolute sum of squares	2.260	0.0126	0.0181	0.0199
$S_{y.x}$	1.063	0.0793	0.0951	0.0997

Table V: Influence of dietary protein levels at different dietary lipid concentrations on specific growth rate (*SGR*), feed conversion ratio (*FCR*) and protein efficiency ratio (*PER*) on juvenile *P. commersonnii* over 60 days

Protein (%)	Lipid (%)	Initial weight (g per fish)	Final weight (g per fish)	*SGR* (% per day)	*FCR*	*PER*
35	6	4.17 ± 2.28	9.65 ± 4.77	4.81^{a} ± 0.43	2.43^{a} ± 0.40	1.05^{a} ± 0.09
35	8	6.37 ± 1.92	13.59 ± 3.61	5.08^{b} ± 0.51	2.27^{b} ± 0.29	1.06^{ab} ± 0.09
35	12	7.78 ± 2.93	16.25 ± 4.61	5.43^{c} ± 0.33	2.43^{a} ± 0.35	1.11^{b} ± 0.12
45	6	6.41 ± 2.16	14.63 ± 3.67	5.51^{cd} ± 0.32	1.86^{d} ± 0.20	1.21^{bc} ± 0.11
45	8	6.67 ± 2.58	16.56 ± 5.32	5.58^{cd} ± 0.31	2.08^{c} ± 0.24	1.29^{cd} ± 0.17
45	12	7.49 ± 2.57	17.83 ± 5.19	5.96^{e} ± 0.36	1.72^{d} ± 0.19	1.25^{cd} ± 0.13
55	6	6.75 ± 2.38	15.59 ± 4.93	5.35^{bc} ± 0.37	1.88^{d} ± 0.12	1.38^{d} ± 0.16
55	8	5.37 ± 1.91	14.05 ± 4.57	5.41^{c} ± 0.32	1.91^{c} ± 0.25	1.37^{d} ± 0.15
55	12	6.44 ± 1.11	18.35 ± 4.25	5.38^{bc} ± 0.36	2.23^{b} ± 0.31	1.30^{cd} ± 0.14

[a, b, c, d, e] Values in the same column with different superscripts are significantly different at $p < 0.05$

species such as turbot *Scophtalmus maximus* (Bromley 1980), red tilapia *Oreochromis* hybrid (De Silva *et al*. 1991), red drum (Ellis and Reigh 1991), Atlantic salmon (Hillestad and Johnson 1994) and juvenile dentex *Dentex dentex* (Tibaldi *et al*. 1996). The lack of a protein sparing effect in the present study is probably because of the narrow range of lipid levels tested (Steffens 1989). It does not, however, imply that such an effect cannot be achieved for juvenile grunter, but rather highlights the need for more detailed investigations on essential fatty acid and amino acid requirements of the species. For example, the protein sparing capabilities of red tilapia and dentex occur at 18% dietary lipid (De Silva *et al*. 1991, Tibaldi *et al*. 1996), although the effect was already apparent in turbot at a dietary lipid inclusion level of 6% (Bromley 1980).

This study has defined the optimum P:E levels for juvenile spotted grunter at which maximum growth is attained under culture conditions. In a detailed study on the feeding biology of spotted grunter, Webb (2001) showed that the preferred diet of the species changes from mysids to sand and mud prawns *Callianassa kraussii* and *Upogebia africana* at an average size of around 200 mm *TL*. Given that the protein content of *C. kraussii* and *U. africana* (39–42%) is lower than for mysids (58% – Irish 1997), it is possible that the total protein requirement of fish >200 mm *TL* would also decrease. To formulate least-cost feeds for the production of fish to a market size of around 500 g would therefore require profiling the P:E requirements for fish >200 mm *TL* (c. 100 g).

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