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The growth of 0-group plaice on artificial diets containing different levels of protein

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EXTRAIT: **La croissance de groupes-0 plies sous l'influence de diètes artificielles contenant différentes proportions de protéines.** Ce travail étudie la croissance de plies, *Pleuronectes platessa,* soumises à des régimes partiellement définis et examine la relation existant entre la richesse en protéines du régime et la vitesse de croissance. Les groupes-0 de plies, individuellement marquées par injection de latex coloré, étaient gardés dans des reservoirs en fibre de verre (approximativement: 90 × 60 × 42,5 de profondeur) sous un système fermé de circulation d'eau de mer. Six groupes de plies recevaient un régime dont la richesse protéique (caséine enrichie d'arginine, cystine, méthionine et tryptophane afin de ressembler à la protéine intégrale de l'oeuf de poule) était différente; respectivement 20, 30, 40, 50, 60 et 70 % du poids sec de la nourriture. Ces différents régimes étaient isocaloriques et contenaient 3.7 calories par gramme. L'expérience a été réalisée à 15° C. Le poids initial moyen des poissons utilisés était compris entre 2 et 3 g. La croissance a été presque linéaire avec chaque régime et les vitesses moyennes de croissance par semaine, pendant la durée de l'expérience (28 semaines) ont été de 0.19, 0.22, 0.49, 0.56, 0.73, 0.75 g avec les régimes contenant respectivement de 20 % à 70 % de protéines. La vitesse de croissance apparait donc augmenter linéairement avec la richesse en protéines du régime jusqu'à la valeur la plus élevée testée. Les taux de conversion (grammes de nourriture sèche nécessaires pour produire une augmentation de poids d'un gramme) ont été de 5.5, 3.5, 2.9, 1.7, 1.6 et 1.6 pour les régimes contenant respectivement de 20 % à 70 % de protéines. Les rapports d'efficacité protéique (P.E.R. = augmentation – en grammes – du poids vif par gramme de protéines ingeré) ont été de l'ordre de 1.0, la valeur optimum étant de 1.16 pour le régime à 50 % protéique. Ces résultats sont discutés et comparés avec ceux trouvés pour le saumon et le poisson chat, *Ictalurus punctatus.*

INTRODUCTION

We have recently shown that the essential amino acid requirements of plaice, *Pleuronectes platessa* L., and of sole, *Solea solea* L., are qualitatively similar to those of Pacific salmon (Cowey et al. 1970). These essential amino acid requirements were determined indirectly by the use of carbon-14 labelled glucose. In order to answer further questions about the basic nutrition of these fish the use of defined or partially-defined diets containing purified nutrients is necessary. The experiment reported here was undertaken to examine the feasibility of using artificial diets in nutritional experiments with plaice and to examine the effects on weight gain and food conversion of different levels of dietary protein.

Studies with other species of fish have shown that their protein requirements are comparatively high when compared with those of man and domestic animals, for instance DELONG et al. (1958) showed that the optimal levels for Chinook salmon were 40 % of the dry diet at 47° F (5° C) and 55 % of the dry diet at 58° F (14.5° C), while DUPREE & SNEED (1966) using Channel catfish at 76° F (24.5° C) found that with casein and wheat gluten diets optimal growth was achieved with 40 % protein in the dry diet. At these levels, the protein source will be the most expensive constituent of fish diets and information about the relation of protein level to growth in flatfish is essential if they are to be cultivated economically and successfully.

MATERIAL AND METHODS

Group-0 plaice, about six months old, were obtained from the White Fish Authority Field Unit, Hunterston, Ayrshire. They were kept in vigorously aerated water in tanks (3′ × 2′ × 1.5′ deep) in a constant temperature room at 15° C; the tanks were individually illuminated with a 6 watt light placed 9 inches above the water surface for 10 h a day. Sea water was recirculated through the tanks from a 10,000 gallon reservoir and the circulation system included a Hanovia sterilisation unit (max. emission at 2537 Å) and a gravel filter supported on a mixture of coarse marble chips and charcoal. The sea water entering the tank room passed through a 500 gallon tank where the temperature was raised to 15° C by means of a 2 kilowatt vitreosil covered element (Thermal Syndicates Ltd., Wallsend, Northumberland). Initially 20 fish were placed in each tank; these fish were individually marked by subcutaneous injection of rubber latex dyes (RILEY 1965) and their growth rates were later followed as individuals within a population.

The composition of the diets is shown in Table 1. On the basis of 9.0 kilocalories/g fat, 4.0 kilocalories/g protein and 4.0 kilocalories/g dextrin these diets are isocaloric containing 3.7 kilocalories/g dry weight. Vitamin free casein (micro pulverized) was from Nutritional Biochemical Corporation, Cleveland. 95 g of casein were supplemented with 2.5 g arginine, 1.0 g cystine, 1.0 g methionine and 0.4 g tryptophan so that the essential amino acid composition of the casein resembled that of whole hens' egg protein. These additional amino acids were mixed with the casein in a ball mill.

The vitamin mixture employed contained the same quantities of vitamins as that which HALVER (1957) devised for use with Chinook salmon; however, choline chloride was replaced by the less hygroscopic choline bitartrate in sufficient quantity to provide an equivalent amount of the base, and vitamin A was supplied preformed in the cod liver oil.

The dry constituents of the diet (casein, dextrin, minerals, vitamins) were thoroughly mixed in a domestic mixer, corn oil and cod liver oil were then added to and mixed with them, these were followed by the addition of the requisite amount of

Table 1

Components of the diets (g)

Component	Protein (%) 20	30	40	50	60	70
Casein (supplemented)	20	30	40	50	60	70
Dextrin	58.5	48.5	38.5	27.3	17.3	7.3
Vitamins	4.5	4.5	4.5	4.5	4.5	4.5
Minerals*	4.0	4.0	4.0	4.0	4.0	4.0
Corn oil	4.0	4.0	4.0	4.0	4.0	4.0
Cod liver oil	3.0	3.0	3.0	3.0	3.0	3.0
α-cellulose flour	0	0	0	2.0	2.0	2.0
Binder	6.0	6.0	6.0	4.0	4.0	4.0
Water**	100	100	100	100	100	100

* To 100 g of Salt mixture No. 2 U.S.P. XIII were added Aluminium chloride 18 mg, Zinc sulphate 357 mg, Cuprous chloride 11 mg, Manganous sulphate 80 mg, Potassium iodide 17 mg and Cobaltous chloride 105 mg.
** Containing the taste attractant mixture in Table 3.

water with further thorough mixing. Finally a binding agent was added to this wet mixture and the whole was again mixed to a smooth homogeneous paste. This diet was fed to the fish as a moist worm by extruding it under pressure from a 50 ml nylon veterinary syringe.

Various binding agents (carboxymethyl cellulose, alginate, gelatine and guar gum) were used to bind this diet but "worms" bound with guar gum showed least tendency to break up when placed in water and in fact maintained their discrete shape for long periods of time. Thus guar gum was used for this purpose throughout the experiment (the actual product being Supercol GF from Tragasol Products Ltd., Hooton, Cheshire).

Table 2

Composition of taste attractant mixture. Methionine, used in the casein supplementation, probably enhances taste producing effect

Component	mg/100 ml water
Inosine 5-phosphate	236
Alanine	20
Glutamic acid	16
Glycine	10
Valine	7
Lysine	33

Only sufficient diet for about 10 days was made up at one time; this was stored at — 15° C in sealed plastic bags.

Some difficulty was encountered initially in persuading the fish to accept this artificial and rather alien diet, and it was felt necessary to include some form of taste attractant in it. The taste producing role of the nitrogenous extractives of a variety of marine products have been investigated by Japanese workers (HASHIMOTO 1967). They showed that certain nucleotides and amino acids each in combination with glutamic acid were responsible for the meaty taste of these products. As a result of their findings a taste producing solution (Table 2) was formulated and substituted for water in the diet. This attractant solution will induce a search behaviour in various marine animals but while its inclusion in the diet markedly improved acceptability for the fish no exhaustive experiments were carried out either on the concentration or variety of substances necessary for maximal taste acceptability, and it is not suggested that this mixture has "optimal" taste attractant properties for the plaice.

Fish were fed three times daily except at weekends when they were fed once per day. Only small quantities of diet were offered at a time and when all the food offered had been consumed more was added to the tank until the fish ceased to show any interest in it, then the small excess of food remaining in the tank was quietly removed from the tank with a siphon some 15 min later.

During the periodic weighings fish were removed singly from the tank with a hand net, drained for 10 sec during which time the colour code was identified, and placed on a piece of hard backed, water absorbent sponge. The weight of sponge + fish was rapidly determined on a Mettler P 1200 top pan balance and the fish was returned to the tank, the weight of the sponge (plus any water it had absorbed) was then ascertained. This method proved satisfactory and reproducible. Fish were not fed for 24 h prior to weighing.

Length measurements of the fish were made periodically by photographing the fish when they were very small, then later, as they increased in size, by use of a measuring board. These length measurements have not yet been fully analysed but in all respects they appear to confirm the conclusions reached from the weight data.

RESULTS

The experiment was commenced on November 11th, 1968; the fish were weighed on 9 subsequent occasions the final measurements being made on May 27th, 1969. It will be observed (Table 3) that the number of fish, for which the complete set of 10 weight readings is available, differs for each dietary group. This was largely because not all the fish on any dietary regime would accept the food offered. These fish slowly starved during the course of the experiment and finally died. Thus only data relating to those fish which survived throughout the experiment and were actively feeding at its end were considered. While it might, at first sight, appear that population density effects would result from the disparity in the numbers of animals shown in Table 3 it should be borne in mind that mortalities did not begin to occur until quite late on in the experiment. For the greater part of the time 20 animals were present in each tank but a variable proportion of these were steadily losing weight. With the exception of the animals receiving the 40 % protein diet the mean weights at the start of the experiment were very similar.

Growth curves for fish fed three of the diets are shown in Figure 1 (others are

Table 3

Growth data on plaice, *Pleuronectes platessa,* fed different levels of protein

1 Protein in diet (%)	2 Number of fish	3 Mean initial weight (g)	4 Average growth increment (g) ± S.E.	5 Average growth rate per week (g) ± S.E.
20	17	2.31	5.25 ± 1.36	0.19 ± 0.05
30	14	2.41	6.47 ± 1.50	0.22 ± 0.06
40	7	3.40	13.51 ± 2.12	0.49 ± 0.08
50	17	2.04	15.63 ± 1.36	0.56 ± 0.05
60	9	2.20	19.89 ± 1.87	0.73 ± 0.07
70	11	2.31	20.45 ± 1.70	0.75 ± 0.06

omitted in the interests of clarity). The average values for absolute growth increment on all diets are shown in Table 3 (column 4). These data clearly indicate a significant increase in absolute growth with increasing dietary protein level the increase being greatest between 30 % and 40 % levels. Although there was evidence that growth was greater for those individuals which were heavier at the beginning of the experiment (as shown by an analysis of covariance of the growth increments with initial weights as covariace) this was outweighed by the effect of diet.

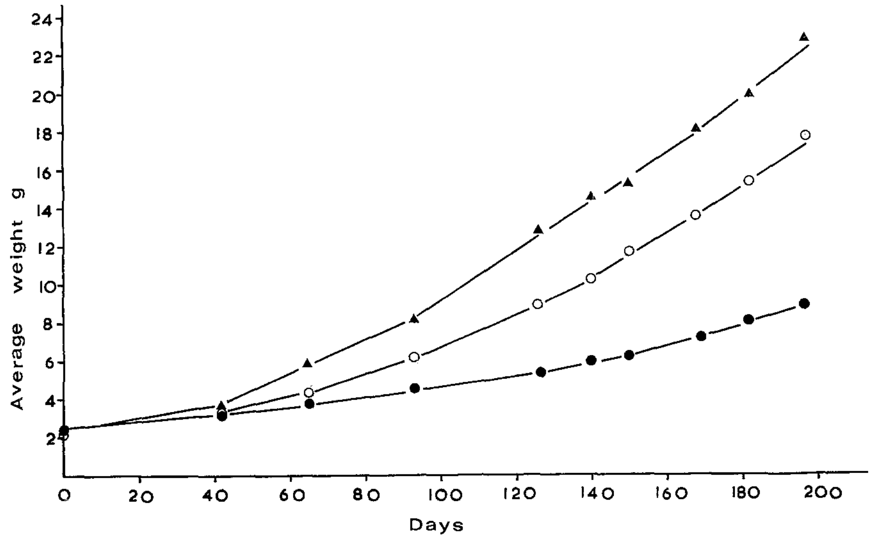

Fig. 1: Growth curves of plaice, *Pleuronectes platessa,* fed three of the dietary levels of protein employed (▲ 70 % protein, ○ 50 % protein, ● 30 % protein)

Absolute growth increments do not utilise the (eight) intermediate weighings and are not therefore the most efficient measure of growth rate. In order to utilise the information provided by all the observations polynomial curves were fitted by least squares regression to the data for each animal and the corresponding regression coefficients compared. Growth appeared to be very nearly linear over the experimental period so that a first degree polynomial, that is a relationship of the form

$$w_t = a + bt$$

where t is the time in days, w_t is the weight at day t, and a and b are constants estimated from the data (b being the growth rate per day) provided a good representation of growth.

The value of b was determined for each fish and the mean values for each dietary treatment are shown in Table 3 (column 5). These results resemble those in column 3 of the same table because growth is reasonably linear over a large part of the experimental period.

At the dietary protein levels used in this experiment growth rate appears to increase linearly. That is there was no falling off or plateauing of growth rate as dietary protein levels were increased to 70 % of the dry diet.

Table 4 shows the conversion rates and protein efficiency ratios (P.E.R.) of the fish on different protein levels for the last eight weeks of the experiment. Measurement of these ratios was restricted to this final period because only by then did we have sufficient experience to be able to feed the fish almost to satiation without at the same time having any (unaccepted) food left in the tank. It was considered that feeding of excess food and then attempting to estimate the uneaten portion of it by drying and weighing, or other means, would lead to gross inaccuracies. In practice, over the period for which conversion ratios were measured, the amount of dry diet consumed by the fish was about 2 % of their body weight per day. Conversion rates fell to a more or less constant level (of about 1.6) with 50 % or more protein in the diet. By comparison lower conversion rates down to 0.9 were obtained for catfish fed casein diets (Dupree & Sneed 1966). The highest P.E.R. (1.16) was obtained with a 50 % protein diet; in measuring the nutritive value of proteins by this method it is recommended that the maximum value obtained in studies at different protein levels be

Table 4

Growth and conversion rates of plaice, *Pleuronectes platessa*, over period March 3rd – May 27th

Parameter	Protein in diet (%) 20	30	40	50	60	70
Total live weight gain (g)	31	43	33	127	75	92
Dry weight food consumed (g)	170	151	96	219	123	148
Conversion rate (g dry diet fed/g live weight gain)	5.5	3.5	2.9	1.7	1.6	1.6
Protein efficiency ratio (g live weight gain/g protein fed)	0.91	0.95	0.87	1.16	1.02	0.89

used. In studies with growing rats the P.E.R. obtained with whole egg protein was approximately 3 while it can be inferred from the data of Dupree & Sneed (1966) that casein diets fed to Channel catfish give a P.E.R. of about 4.

DISCUSSION

The diets fed were isocaloric on a total energy basis. Earlier findings had indicated that high levels of digestible carbohydrate were deleterious to trout causing liver damage (Phillips et al. 1948). Later experiments with salmon (Buhler & Halver 1961) showed that with an adequate vitamin supplement salmon could tolerate high levels of dietary carbohydrate. The vitamin levels used by us were probably far in excess of the requirements of the fish; gross examination of the livers revealed no signs of damage while chemical analyses of the livers (which will be reported on later) revealed that carbohydrate levels were similar in fish on different dietary regimes.

The rates of growth reported here are not optimal for plaice – clearly variation in factors such as tank size, population density, light intensity, provision of a sandy substratum might produce higher growth rates. In fact a particular set of conditions have been used to determine the effect of dietary protein level on growth rate. The results obtained contrast with recent findings on other fish (DeLong et al. 1958, Dupree & Sneed 1966) in that no optimal protein level was found with up to 70 % of protein in the dry diet. Protein levels of this order actually produce depression of growth in the catfish, where the optimum level with casein is 40 % of the dry diet at 76° F (24.5° C).

The casein used in the experiment was supplemented by the addition of four amino acids so that the protein fed more nearly resembled whole egg protein. This latter material has given highest growth rates with mammals and has frequently been used as a standard in comparative tests of biological value. It seemed therefore that such a protein must most nearly meet the protein requirements of the plaice. However, the values for P.E.R. indicate that plaice utilize protein less efficiently than do catfish or mammals.

SUMMARY

1. Group-0 plaice, *Pleuronectes plattessa* L., were grown on partially defined diets containing different levels of protein.
2. The growth rate appeared to increase linearly as the protein content of the diet was increased up to the highest level used (70 % of the dry diet).
3. A conversion rate of 1.6 was obtained with diets containing 50 % or more of protein.
4. The protein efficiency ratio on all diets was near unity. The maximum value obtained being 1.16 with the 50 % protein diet.

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