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Usefulness of feeding protein and non-protein calories apart in studies on Energy-Protein interrelationships in rainbow trout

S.J. KAUSHIK, P. LUQUET, Denise BLANC
I.N.R.A., Laboratoire de Nutrition et d'Elevage des Poissons
Centre de Recherches hydrobiologiques
Saint-Pée-sur-Nivelle, 64310 Ascain (France)

Summary

Groups of rainbow trouts were fed fixed levels of a high-protein diet immediately followed by a non-protein energy complement to satiation. Daily voluntary intake of the non-protein diet by the different groups of trout was followed during the whole course of the experiment that lasted nine weeks. Growth, gross energy intake, efficiencies of protein and energy utilization were followed. The results presented show that there is an adjustment of the voluntary intake of the non-protein energy complement in relation to the amount of protein made available to rainbow trout and that the gross energy intake is however relatively constant beyond a certain level of protein intake (about 1.7 g prot./kg BW/day). Protein efficiency ratio as well as protein productive value of the groups of fish fed protein and non-protein energy separately were comparable or even better than the controls fed a complete diet. Energy efficiency was found to be related to the proportion of protein energy intake. Maintenance protein needs of trout at a given stage of growth were also estimated. This method of feeding the two major components separately can be employed in fish with success for future studies on energy-protein relationships.

Introduction

Compared to homeotherms, fish do not seem to exhibit any qualitative specificity in their protein or amino acid needs for growth ; but the preferential use of ingested protein for energetic purposes is a well-documented phenomenon. It has however been shown by many that considerable economy of protein can be made by the judicious incorporation of other sources of energy in diets of growing fish. Consequently, the importance of protein-calorie interrelations in the optimal utilization of a diet for growth purposes has also been recognised in fish by many authors (LEE & PUTNAM, 1973 ; GARLING & WILSON, 1976 ; TAKEUCHI, WATANABE & OGINO, 1979).

In homeotherms, such interactions have been studied by offering animals energy and protein parts of the diet separately, with either fixed protein rations or on a free-choice basis. Since the early works by ABRAHAM *et al.* (1961) and CALET, JOUANDET & BARATOU (1961), the usefulness of such methods of « separate feeding » has been well demonstrated in these animals (see RERAT, 1971). This method of feeding of protein and non-protein energy components apart was employed with growing yearling rainbow trout ; observations on the efficiency of such a method in fish alongwith preliminary results obtained are reported here.

Material and methods

Principle of the method

A given quantity (X) of protein satisfying the maintenance and growth needs of fish of a given body weight W_0 would become insufficient to support growth as fish gain weight (ΔW). At this stage ($W_t = W_0 + \Delta W$), the same amount (X) of protein would satisfy only the maintenance needs and zero growth would occur. By varying the quantities of protein intake of different groups of fish (of identical body weight), and by following for each group, the stage of growth when no more weight gain is possible, the maintenance needs of fish at different stages of growth (W_t) can be estimated. The absolute quantity of protein given to any group of fish is kept constant all through the trial period and a non-nitrogenous diet is simultaneously given *ad libitum,* thus enabling fish to complement their energy needs in relation to their protein intake.

TABLE 1

Composition of the experimental diets
Composition des régimes expérimentaux

	g/kg
Diet BP	
Fish meal	980
Vitamin mix (1)	20
Diet EO	
Raw maize starch	650
Cellulose (2)	80
Fish-oil	250
Vitamin mix (1)	20

Chemical composition of the diets (3)

Diets	BP	EO	Control
Dry matter (g/kg)	843.5	896.8	883.3
Protein (N × 6.25)	731.5	6.9	491.4
Energy	4 976	5 720	5 028
Fat content	100.4	283.7	114.8
Ash content	183.5	3.0	130.2

(1) E.I.F.A.C., 1971.
(2) Durieux (C.E.P.O., SS/200).
(3) In g/kg of dry matter for protein, fat and ash contents ; in Kcal/kg d.m. for energy content.
1 Kcal = 4.18 KJ.

Test diets

The composition of the high-protein (BP) diet and of the non-protein energy complement diet (EO) is given in table I. The diet BP was pelleted dry (2.5 mm diameter) in two different lengths (3 and 10 mm). To the constituents of the diet EO, as shown in table I, was added about 25 p. 100 water and 1 p. 100 sodium alginate binder ; they were mixed well in a domestic blendor and then drawn into pellets of 2.5 mm diameter. The wet pellets were freeze-dried and stored air-free, in plastic bags.

Fish and feeding

Ten groups of 100 rainbow trout each (*Salmo gairdneri* R.), having a mean initial weight of 47.0 ± 1.2 g were kept in 10 circular plastic tanks (holding capacity : 300 l ; flow rate : 30 l/min). The temperature of the spring water remains constant throughout the year at 17 ± 0.5 °C. The tanks kept outdoors were partially covered with opaque lids and natural photo-period was maintained (january-march, Landes, France).

Of the ten groups, 8 served as experimental lots (lots 0 to 7) and 2 as controls. All fish were fed twice daily (9 a.m and 4 p.m). Lot 0 received no BP and was fed the diet EO to satiation at every meal. Lots 1 to 7 received increasing amounts of the diet BP (5, 10, 20, 30, 40, 50 and 60 g diet BP/lot/day) during the whole course of the experiment. To fish in these lots, after one half of the prescribed amount of BP was distributed at every meal of the day, diet EO was given to satiation. Feed intake of EO was noted for each lot every day.

Fish were weighed in lots every third week of the trial that lasted 9 weeks. Feed intake of the control fish were recorded weekly. Data recovered at the end of every weighings were used for calculations, but only averages for the whole experimental period are reported in table II and fig. 1. Proximate analyses (dry matter, Kjeldahl nitrogen and Energy content through Bomb calorimetry) of a single representative sample of ten fish at the start of the experiment and of ten fish from each lot at the end were also made. Statistical analyses were made following methods outlined by SNEDECOR & COCHRAN (1956).

Results and discussion

Feed intake

Feed intake of the control lots averaged 22 g.d.m/kg BW/day over the nine-week experimental period. This is a rather low value for our hatchery conditions where it is not uncommon to observe feed intakes of more than 30 g/kg BW/day. This is probably attributable to the type of tanks used to which the fish did not acclimate well. Data obtained are however comparable among themselves as all fish were grown under identical conditions. Much day to day variability was noticed in the voluntary energy intake (diet EO) of all experimental groups, the coefficient of variation of EO intake ranging between 10 and 28 p. 100. This phenomenon is frequent in fish fed *ad libitum* and the duration of the trials should be sufficiently long to mask such fluctuations.

As can be seen from fig. 1, the total feed intake (BP + EO) of almost all groups of fish, except lots 0 and 1, was uniform at 17 g d.m/kg BW/day, irrespective of the quantity of protein distributed in the form of diet BP. The voluntary intake of the energy complement diet is also represented in fig. 1. At low levels of protein intake, the voluntary intake of this non-protein energy diet increases linearly upto a small extent falling sharply then onwards.

TABLE 2

Mean values on intake (g or Kcal/kg BW/day) of protein or gross energy, growth and feed efficiency of groups of rainbow trout fed different prescribed amounts of protein daily for nine weeks

Valeurs moyennes de consommation de protéines, énergie brute, croissance et efficacité alimentaire de groupes de truites arc-en-ciel alimentées avec des quantités journalières différentes de protéines pendant 9 semaines

Lot n°	0	1	2	3	4	5	6	7	T_1 and T_2
Diet	EO alone	BP (g/lot/day) + EO *ad libitum*							Controls
Quantity	ad lib.	5	10	20	30	40	50	60	ad lib.
Intake									
Protein (g)	—	0.86	1.66	2.81	3.87	4.72	5.50	6.39	10.78
Gross Energy (Kcal)	57	78	92	97	86	89	86	90	110
Non-protein Energy (Kcal)	57	72	81	79	60	58	49	47	49
Protein-calorie ratio (mg Prot/Kcal)	—	11	18	29	45	53	64	71	98
Mean Initial Weight (g)	48.0	46.3	46.8	46.3	46.4	45.1	46.6	46.5	48.8
Final Body Composition									
Mean Weight (g)	34.9	41.2	48.8	62.3	61.7	74.1	73.9	79.8	107.0
Dry Matter (%)	23.97	25.37	24.73	24.26	26.55	24.40	27.12	27.56	27.30
Protein Content (% d.m)	66.23	67.50	65.46	70.33	65.21	73.77	62.22	66.90	67.31
Energy Content (Kcal/g d.m)	4.954	5.348	5.676	5.603	5.798	5.313	5.729	5.682	5.689
Utilization									
Weight Gain (g/kg BW/day)	—7.2	—3.1	0.7	3.3	4.0	7.6	7.9	8.4	12.5
Food Conversion Ratio (F.C.R.)	—	—	—	4.69	3.85	2.15	2.04	2.00	1.76
Protein Efficiency Ratio (P.E.R.)	—	—	0.12	1.20	1.06	1.70	1.46	1.37	1.15
Protein Productive Value (P.P.V. %)	—	—	—	29.38	20.97	34.96	22.30	28.28	22.68
Energy Efficiency (%)	—	—	4.40	9.28	14.31	13.26	21.66	20.02	21.31

Diets EO and control were given *ad libitum* at every meal. In groups 1 to 7, half of the prescribed amount of diet BP was given at every meal, immediately followed by the diet EO; lots 1 to 3 received 3 mm long pellets of BP and lots 4 to 7 had pellets of 1 cm in length.
F.C.R. = dry feed intake / weight gain.
P.E.R. = weight gain / protein intake.
P.P.V. = protein retained / protein intake (apparent net protein utilization).
Energy efficiency = energy deposited / gross energy intake.

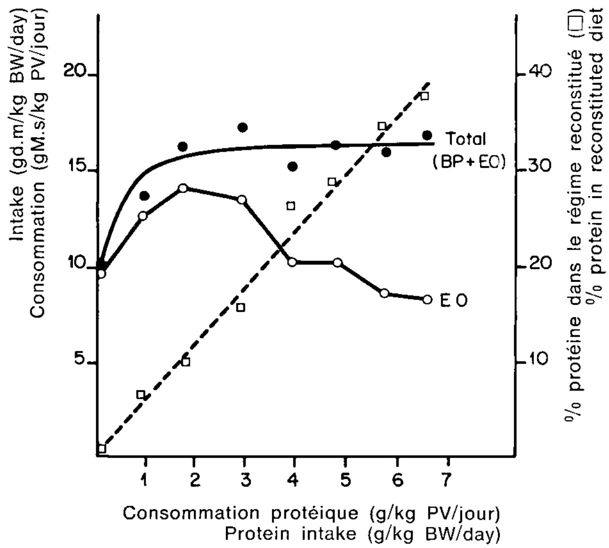

FIG. 1

Total feed intake (●), voluntary intake of the non-protein diet (○)
and adjustment of protein level in reconstituted diet (□) in relation to protein intake
Quantité totale ingérée (●), consommation spontanée de l'aliment non protéique (○)
et ajustement du taux protéique dans le régime reconstitué (□)
en fonction de la consommation protéique

Gross energy intake (GEI) of the controls (under mixed feeding of protein and non-protein energies in the same diet) averaged 110 Kcal/kg BW/day. The group fed the non-protein diet alone (lot 0) had a GEI of only half this amount (57 Kcal). Maximum GEI (around 90 Kcal) in the experimental lots was attained even by fish in lot 2 and this level was found to be maintained in other fish fed even greater amounts of protein daily. It was also found that the GEI of fish (Kcal/fish/day) was linearly related to the mean body weight of each period, within the range of body weights (35-80 g) encountered during the trial ($r = 0.79$; $p < 0.01$). Protein calorie ratio (mg Prot/Kcal) of the control diet was 98 and those of the diets freely reconstituted by fish ranged between 11 and 71, in a linear manner.

Control lots fed to satiation had a mean protein intake of about 11 g/kg BW/day, which was not found to vary during the course of the experiment. On the contrary, in the experimental lots, the mean daily protein intakes per unit body weight were found to vary due to changes in body weight during the trial. This was only intentional and was exploited for the estimation of the maintenance need. Further, there was found an adjustement of the protein concentration (p. 100) of the diet reconstituted by fish to the absolute quantity of protein made available. A strict linear relationship was observed between protein intake (X) and the percentage protein content (Y) in the reconstituted diet :

$$Y \text{ (p. 100)} = 0.390 + 6.176 \text{ X}$$

with a highly significant correlation coefficient (0.973). This relation is also depicted in fig. 1. Such adjustments have already been shown to exist in higher animals (GUILLAUME & FENDRY, 1964).

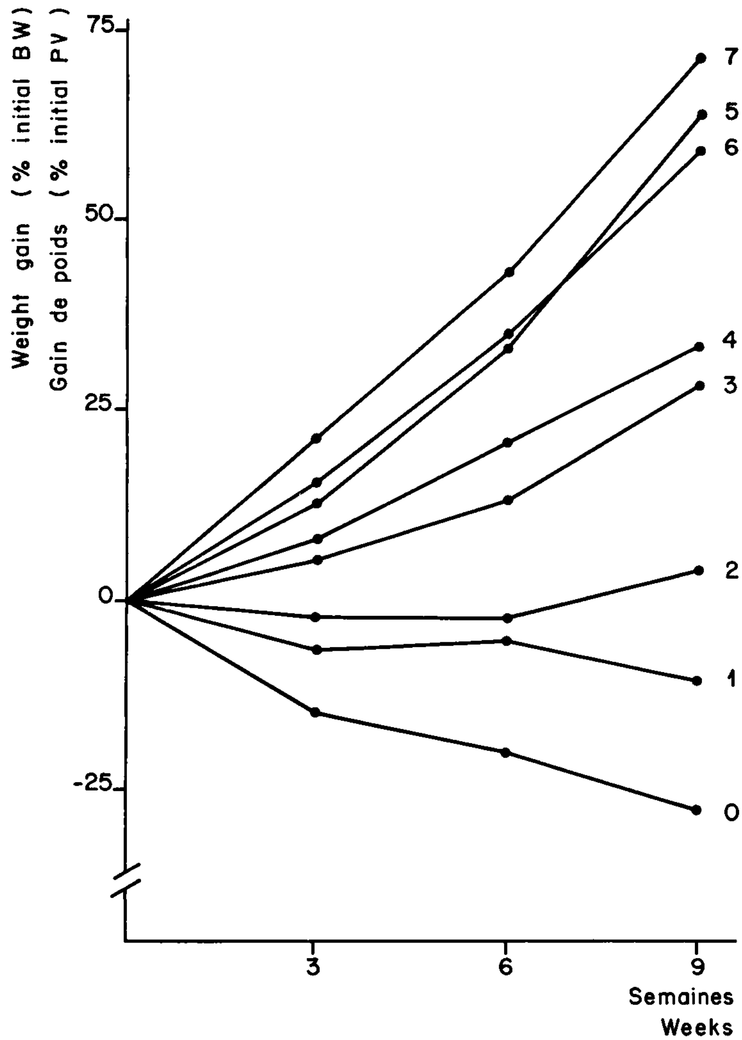

FIG. 2

Body weight changes in Rainbow trout (lots 0 to 7) fed varying amounts of protein daily

Changement de poids corporel chez la Truite arc-en-ciel alimentée avec différentes quantités journalières de protéines

Growth criteria

1. - *Weight gain*

Weight gain of the controls averaged 12.5 g/kg BW/day over the whole period. Fish fed the non-protein diet (lot 0) and those fed daily a ration of only 5 g of diet BP (lot 1) were found to lose weight. Fish receiving 10 g BP daily were found to lose weight during the first six weeks and then appeared to gain weight. The other lots (3 to 7) had mean weight gains ranging from 3 to 8 g/kg BW/day (see fig. 2 and table II).

2. - *Feed efficiency*

Food conversion ratios (FCR) of the control lots were satisfying (1.7) and those of the experimental groups were all relatively higher. The best FCR among these lots was noted for that group of fish fed the greatest amount of protein daily. The protein efficiency ratio (PER) of the control lot was on the contrary, rather poor, being only 1.15. PER of the experimental lots were variable, but were on the whole better than in the controls (except for lots 1, 2 and 4). On regrouping individual data for all lots and for all periods, there was found a logarithmic relationship between protein intake and PER with a highly significant correlation (0.834).

3. - *Protein and energy utilization*

Initial body composition was as follows : water content — 76.49 p. 100 ; protein (N × 6.25) content — 74.88 p. 100 ; fat content — 13.51 p. 100 and energy content — 5 208 cal/g d.m. The protein productive values (PPV = apparent net protein utilization) and energy efficiencies were variable within the experimental groups. The PPV of the experimental (separate feeding) lots were greater or equal to the controls, except for lot 4 ; energy efficiencies were found to increase with increasing levels of protein intake upto a certain extent beyond which they were stable around 21 p. 100.

If the FCR alone were considered, there is no doubt that the experimental groups were less efficient than the controls fed a complete diet. On the other hand, both PER and PPV data indicate that even those fish receiving only 2.8 g protein/kg BW/day convert protein more efficiently than the controls which had a daily protein intake of more than 10 g/kg body weight. Under protein restriction with non-protein energy available freely, efficiency of conversion of dietary protein into body protein is apparently improved. A rapid calculation shows that energy efficiency is also similarly affected by the proportion of GEI available as protein energy.

Although protein-gross calorie ratios are given, no pertinent conclusions can be drawn from such information as unfortunately no digestibility measurements were made on the non-protein diet (the apparent digestibility of protein and of energy of the high protein diet are known to be above 91 p. 100). This energy complement diet contains besides cellulose which is indigestible by rainbow trout, considerable amount of raw starch, the apparent digestibility of which is low (never exceeding 40 p. 100) and is subject to variations (Bergot, 1979). Since intake of this non-protein diet varies in response to protein intake, energy intake in the form of carbohydrates is also variable increasing the difficulties of digestibility measurements.

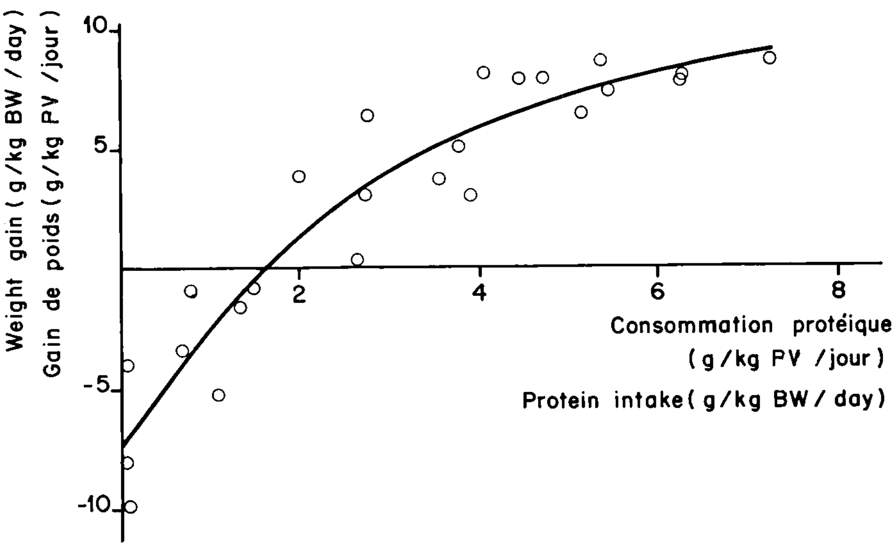

FIG. 3

Relationship between daily protein intake and weight gain
Relations entre la consommation protéique quotidienne et le gain de poids

Maintenance needs

As can be seen from fig. 2, the duration of 9 weeks is evidently too short to demonstrate stages of zero growth for all groups of fish receiving different daily rations of protein. Indeed, zero growth is noted in fish of lots 1 and 2 receiving about 0.77 to 1.47 g protein/kg BW/day during the second period of 3 weeks. At this stage, the mean body weights of these fish were 44 and 45.8 g respectively in the two lots. Accidental loss of few fish in lots 0, 1 and 2 at the end of the trial could have resulted in differences in body weight changes between the 2nd and 3rd periods. Analysis of overall data (fig. 3) using the model proposed by MORGAN, MERCER & FLODIN (1975) permits us to reach to similar conclusions as regards the maintenance needs of about 1.6 g protein/kg BW/day for the range of fish body weights encountered here. Caloric requirements could also be calculated similarly and were found to vary little, being about 64.9 $\pm$ 1.3 Kcal (GEI)/kg BW/day. It is however possible that, with other methods of estimation of maintenance needs, results are different.

Although the animal husbandry criteria retained here (FCR, PER, PPV, etc.) reflect only the overall efficiency of the diets, they do well serve the purpose of demonstrating the validity of this method in studies on protein-calorie interactions in fish. Experiments of longer durations alongwith digestibility studies should allow us to establish maintenance requirements of rainbow trout at successive stages of growth. Feeding protein and non-protein energy sources separately to fish can be an useful tool for further research in these lines.

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Résumé

Validité de la méthode d'alimentation en repas séparés dans les études de relations calorico-protéiques chez la truite arc-en-ciel

Des lots de truites arc-en-ciel ont été nourris avec des taux croissants d'un régime (BP) riche en protéines suivi d'un aliment non-protéique (EO) distribué *ad libitum*. La consommation spontanée de l'aliment non-protéique a été suivie quotidiennement pendant la durée expérimentale de 9 semaines. Le gain de poids, la consommation énergétique et les efficacités d'utilisation protéique et énergétique ont été enregistrées. Les résultats obtenus indiquent qu'il y a un ajustement de la consommation spontanée de l'aliment non-protéique en fonction de la consommation protéique et que la consommation totale de l'énergie brute se stabilise à partir d'un seuil de taux d'ingestion protéique. Les coefficients d'efficacité protéique ainsi que les coefficients d'utilisation protéique sont comparables sinon meilleurs chez les truites nourries en régime repas séparés que chez celles nourries avec un régime témoin. L'efficacité d'utilisation énergétique est liée à la proportion de l'énergie protéique ingérée. Le besoin en protéine pour l'entretien de la truite à un stade de croissance donné est également estimé. Cette méthode d'alimentation en repas séparés semble applicable aux études de relations énergie-azote chez le poisson.

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