Relationship between dietary protein quality, feeding pattern and growth in seabass (Dicentrarchus labrax) fry

Langar H.

in

Tacon A.G.J. (ed.), Basurco B. (ed.).
Feeding tomorrow's fish

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 22

1997
pages 265-274

Relationship between dietary protein quality, feeding pattern and growth in seabass (*Dicentrarchus labrax*) fry

H. LANGAR
INSTITUT NATIONAL DES SCIENCES
ET TECHNOLOGIES DE LA MER (INSTM)
28, RUE DU 2 MARS 1934
2025 SALAMMBO,
TUNISIA

SUMMARY - Several protein sources were formulated within isoproteic and isocaloric diets and fed at the same level to seabass fingerlings. The diets resulted in different growth rates which could not be explained either by digestibility or by a deficiency or imbalance of essential amino acids. The measurement of feed intake and gastrointestinal transit was performed using a new fast and accurate technique, but did not shed any light to explain the growth differences observed; although it did allow a calculation of the maximal daily ration for the seabass fingerlings. During these experiments, feeding frequency appeared to be another factor influencing growth; the sequential distribution of the ration inducing a faster growth than continuous feeding of the same amount of food. However, the differences in growth could be explained by mechanisms concerning protein metabolism due to difference in protein quality and method of feeding. For example, protein quality influences protein accretion by acting on the retention of synthesized protein (decreasing its degradation), rather than on the synthesis which is bound to the body RNA content; the ribosomal activity remaining unchanged. Moreover, under the action of sequential feed distribution, protein degradation increases while synthesis is stimulated under the effect of an increase of RNA content and ribosome activity.

Key words: Fish, protein, feeding pattern, growth, turnover.

RESUME *- "Relation entre la qualité de la proteine du régime, le mode de nourrissge et la croissance des alevins de bar (*Dicentrarchus labrax*)." Avec des régimes isoprotéiques et isoénergétiques ne différant entre eux que par la source des protéines (choisie à dessein), la croissance du jeune alevin de bar était effectivement variable. Cette différence n'a pas pu être expliquée ni par la digestibilité ni par une déficience ou un déséquilibre en acides aminés essentiels de ces protéines. La mesure de l'ingéré et du transit, par une nouvelle méthode rapide et précise, n'a fourni aucune donnée explicative supplémentaire. Elle a cependant permis de calculer la ration journalière maximale pouvant être ingérée par l'alevin de bar. Au cours de ces expériences, le mode de distribution de l'aliment s'est avéré être un deuxième facteur influençant la croissance : une distribution séquentielle de la ration alimentaire induit une croissance supérieure à celle obtenue par une distribution continue. Les différences de croissance, sous l'effet de la qualité de la protéine et du mode de distribution de l'aliment, ont pu être expliquées par des processus ayant trait au métabolisme des protéines. La qualité de la protéine alimentaire agit sur le degré de rétention des protéines synthétisées, en diminuant leur dégradation, plutôt que sur la synthèse qui est liée à la teneur corporelle en ARN ; l'activité des ribosomes est inchangée. Sous l'effet de la distribution séquentielle de l'aliment, la dégradation est augmentée, tandis que la synthèse est stimulée à la suite d'une augmentation de la teneur en ARN et de l'activité ribosomale.*

Mots-clés *: Poisson, protéine, mode de nourissage, croissance, turnover.*

INTRODUCTION

Fishmeal is generally recognized as the best source of dietary protein for most carnivorous fish species. Traditionally, high levels of fishmeal protein are used in

fish diets. This is justified by the fact that these proteins are readily transformed by fish into new fish tissue protein of a similar composition. However, the high cost of good quality fishmeal poses real problems for cost-effective feed formulation. The common practice in seabass (*Dicentrarchus labrax*) husbandry is to partially replace fishmeal proteins with less expensive protein sources to obtain least cost without lowering the quality of the feed ; in other terms without adversely affecting growth and production.

Many authors (Métailler et al 1977; Alliot et al 1979; Alliot 1982; Langar and Métailler 1989; Spyridakis 1989) have worked on the possibility of partially substituting fishmeal by other protein sources within seabass diets; the authors showing that growth was directly influenced by the protein source used. The aim of this paper was to investigate the factors affecting growth when seabass fry are fed diets differing only in protein quality and the physiological and metabolic process of nutrition that are influenced by these factors.

BIOLOGICAL VALUE OF DIETARY PROTEIN

A fishmeal-based control diet (diet REF) was compared against different protein sources, including greaves meal (a defatted collagen meal, obtained as a by-product of the tallow and lard industry), squid meal and soya protein concentrate with in isoproteic, isolipidic and isocaloric diets containing 50 % crude protein, 12 % lipids, 15 % carbohydrates and 19 MJ/kg gross energy. Each protein source was incorporated at a level of 30 or 50 % of total crude protein content of the diet (ie. GR30 and GR50 for greaves meal, H30 and H50 for fish protein hydrolysate, SQ30 and SQ50 for squid meal and diets S30 and S50 for soya protein concentrate, respectively). All diets were fed at 3, 3.5 or 4% of body weight per day to 1.3 g seabass fry over 50-day feeding period and induced different growth responses (Langar, 1992 ; Figure 1). All these experiments were carried out in 60l indoor sub-square tanks within sand filtered running sea water (S = 35 ‰, pH = 8) maintained at 18 ± 1 °C.

Increasing feeding rate from 3 to 3.5 % body weight per day significantly improved growth and food conversion ($p < 0.05$) without changing the relative performances of diets (Figure 1). However, increasing the ration from 3.5 to 4 % induced an increase in food conversion without enhancing growth; reflecting overfeeding with 4 % ration (Figure 1).

Since growth is dependent of amino acid availability, differences in growth observed with a given feeding level could supposedly be due to differences between dietary protein digestibility and/or differences between the essential amino acid (EAA) balances of the dietary proteins.

However, the observed apparent protein and energy digestibilities of the above diets (measured according to the method of Spyridakis *et al*, 1988), were almost identical (close to 90 %) and therefore could not explain the differences obtained in growth (Langar, 1992; Figure 2).

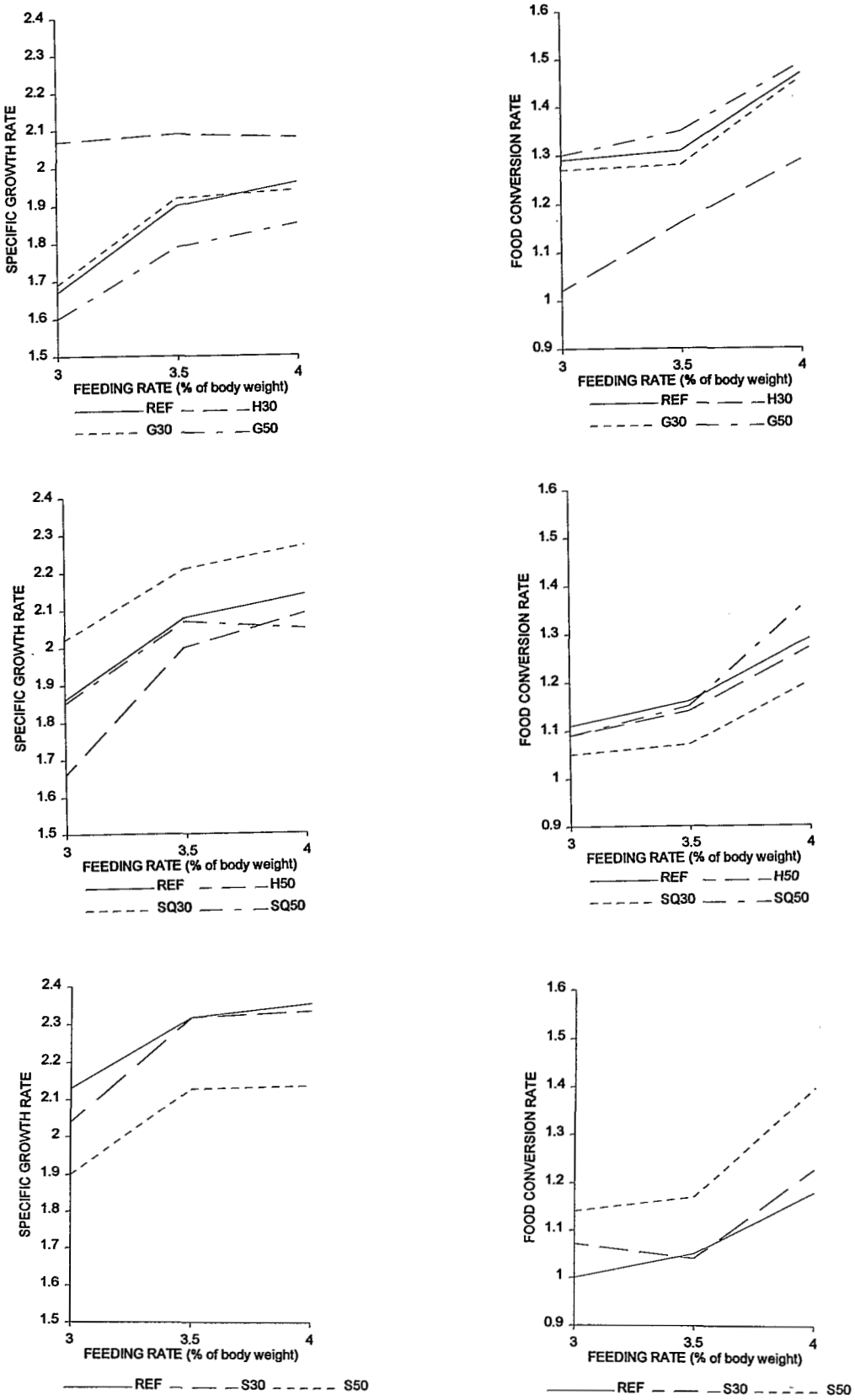

Fig. 1. Effect of feeding rate on specific growth rate and food conversion ratio of seabass fed over 50-day period on different diets. (Data from Langar, 1992).

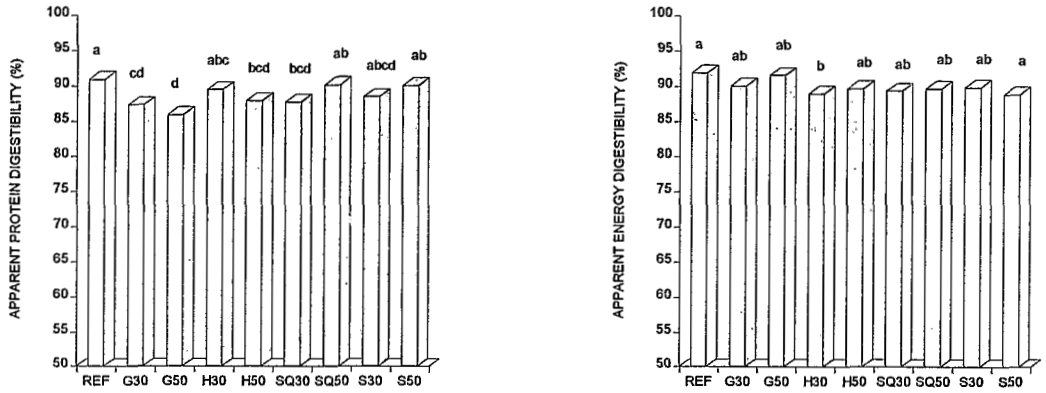

Fig. 2. Apparent protein and energy digestibilities of experimental diets REF, G30, G50, H30, H50, SQ30, SQ50, S30 and S50. Bars of a given parameter with a common letter are not significantly different ($p > 0.05$) (Data from Langar, 1992).

Similarly, the essential amino acid balance of the different dietary protein sources were checked using the EAAI method of Oser (1951) and by the chemical index method of Mitchell and Block (1946) and yielded no more information which could explain the differences in growth observed (Langar, 1992; Table 1).

Table 1. Chemical indexes and essential amino acid index (EAAI) of the dietary proteins evaluated[1] (Data from Langar,1992)

Diet	Limiting amino acid	Chemical index	EAAI[2]
REF	Arg or (Phe+Tyr)	84.77	1.00291
H30	(Phe+Tyr)	80.21	1.00450
H50	(Phe+Tyr)	82.05	0.99992
G30	Val	82.52	1.00523
G50	(Phe+Tyr)	79.31	1.00784
SQ30	Val	82.52	1.00226
SQ50	Val	79.15	1.00428
S30	Val	88.42	1.00500
S50	Val	83.12	1.02979

[1] Essential amino acids checked according to the method of Moore *et al.*, (1958) after Hcl hydrolysis (Hcl 6N, 24h, 110 °C) which destroyed partially Meth and Cyst and completely Trp ; Met, Cys and Trp were, then, not taken into account for the calculation of these indices.

[2] Using egg's protein as reference.

It follows from the above results that, in certain cases, chemical indices and protein digestibility alone are not always a good indicator of protein quality. Growth response tests remain the best method for estimating the nutritional value of dietary protein. Despite this, chemical indexes and digestibility are still generally considered as a good first step to pre-select feed stuffs from a large variety of raw materials available on the market.

Thus, the differences in growth observed in the seabass fingerlings fed the different experimental diets, could have been due to differences in real food intake or to other factors controlling ingestion such as gastrointestinal evacuation; food intake and gastro-intestinal transit probably being affected by the palatability and physical state of dietary protein source, respectively.

FOOD INTAKE AND GASTRO-INTESTINAL TRANSIT TIME

Using a radioisotope technique with 1 g seabass for measuring food intake, Langar and Guillaume (1993) found that fish accustomed to two of the experimental diets (REF and GR50; differing only in their protein biological value) regained their appetite about 90 minutes after the first daily feeding. Ration ingested at the time of the return of appetite was not significantly different ($p > 0.05$) from that ingested at the first daily feeding irrespective of food used. According to this result and to the feeding method employed (i.e. hand feeding, three times per day, with inter-feeding intervals exceeding 3 hours) total daily feed intake was estimated for three batches of 1.3 g seabass (fed diet REF, GR30 and GR50 and reared at a temperature of 18 $\pm$ 1 °C) and found to be 3.5 % of body weight per day irrespective of the protein source used (Langar and Guillaume, 1994b). This confirms the results obtained for the dose-response tests described above, which showed that the optimal ration for growing seabass reared at a temperature of 18° $\pm$ 1 °C, was 3.5 % of body weight irrespective of diet.

Using the same radioisotope method with seabass of 2.4 g body weight and adapted on diets REF, GR30 and GR50, Langar (1992) found that the gastro-intestinal evacuation rate of a meal consumed at a time zero strictly followed a square root model, beginning 6 hours after feeding and finishing at 95 %, 26 hours later (i.e. 32 hours after taking the meal) independently of dietary protein. If it is considered that dietary protein has the same effect on gastric as on intestinal transit, and, since the beginning of gastro-intestinal evacuation and the whole duration of the transit are similar in fish fed the three diets with differing protein quality, then gastric evacuation would be independent of dietary protein source. This conclusion is in agreement with the results of Spyridakis (1989) with 70 g seabass.

Bearing in mind this hypothesis, and the hypothesis of Fletcher (1984) that the time of 'return of appetite' is a direct consequence of gastric evacuation, it follows therefore that 'return of appetite' would also be independent of dietary protein source. This confirms results of Langar and Guillaume (1993).

Although the investigations on food intake and gastro-intestinal transit time did not explain the differences in fish growth obtained, they show that other factors apart from dietary protein could effect growth. In fact, Langar (1992) tried to

compare the food intake of two batches of seabass fry adapted to continuous feeding and meal feeding and found that there was a tendency of growth improvement with meal feeding over continuous feeding.

PROTEIN SYNTHESIS AND DEGRADATION

In addition to the above diets (REF, H30, H50, GR30 and GR50), Langar *et al.* (1993) also used diet F50 in which 50 % of the dietary crude protein was provided from fishmeal and 50 % from hydrolyzed feather meal, so as to check the effect of dietary protein quality on protein synthesis; hydrolyzed feather meal used as a negative control since its dietary inclusion at a level of 30 % of crude protein induced a marked depression in fish growth (Langar and Métailler, 1989).

Apparent protein and energy digestibilities for diet F50 were found to be very similar to those reported for the other diets used (88.3% and 89.4% respectively; Langar *et al.,* 1993).

Using the flooding dose of labelled phenylalanine method (Garlick *et al.*, 1980), for studying protein turnover of young seabass, Langar *et al.* (1993) found that poor dietary protein quality involves high protein synthesis linked to an increase in whole body RNA level, the ribosomal activity remaining constant (Figure 3). Moreover, the higher rate of protein synthesis was always accompanied with a higher rate of protein degradation and a lower efficiency of protein retention (Figure 3).

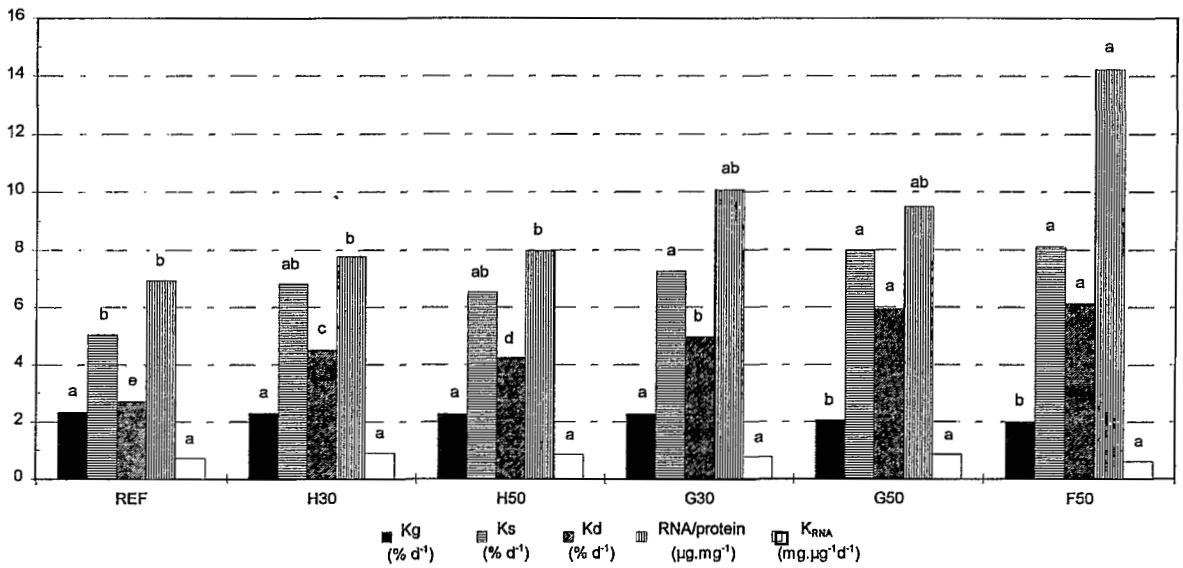

Fig. 3. Fractional protein specific growth rates (Kg), fractional whole body protein synthesis (Ks), fractional whole body protein degradation (Kd), RNA/protein ratios and ribosomal activities (K_{RNA}) of fish fed on diets REF, H30, H50, G30, G50 and F50 for 50 days. Bars of a given parameter with a common letter are not significantly different ($p > 0.05$). (Data from Langar *et al.*, 1993).

The higher rates of protein degradation obtained with fish fed diet H30 and H50 were attributed to a probable faster amino acid absorption from the digestive tract within fish fed diets containing hydrolyzed fish protein compared with diet REF. The same effect of poor dietary protein quality was also found by Langar and Guillaume (1994a) with diet REF, GR30 and GR50 in two batches of seabass fed either continuously or by meal feeding (Figure 4).

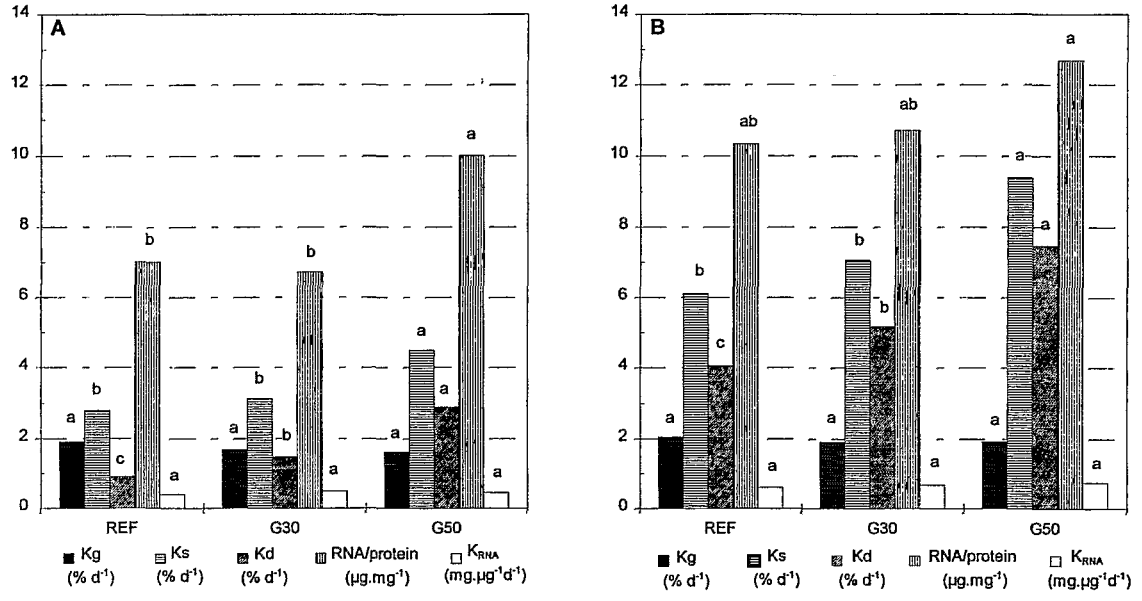

Fig. 4. Fractional protein specific growth rates (Kg), fractional whole body protein synthesis (Ks), fractional whole body protein degradation (Kd), RNA/protein ratios and ribosomal activities (K_{RNA}) of fish fed on diets REF, G30 and G50 for 30 days. A : Continuous feeding ; B: Meal feeding. Bars of a given parameter with a common letter are not significantly different ($p > 0.05$). (Data from Langar et al., 1994a).

The enhancement of fish growth by meal feeding is believed to be due to a higher stimulation of protein synthesis ; the high level of protein synthesis being linked to an increase in whole body RNA level and ribosomal activity (Langar and Guillaume, 1994a). Moreover, since the increase of protein synthesis is also accompanied by a marked increase in the rate of protein degradation (Langar and Guillaume, 1994a), it follows therefore that the efficiency of protein retention decreases and protein growth is not improved in keeping with the higher rate of protein synthesis (Figure 5).

CONCLUSION

In contrast to higher vertebrates in which the biological value of dietary proteins can be readily determined by digestibility and chemical methods, the results of the present study with fish have shown that the growth response test remains as the only accurate method for characterizing the dietary protein quality.

The low growth response observed in fish fed a diet containing a "poor" (ie. low quality) protein source was found to be linked to a low efficiency of protein retention rather than to a lack of protein synthesis.

Clearly considerable further research on protein degradation is required so as to elucidate its nature and role in protein metabolism and fish growth.

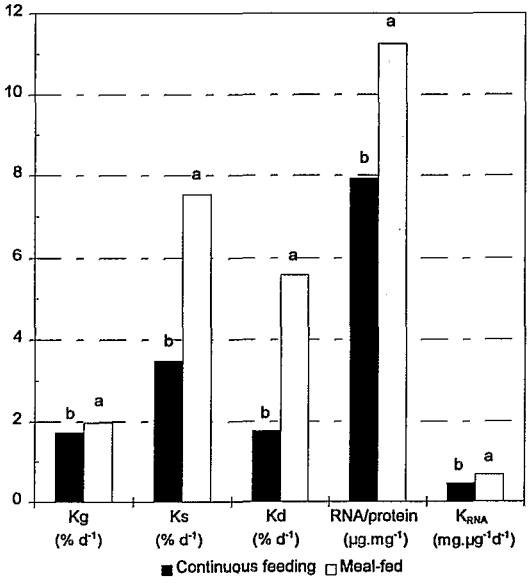

Fig. 5. Effects of feeding pattern on growth and on parameters of protein metabolism of fish fed on diets REF, G30 and G50 for 30 days. Bars represents the values obtained by the two-way analysis of variance used to compare the effect of diets and of the feeding sequence. Bars of a given parameter with a common letter are not significantly different ($p > 0.05$). (Data from Langar et al., 1994).

ACKNOWLEDGMENTS

This work was part of a doctorate thesis of Université de Breragne Occidentale of Brest (France), achieved at IFREMER center of Brest (France). The author offers his thanks to Drs J. GUILLAUME (IFREMER/INRA Brest, France), B. FAUCONNEAU (Laboratoire de physiologie des poissons, INRA, Rennes, France) and S. J. KAUSHIK (INRA, St-Pée-sur-Nivelle, France) for their supervision and advises.

REFERENCES

Alliot, E., Pastoureaud, A., Pelaez Hudlet, J. et Métailler, R. (1979). Utilisation des farines végétales et des levures cultivées sur alcanes pour l'alimentation du bar. In *Finfish Nutrition and Fishfeed Technology*, Berlin, 1979, Halver, J.E. and Tiews, K. (eds), Vol. II, pp. 229-238.

Alliot, E. (1982). *Aspects nutritionnels chez le loup, Dicentrarchus labrax, en milieu controlé. Influence des régimes alimentaires et des facteurs de l'environnement sur le bilan azoté*. Thèse Doct. Etat, Univ. Aix Marseille II. Fac. Sci. de Luminy.

Fletcher, D.J. (1984). The physiological control of appetite in Fish. *Comp. Biochem. Physiol.*, 78A: 617-628.

Garlick, P.J., McNurlan, M.A. and Preedy, V.R. (1980). A rapid and convenient technique for measuring the rate of protein phenylalanina. *Biochem. J.*, 192: 719-723.

Langar, H. (1992). *Effets physiologiques et métaboliques de la qualité nutritionnelle des protéines chez le jeune alevin de bar (Dicentrarchus labrax)*. Thèse Doct., Université Bretagne Occidentale, Brest.

Langar, H. et Guillaume, J. (1993). Mise au point d'une technique de mesure de l'ingéré chez le jeune alevin de bar (*Dicentrarchus labrax*) à l'aide d'iodure d'argent marqué. In *Fish Nutrition In Practice. Proceedings of the IVth International Symposium on Fish Nutrition and Feeding*. Biarritz, France, 24-27 June 1991, Kaushik, S.J. and Luquet, P. (eds). INRA, Paris, pp. 629-632.

Langar, H. and Guillaume, J. (1994a). Effect of feeding pattern and dietary protein source on protein synthesis in European sea bass (*Dicentrarchus labrax*). *Comp. Biochem. Physiol.*, 108A: 461-466.

Langar, H. and Guillaume, J. (1994b). Estimation of the daily ration of fingerlings sea bass, *Dicentrarchus labrax*, using a radioisotope method.- *Aquaculture*, 123: 121-126.

Langar, H., Guillaume, J., Métailler, R. and Fauconneau, B. (1993). Augmentation of protein synthesis and degradation by poor dietary amino acid balance in European sea bass (*Dicentrarchus labrax*). *J. Nutr.*, 123: 1754-1761.

Langar, H. and Métailler, R. (1989). Use of some protéin from animal origin for european sea bass (*Dicentrarchus labrax* L.), feeding. *E.A.S., Special Publication*, 10: 139-140.

Métailler, R., Mery, C., Depois, M.N. et Nedelec, J. (1977). Influence de divers aliments composés sur la croissance et la survie d'alevins de bar (*Dicentrarchus labrax*). *3rd meet. ICES working group Mar.*, Brest, France, 10-13 May. Actes de Colloques CNEXO, 4 : 93-109.

Moore, S., Spackman, D.H. and Stein, H.W. (1958). Chromatography of amino acids on sulfonated polystyrene resins.- *Analyt. Chem.*, 30: 1185-1190.

Mitchell, H.H. and Block, R.J. (1946). Dome relatioships between the amino acid contents of proteins and their nutritive values for the rat. *J. Biol. Chem.*, 163: 599-620.

Oser, B.L. (1951). *J. Am. Dietet. Assoc.*, 27: 296. Quoted by Oser, B.L. (1959). An integrated essential amino acid index for predicting the biological value of

proteins. In Protein and amino acid nutrition. Albanese, A.A. (ed). New York, NY, Academic Press, pp. 281-295.

Spyridakis, P. (1989). Utilisation des protéines alimentaires chez le bar (*Dicentrarchus labrax*) : aspects digestifs et métaboliques. *Thèse Doct. Univ. Bretagne Occidentale, Brest.*

Spyridakis, P., Gabaudan, J., Métailler, R. et Guillaume, J. (1988). Digestibilité des protéines et disponibilité des acides aminés de quelques matières premières chez le bar (*Dicentrarchus labrax*). *Reprod. Nutr. Dévelop.*, 28 (6A): 1509-1517.