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Advances in the Study on Nutrient Requirements of Grouper (*Epinephelus* sp.): a Review

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Abstract The paper reviews the recent advances in studying grouper nutrition requirement for the development of cost-effective and environmentally friendly artificial diets. It consists of seven parts: protein and amino acid, lipid and essential fatty acid, carbohydrate, vitamin, mineral, alternative protein source, broodstock and larval nutrition. The review provides some basic information for further investigation of nutrient requirements of groupers.

Key Words *Epinephelus*; grouper; nutrient requirement; fish nutrition

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1 Introduction

Groupers are coastal warmwater carnivores and widely distributed in the world. Because of their fast growth, high feed efficiency, hardiness in a crowded environment and very high market value, groupers are considered to be the most popular candidate species for intensive aquaculture (Chen and Tsai, 1994; Boonyaratpalin, 1997). They are also amongst the most important finfish species cultured in saltwater ponds or sea cages in southern China, mainly consisting of *E. coioides*, *E. malabaricus*, *E. awoara* and *E. akaara*. Recently, there has been an increasing interest in grouper nutrition and formulated diet development (Lin and Zhang, 1996; Lin *et al.*, 1995; Jia *et al.*, 1994; Wang *et al.*, 2003; Yu *et al.*, 2004) although compounded feed developed specifically for the fishes are still in its infancy. Different aspects of grouper nutrition are frequently studied by different research groups. The aim of the paper is to review the recent advances in the study of grouper nutrition requirements including some results recently obtained in our laboratory.

2 Grouper Nutrition Requirements

2.1 Protein and Amino Acid

Groupers are carnivorous fishes in natural habitats, so their dietary protein requirements, like other carnivorous marine species, are normally high. About 50% protein content in the diet was needed to promote maximum growth of groupers regardless of the protein sources used in these studies (Table 1). However, a protein level of 40%–45% is suggested to be the most economical in practical feed formulation. The optimal dietary protein level is usually higher at the larval and fry stages and lower at the grow-out stage. Thus, the requirement estimates shown in Table 1 will also be useful for formulating grow-out and larval diets for groupers. Dietary energy content has been reported to modulate the protein requirements of groupers. Shiau and Lan (1996) reported that protein could be lowered from 50% to 44% while the energy level of 1423.512 kJ per 100 g diet was maintained, suggesting that protein could be spared by lipid as long as energy requirements were met. In other studies, decreasing the dietary protein level from 460 g kg^{-1} to 420 g kg^{-1} by increasing the starch content in the diet from 195 g kg^{-1} to 246 g kg^{-1} did not reduce the weight gain and food efficiency, suggesting that starch could spare some protein when the dietary protein level was low (Shiau and Lin, 2001).

Fishes do not have absolute protein requirements, but require the amino acids that synthesize proteins. Groupers can be assumed to require the same 10 essential amino acids (EAAs) as other species. Chen (1995)[①] determined the methionine requirement of *E.*

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① Chen, C.H., 1995. Total sulfur amino acid requirement and its effect on nitrogen metabolism for grassy grouper (*Epinephelus malabaricus*). National Taiwan Ocean University, Keelung, Taiwan. 135 pp.

Table 1 Optimal protein requirements of several grouper species

Fish species	Size/g	Protein sources	Requirement/%	References
E. malabaricus	65–170	Tuna muscle and fish meal	40–50	Teng *et al.*, 1978
	3.79	Casein	47.8	Chen and Tsai, 1994
	9.22	Fish meal	50.2	Shiau and Lan, 1996
E. akaara	126.4	Fish meal and casein	48.37–49.24	Chen *et al.*, 1995
E. coioides	10.7	Fish meal and casein	48.1	Luo *et al.*, 2004a

malabaricus by adding graded levels of crystalline methionine to a basal diet in which protein was supplied by a mixture of casein, gelatin, and amino acids. He found that the optimal methionine requirement of this grouper (initial weight: 5.4 g) was 9.7 $g\,kg^{-1}$ diet (or 25.6 $g\,kg^{-1}$ protein) with 0.7 g cystine kg^{-1} diet. Chen (1995)① also demonstrated that supplementary cystine could spare as much as 50% of methionine requirement for grouper. Other EAA requirements of groupers remain to be determined. In the absence of amino acid requirement data, the amino acid profile of the whole body of the animal could be used as a reference of the EAA requirements (Wilson and Poe, 1985). According to the assumption, the amino acid profiles of several grouper species shown in several documents (Chen *et al.*, 1994; Zhang and Chen, 1996; Liu *et al.*, 2001; Millamena, 2002) can be used as a guideline for practical diet formulation. On the other hand, as amino acid composition does not change significantly among fish species, inter-species differences in amino acid requirements are not expected, particularly if requirements are estimated by the ideal protein method (Akiyama *et al.*, 1997). Therefore, the EAA requirements of groupers can also be assessed by available data on the amino acid requirements of other species until trials are conducted to determine their true requirements.

2.2 Lipid and Essential Fatty Acid

Lipid serves both as an energy source and the source of essential fatty acid, but a higher lipid level will reduce the growth and shelf-life of the final product, so an adequate lipid level in the diet is very important. Lin and Shiau (2002) conducted a trial to determine the optimal dietary lipid level for *E. malabaricus* (initial weight: 4.43 ± 0.07 g) using fish oil and corn oil (1:1) as lipid sources. The results showed that 9% of dietary lipid was needed to obtain the best growth rate and feed efficiency but 4% of dietary lipid appeared to meet the minimal requirement. In our recent study (Luo *et al.*, 2004)②, 10% of dietary lipid was considered to be optimal for maximum growth for *E. coioides* (initial weight: 10.9 ± 0.1 g) cultured in floating netcages with fish oil as a unique lipid source. Based on the results of these two experiments, it seemed that inter-species differences in the lipid requirements of groupers might be minimal.

To our knowledge, the essential fatty acid requirements of groupers are not known yet. However, Wu and Chen (2001) investigated the combined effect of dietary docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on the growth performance and immune responses of juvenile *E. malabaricus*. The juvenile groupers were fed test diets containing a dose of 10 $g\,kg^{-1}$ diet of DHA and EPA in combinations of 3:1, 2:1, 1:1, 0.7:1 and 0.3:1, respectively. The results indicated that higher DHA: EPA ratios resulted in better growth and that fish fed the diet with the least amount of DHA (0.3:1) showed a weight gain significantly lower than any other dietary group. Dietary DHA also enhanced the immune response of grouper. In another study, Wu *et al.* (2002) pointed out that DHA was superior to EPA as an essential fatty acid for the growth performance of groupers.

2.3 Carbohydrate

Carbohydrate is the least expensive form of dietary energy, but fishes do not use carbohydrate as efficiently as mammals and birds, as fishes have a limited capability to digest and metabolize carbohydrates (Wilson, 1994). Besides, carbohydrate digestibility is affected by the complexity of the molecule, concentration in the diet, amount ingested and technological treatments applied to carbohydrate (Kaushik and Medale, 1994). Humpback grouper has a very limited capacity to digest carbohydrate-rich products (Laining *et al.*, 2003). In the diet for *E. malabaricus*, Shiau and Lin (2002) found that starch was better utilized than glucose at water temperature 23℃ based on weight gain, feed efficiency and protein efficiency ratio. Grouper fed glucose diet had higher hepatic glucose-6-phosphatase activity than fish fed starch diet (Shiau and Lin, 2002). Increasing dietary glucose levels and decreasing dietary protein contents resulted in reduced weight gain (Shiau and Lin, 2001). However, water temperature could affect carbohydrate utilization. *E. malabaricus* utilized glucose as well as starch when the water temperature increased from 23℃ to 29℃ (Shiau and Lin, 2001). Inclusion lev-

① Chen, C. H., 1995. Total sulfur amino acid requirement and its effect on nitrogen metabolism for grassy grouper (*Epinephelus malabaricus*). National Taiwan Ocean University, Keelung, Taiwan. 135 pp.

② Luo, Z., Y. Liu, K. Mai, L. Tian, D. H. Liu, *et al.*, 2004. Effect of dietary lipid level on growth performance and body composition of grouper *Epinephelus coioides* juveniles fed isonitrogenous diets in floating netcages. *Aquaculture International*, in press.

els of carbohydrate in the diet also influence carbohydrate and other macronutrient utilization. However, there is little information available for determining the optimal concentration of carbohydrate in the diet for groupers and more work is needed for providing insight into its utilization by the fish species.

2.4 Vitamins

It is difficult to exactly determine vitamin requirements because measurable amounts of certain vitamins may be present in feedstuffs. They are likely to bind with macromolecules and not wholly bioavailable (Cowey, 1992). In addition, vitamins, especially some fat-soluble ones, can be stored in the tissue, which could clearly alter the time taken for a deficient diet to affect growth performance and deficiency signs. So far, vitamin A and vitamin C have been the two kinds of vitamins with requirements defined for grouper. Vitamin C has been reported to play a special role in immunity of groupers (Qin *et al.*, 2000a, b). Boonyaratpalin (1997) suggested the the minimum level of L-ascorbyl-2-phosphate-Mg required for normal growth of *E. tauvina* was 30 mg kg^{-1} dry diet. Fish fed diets without supplemental L-ascorbyl-2-phosphate-Mg showed deficiency signs, such as loss of appetite, short snout, erosion of the opercula and fins, hemorrhaging eyes and fins, exophthalmia, swollen abdomen, abnormal skull, falling pharyngobranchials, severe emaciation, scoliosis and lordosis. Dietary vitamin C sources influenced the estimation of vitamin C requirements. Lin and Shiau (2004) reported that the adequate dietary vitamin C concentration for *E. malabaricus* is 17.9 mg ascorbic acid (AA) equivalent of 2MP-Mg kg^{-1} and 8.3 mg AA equivalent of C2MP-Na kg^{-1}, respectively. C2MP-Mg was about 46% as effective as C2MP-Na in meeting the vitamin C requirement of grouper (Lin and Shiau, 2004). On the other hand, the vitamin A requirement of juvenile greasy grouper (5.84 ± 1.5 g) has been determined to be a minimum of 310 IU vitamin A kg^{-1} diet for maximum growth based on broken-line regression analysis of weight gain (Mohamed *et al.*, 2003). Fish fed the basal diet (210 IU vitamin A kg^{-1} diet) developed haemorrhages in the skin overlaying the base of the fins and erosion on the caudal peduricle (Mohamed *et al.*, 2003).

2.5 Mineral

Minerals are responsible for skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium and for the functioning of biologically important compounds such as hormones and enzymes. Mineral deficiencies can cause biochemical, structural and functional disorders (Watanabe *et al.*, 1997). However, it is very difficult to effectively determine the mineral requirements of fishes because many minerals are required in small quantities and can be obtaind partly from water by exchange across the gill membrane or ingestion and by absorption across the gut. Ca, Mg, Na, K, Fe, Zn, Cu and Se can all be obtained from the water; PO_4 probably together with Cl and SO_4 is best supplied from food (Cowey, 1992). So one should keep cautious when comparing the mineral requirements of fish obtained in different laboratories or under different experimental conditions. At present, requirement studies normally involve experiments dealing with animal responses or performances relating to feeding graded levels of an essential mineral over a wide range, from zero to levels far beyond optimal (Watanabe *et al.*, 1997). For groupers available data are limited to dietary zinc and phosphorus requirements. The requirement of zinc for *E. malabaricus* was demonstrated ranging from 28 to 36 mg kg^{-1} diet based on broken-line analysis of weight gain, food conversion, and body tissue zinc concentration. Increment of dietary zinc level significantly enhances zinc abundance in fish serum, muscle, vertebrate and scales (Hu *et al.*, 2001). On the other hand, Zhou *et al.* (2004)① determined a dietary level of 0.86% total phosphorus based on relative weight gain, specific growth rate and bone phosphorus. In their study dietary total phosphorus levels (consisting of the contents of ingredients and supplemental monocalcium phosphate) were 0.68%, 0.77%, 0.86%, 1.02% and 1.21%, respectively. No published information is available on the evaluation of other mineral requirements of groupers.

2.6 Alternative Protein Sources

In fish culture, there is an increasing emphasis on developing cost-effective fish meal substitutes using less expensive terrestrial animal-and plant-based protein sources. Based on information available, it seemes that digestibilities of feedstuffs of animal origin for groupers are higher than that of plant origin (Eusebio *et al.*, 2002, 2004; Laining *et al.*, 2003; Lin *et al.*, 2004). Accordingly, animal protein sources can often attain higher inclusion levels in grouper diets than plant protein sources without adverse influence on growth and feed efficiency. Table 2 lists the substitution levels of several alternative protein sources of fish meal in the diet for grouper. Generally speaking, different protein sources used in combination can contribute to the compensation of amino acid imbalance in a single feedstuff and can replace accordingly more fish meal than a single protein source. Tsai (2003)②sub-

① Zhou, Q., Y. Liu, K. Mai, and L. Tian, 2004. Effect of dietary phosphorus levels on growth, body composition, muscle and bone mineral concentrations for orange-spotted grouper *Epinephelus coioides* reared in floating cages. *Journal of the World Aquaculture Society*, in press.

② Tsai, H. C., 2000. Studies on improvement of grouper feed-study of partial substitution of fish meal with different protein sources. National Taiwan Ocean University, Keelung, Taiwan, 120 pp.

Table 2 Optimal replacement levels of fish meal by alternative protein sources in grouper diet

Fish species	Alternative protein sources	Replacement level/%	References
E. coioides	Meat solubles	40	Millamena and Golez, 2001
	Meat meal and blood meal (4:1)	80	Millamena, 2002
	Lupin meal	50	Millamena and Toledo, 2002
	Fermented soybean meal	10	Luo *et al.*, 2004 b

stituted white fish meal in the diet for *E. coioides* with either poultry meal or soy protein concentrate, or both. He found that only a quarter of white fish meal could be replaced by either poultry meal or soy protein concentrate, but a half of fish meal could be replaced when a mixture of equal poultry meal and soy protein concentrate was used for the replacement. On the other hand, the inclusion level of a protein source also depends on the nutritional value. Feedstuff digestibility assessment for fish was an essential prerequisite for determining nutrient requirements, for screening the potential nutritive value of alternative feed ingredients and for the development of nutritionally adequate diets at the least cost (Hajen *et al.*, 1993). White fish meal and brown fish meal showed higher protein digestiblities by *E. coioides*, compared to other ingredients such as soybean meal, peanut meal and yeast. The amino acid availability values of white fish meal and brown fish meal were also higher than those of others. The apparent digestibilities of dry matter, crude protein, lipid, gross energy, amino acids, and fatty acids in yeast were among the lowest (Lin *et al.*, 2004). The protein of both plant and animal feed ingredients was well digested by humpback grouper, while the dry matter and gross energy of the protein-rich animal feed ingredients were more digestible than the carbohydrate-rich plant feed ingredients such as many plant feedstuffs and shrimp head meal (Laining *et al.*, 2003). Because of this poor digestive capacity, the potential of using carbohydrate-rich meals as partial substitutes for fish meal in diets for humpback grouper appeared to be quite low and further researches are needed to ascertain it. Some processing methods have been reported for enhancing the digestibility of feedstuffs, and accordingly allowing higher levels of replacement. For example, preservation of blood by treatment with either propionic acid or formic acid significantly enhanced the apparent digestibility of dry matter and crude protein of blood meal from 48.1 ± 0.85, 55.2 ± 1.35 to 61.7 ± 2.60, 84.2 ± 0.69 (propionic acid-treated) and 67.9 ± 1.63, 87.5 ± 0.55 (formic acid-treated), respectively (Laining *et al.*, 2003).

2.7 Broodstock and Larval Nutrition

Broodstock and larval nutrition requirements of grouper are two neglected aspects and related data are lacking. According to previous studies of other fish species, broodstock nutrition is known to have a profound effect upon gonadal development, spawning, egg quality and fecundity. However, precise information on broodstock nutrition is confined to only several nutrient requirements, such as essential fatty acids, tocopherol and ascorbic acid. Few researches have been conducted to determine the effect of variation of dietary macronutrient and mineral contents on the reproduction of broodstock, which may play an equal important role during the process of reproduction. At present, information available on the nutrient requirements of grouper broodstock is extremely scarce. Zou *et al.* (2003 a) reported the rearing of broodstock grouper, their spawning, fertilization and embryonic development, which might provide some basis for the estimation of nutrient requirements of broodstock. Xiao *et al.* (2003) suggested that fresh trash fish supplemented with 0.2% vitamin E could improve the quality of the eggs and the quality of the larvae, but 0.5% vitamin C could not apparently affect the diameter of eggs, the constitute of fatty acid and the body length of larvae.

At present, most larval nutritional requirements are estimated indirectly by manipulating the live prey composition (Rainuzzo *et al.*, 1997; Sargent *et al.*, 1997). However, the provision of live food (*i.e.* microalgae, rotifer and brine shrimp) is characteristically plagued with variable supply and nutritional quality (Watanabe *et al.*, 1983). This will essentially bring many inconveniences to the estimation of larval nutrient requirements. The development of inert compound diets (such as microdiets) will contribute to a better evaluation of their nutritional requirements, which has been proved to be a useful tool for studying the nutritional requirements of marine fish larvae (Rosenlund *et al.*, 1997; Yufera *et al.*, 1999). However, for grouper, there is a shortage of studies dealing with the nutrient requirements of larval grouper, which limits the massive production of grouper fries and grownout. Several researches have been conducted to determine the development of the digestive system in larval grouper (Wu and Lin, 2003; Zou *et al.*, 2003 c), which would improve our understanding of larval digestion physiology and promote the development of artificial food for larvae. The larval digestive system of *E. coioides* was morphologically ready to process external food at the time of mouth opening (4 d after hatching) (Wu and Lin, 2003). Zou *et al.* (2003 b) reported the feeding and growth of the larvae from *E. malabaricus* in the process of nourishing transformation. Wang *et al.* (2003) achieved limited success in using microdiets for the nursery of grouper larvae (from hatching to post-hatched day 25) when compared to using biodiets (fortified or not fortified). Zheng (2004) reported that the content ratios of DHA to EPA were a factor more important in influencing the growth speed of larval grouper. The growth rates of larval grouper fed the diet with a DHA:EPA ratio of 6.3 were higher than those of groups with a DHA:EPA of 1.73–2.75.

3 Conclusion

The current review covers a wide spectrum of grouper nutrient requirements and all the aspects of nutrient needs are studied. Apparently, more work is needed to determine other unknown nutritional requirements. Grouper broodstock and larval nutrient requirements should be further studied as they have been determined for other fish species.

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