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*Review*

# Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices

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**ABSTRACT.** Supplementing high levels of dietary crude protein in the absence of amino acid balance and enough energy in fish diets may results in reduced growth, improper feed intake and poor protein utilization efficiency coupled with unviable feed costs and adverse environmental effects due to high nitrogen excretion. It is necessary to improve the dietary protein quality to avoid unnecessary nitrogen excretion, and the use of protein as an energy source by fish, quality and determine proper dietary protein to energy (DP:DE) ratios in order to maximize protein utilization efficiency. It will help the aquaculture sector to obtain better growth response at a low-cost and without any adverse environmental effects. In the present article, we have deliberated in detail the previous and ongoing researches about protein research in fish nutrition. Besides, we have made a comparison between two so far commonly used methods in protein research, *i.e.*, the graded supplementation and diet-dilution technique. According to the data presented here, is focusing on the formulation of balanced diets, the diet-dilution technique seems to be more accurate than the graded supplementation method. Thus, future studies should be focused on the use of diet-dilution technique along with proper dietary protein to energy ratios in order to formulate well-balanced diets. These attempts will significantly improve the protein and energy research in aquaculture nutrition. Moreover, feed industries will become able to formulate biologically balanced and environment-friendly diets at a low cost.

**Keywords:** feed formulation; effective protein to energy ratios; better biological response; low-cost balanced diets; aquaculture nutrition

Dietary protein is an essential but most expensive nutrient in fish diets that directly affect fish growth, feed intake and feed costs (Halver & Hardy, 2002; Lee *et al.*, 2002). Its insufficient amounts in diets cause reduced growth and unregulated feed intake, while its excessive amounts make the diet unbalanced and may result in extra feed costs, nitrogen excretion and aquatic pollution (Alam *et al.*, 2008). Therefore, it is necessary to improve the protein quality of diets in order to achieve balanced diets and control the increasing feed costs and aquatic pollution.

Protein requirement is closely related to energy amounts in diets due to the protein-sparing effect of dietary energy (Ai *et al.*, 2004). The supplementation of high protein with insufficient energy is responsible for decreased protein utilization efficiency (Robinson & Li, 1997; Ai *et al.*, 2004). Since, protein should be used only for tissue synthesis rather than as a source of energy (Walton & Cowey, 1982; Li *et al.*, 2012). The supplementation of the proper dietary protein of good quality coupled with proper energy is a critical factor for obtaining optimum growth, feed intake and maintenance of fish (Lemos *et al.*, 2014).

In protein requirement research, the main problem is the use of unbalanced diets (Kesamura *et al.*, 1982; Silva & Perera, 1985; Teshima *et al.*, 1985; Wang *et al.*,

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1985a,b; Siddiqui *et al*., 1988). High protein diets need adequate energy to be metabolized properly, and it has been recommended as an important approach to utilize better the available protein by enhancing the protein utilization and reduces the extra supplementation of nitrogen and consequently the aquatic pollution (McGoogan & Gatlin, 1999). Diets with amino acid balance are usually known as well-balanced and such diets should be provided with proper energy in order to avoid the use of amino acids as a source of energy. For this purpose, dietary lipids are usually considered good sources of energy and essential fatty acids which are necessary for proper growth and development of fish. The use of lipids as compared to carbohydrate as a source of non-protein energy is usually considered more efficient because it is an energy-rich nutrient and is happily metabolized by fish (NRC, 2011). Proportionally more of the dietary amino acids oxidize and less partition into synthesis when diet with a higher protein to energy ratio offer to cod which explains the lower anabolic stimulation of cod. Thus, it is notable that omnivorous and herbivorous fish can utilize a broader range of diets than carnivores (Carter *et al*., 1994).

## Dietary protein quality

Most of the previous literature (Table 1) is based on graded supplementation methodology using crude values for the formulation of diets and thus, showing higher dietary protein and energy requirements for several fish species. The higher protein and energy requirements may be due to the use of crude protein (lack of amino acids balance) or poor quality protein ingredients (Gous *et al*., 2018). Diets with poor quality protein sources and without amino acid balance and insufficient energy are usually considered unbalanced (Wethli *et al*., 1975). Sometimes while using high protein contents, the energy may become a limiting factor which results in the determination of improper protein requirements (Gous *et al*., 2018).

In the aquaculture industry, the formulation of economically and biologically viable and environment-friendly diets is getting popularity. In this regard, researchers and feed industries are working together on different feed approaches and techniques in order to minimize the use of high protein levels by improving the dietary protein quality and energy levels. So far, two types of techniques have been used in animal research (Fisher & Morris, 1970; Gous & Morris, 1985) and fish nutrition (Abboudi *et al*., 2007; Liebert & Benkendorff, 2007). One is the graded supplementation technique which describes the graded supplementation of synthetic amino acid/protein to a basal diet deficient in amino acid/protein. The disadvantage of this method is that a systematic change occurs in amino acid balance in

**Table 1.** Previous literature-based protein and energy requirements for different fish species.

| Fish species | Dietary protein and energy requirements/ratios | References |
|---|---|---|
| Grouper *Epinephelus malabaricus* juveniles | 44% dietary protein, 340 to 375 kcal $kg^{-1}$ dietary energy | Shiau & Lan (1996) |
| Goldfish *Carassius auratus* | 38-40% dietary protein, 3,200 kcal $kg^{-1}$ dietary energy, 119 to 125 mg $kcalP^{-1}$ E $ratio^{-1}$ | Souto *et al.* (2013) |
| Siamese fighting *Betta splendens* | 28% digestible protein, 3,200 kcal $kg^{-1}$ digestible energy | Lemos *et al.* (2014) |
| Arctic char *Salvelinus alpinus* | Increasing energy levels showed a positive response at increasing dietary protein levels | Amoah (2012) |
| Parrotfish *Oplegnathus fasciatus* juveniles | 45.2% dietary protein, 4.42 kcal $kg^{-1}$ dietary energy | Kim *et al.* (2016a) |
| Barred knifejaw *Oplegnathus fasciatus* juveniles | 45% dietary protein, 3.49 kcal $kg^{-1}$ dietary energy, 31.1 mg $kcalP^{-1}$ E $ratio^{-1}$ | Kim *et al.* (2016b) |
| African catfish *Clarias gariepinus* fingerlings | 43% dietary protein, 5.07 kcal $kg^{-1}$ dietary energy, 20.5 mg $kcalP^{-1}$ E $ratio^{-1}$ | Ali & Jauncey (2005) |
| Nile tilapia *Oreochromis niloticus* | Tolerate a wide range of dietary energy to protein levels without any side effects on fish | Li *et al.* (2013) |
| Spotted babylon *Babylonia areolata* | 35% dietary protein, 4.0 kcal $kg^{-1}$ dietary energy, 85.99 mg $kcalP^{-1}$ E $ratio^{-1}$ | Chaitanawisuti *et al.* (2010) |
| Spotted babylon *Babylonia areolata* juveniles | 45% dietary protein, 8% dietary lipid (energy) | Chi *et al.* (2010) |
| Pacu *Piaractus mesopotamicus* juveniles | 22% dietary crude protein, 4,200 kcal $kg^{-1}$ dietary crude energy | Fernandes *et al.* (2001) |
| Pacu *Piaractus mesopotamicus* fingerlings | 26% dietary crude protein, 4,200 kcal $kg^{-1}$ dietary crude energy | Fernandes *et al.* (2000) |
| Tilapia *Oreochromis aurea* | 56% dietary protein, 4,600 kcal $kg^{-1}$ dietary energy, 123 mg $kcalP^{-1}$ E $ratio^{-1}$ | Winfree & Stickney (1981) |
| Cachama *Piaractus brachypomus* juveniles | 31.6% dietary crude protein, 3,080 kcal $kg^{-1}$ dietary crude energy | Vásquez-Torres *et al.* (2011) |
| Pacu *Piaractus mesopotamicus* | 27% dietary crude protein, 2,220 Autor revisar este valor mg $kcalP^{-1}$ E $ratio^{-1}$ | Bicudo *et al.* (2010) |
| Florida pompano *Trachinotus carolinus* | 32.5-36.6% dietary protein, 3.68 kcal $kg^{-1}$ dietary energy, 23.8 to 25.1 mg $kcalP^{-1}$ E $ratio^{-1}$ | Riche (2009) |
| Angelfish *Pterophyllum scalare* | 26% dietary protein, 3,100 kcal $kg^{-1}$ dietary energy | Zuanon *et al.* (2009) |
| Giant trahira *Hoplias lacerdae* fingerlings | 47% dietary crude protein showed better response independently of dietary energy levels | Veras *et al.* (2010) |
| Barramundi *Lates calcarifer* juveniles | 50% dietary protein, 5.01 kcal $kg^{-1}$ dietary energy | Nankervis *et al.* (2000) |
| Pacu *Piaractus mesopotamicus* | 25% dietary crude protein, 4% g $kg^{-1}$ lipid (energy), 46% carbohydrates | Abimorad & Carneiro (2007) |
| Meagre *Argyrosomus regius* | 50% dietary protein, 15-18% lipid (energy) | Fernandes (2013) |
| Gilthead Seabream *Sparus aerates* | 13.33 kcal $BW(kg)^{-0.83}$ $d^{-1}$ energy for maintenance, 0.086% BW $(kg)^{-0.70}$ $d^{-1}$ protein requirement | Lupatsch *et al.* (1998) |
| Blackfin Seabream *Acanthopagrus berda* juveniles | 40-42% dietary protein, 12.7 mg $kcalP^{-1}$ E $ratio^{-1}$ | Rahim *et al.* (2016) |

different dietary treatments which may affect the growth response and feed intake (Fisher & Morris, 1970; Gous & Morris, 1985). The other problem is faced during the determination of amino acid requirements for maintenance where amino acids to be tested usually used at low amounts than their optimum requirement for growth. Diets with too much synthetic amino acid (AA) are unable to maintain fish growth as they restrict the feed intake (Cowey, 1992).

The second is diet dilution technique which, for the first time, was established for the evaluation of growth responses to methionine intake in laying hens (Fisher & Morris, 1970). The same method with some modifications was then used by Pilbrow & Morris (1974) to determine laying hen response to dietary lysine intakes. Freeman (1979) used it for the determination of tryptophan requirement in chicks. Similarly, Gous & Morris (1985) used the same technique for evaluating the response of broiler chickens to increasing levels of lysine.

Later on, it was also tested in fish species like in Atlantic salmon (*Salmo salar* L.) to determine its threonine requirement for maintenance by Abboudi *et al.* (2007) and in *Oreochromis niloticus* to determine lysine requirements by Liebert and Benkendorff (2007). This technique is based on the formulation of two basal diets, a high protein diet and the protein free or low protein diet (Fisher & Morris, 1970). The high protein diet usually contains all essential amino acids in excess than its requirement, and the low protein diet provides an amount of EAAs less than their requirement. It is based on the hypothesis that AA utilization is independent of the dietary protein level and it avoids the problem of the change of amino acid balance in successive dietary treatments. Moreover, the high-protein and low-protein diets supply the dietary amino acid ratios on the basis of protein-bound AA. Therefore, it also avoids the problem regarding the restricted feed intake and low utilization of diets provided with too much synthetic AAs, which has been reported as an important limitation of the graded supplementation method (Hauler and Carter, 2001). Thus diets formulated by the dilution technique significantly improve the feed intake and maintain proper growth (Abboudi *et al.*, 2007). This method needs to be further tested in fish nutrition, in order to decide which method is accurate. However, on the basis of the above-mentioned facts and results, diet dilution technique appears more valid than the graded supplementation method as it makes possible the development of well-balanced diets and also provides an excellent design to study the amino acid requirements for maintenance (Fisher & Morris, 1970; Gous & Morris, 1985; Halver & Hardy, 2002; Abboudi *et al.*, 2007; Liebert & Benkendorff, 2007).

**Dietary protein to energy (DP:DE) ratios**

Optimum protein requirements cannot be determined until sufficient energy is provided in the diet. On the other hand, the animal will use part of the dietary protein as an energy source which may lead to the assessment of inaccurate protein requirements. Thus, the graded supplementation approach is somewhat controversial in fish nutrition since the proper ratio between dietary protein and energy is essential to be maintained during the formulation of diets (Li *et al.*, 2013). Diets supplemented with high protein, but low energy levels, restrict the feed intake and result in reduced performance of animals including fish (Morris *et al.*, 1987; Gous *et al.*, 1990; Li *et al.*, 2013). Such a relationship between protein and energy has been documented in several animals such as pigs and broilers (Campbell *et al.*, 1985; Morris & Njuru, 1990).

According to Kyriazakis & Emmans (1992), the protein utilization efficiency is affected negatively by the abnormal protein to energy ratios. The importance of proper dietary protein to energy ratio in the formulation of fish diets has been reported in several previous pieces of research (Webster *et al.*, 1995; Hernández *et al.*, 2001; Ai *et al.*, 2004; Li *et al.*, 2013). These ratios may vary due to several factors such as fish species, size, growth phase, dietary protein source, amino acid profile, environmental conditions, experimental design and feeding habit (Ai *et al.*, 2004; Wang *et al.*, 2013).

The optimum dietary protein requirement and P:E ratios have been determined for several fish species (Tacon & Cowey, 1985; Teshima *et al.*, 1985; Hepher, 1988; Moon & Gatlin, 1991; Okorie *et al.*, 2007; NRC, 2011). It has been documented that the use of proper dietary protein: energy ratios (mg protein $kcal^{-1}$) had improved the growth and feed utilization efficiency of channel catfish, *Ictalurus punctatus* (Garling & Wilson, 1976), Indian major carp, *Labeo rohita* (Das *et al.*, 1991), Nile tilapia, *Oreochromis niloticus* (El-Sayed & Teshima, 1992), red hybrid tilapia, *Oreochromis* sp. (Santiago & Laron, 1991), blue tilapia, *Tilapia aureus* (Winfree & Stickney, 1981), Asian seabass, *Lates calcarifer* (Catacutan & Coloso, 1995), Korean rockfish, *Sebastes schlegeli* (Kim *et al.*, 2004), bagrid catfish, *Pseudobagrus fulvidraco* (Kim & Lee, 2005), Japanese eel, *Anguilla japonica* (Okorie *et al.*, 2007), and Persian sturgeon, *Acipenser persicus* (Mohseni *et al.*, 2013).

If proper DP:DE ratios are not provided in diets, then amino acids which are the building blocks of protein are utilized by fish as an energy source. Consequently, the balance between protein and energy is lost and brings high nitrogen releases into the aquatic environment due to the deamination of surplus amino acids. Low protein to energy ratios may reduce the growth performance due to greater metabolic need of energy to eliminate extra nitrogen and thus result in the deterioration of water quality, which is not suitable for a healthy aquatic environment (Catacutan & Coloso, 1995; Tibbetts *et al*., 2000; Alam *et al*., 2008). While high protein to energy ratios perhaps cause an increased lipid deposition in the body and reduce the feed intake and may lead to the scarcity of some nutrients (Cho, 1992). Therefore, the use of proper protein to energy ratios plays an important role in the formulation of practical diets in the fish industry.

Some fishes efficiently utilize dietary nutrients over a wide range of dietary protein to energy (DP:DE) ratios and energy quantities (Carter *et al*., 1994). They attempt to improve their body energy status as compared to maximizing growth at the expense of lipogenesis patenting from dietary protein without inducing any harmful effects on the proximate composition or performance. The extra energy deposition may be used for seasonal, migratory or maintenance purposes. Moreover, low DP:DE ratios but high energy levels can improve the feed conversion ratio (FCR) of fish. The positive link between dietary energy and FCR has been reported in various aquatic species (Hillestad & Johnsen, 1994; El-Mowafi *et al*., 2010; Ekmann *et al*., 2013) when growth was not restricted via dietary protein level. Similar results were obtained for gilthead sea bream which has shown an efficient utilization of diets with low DP:DE ratios but higher energy levels (Ekmann *et al*., 2013). This information may be important and could be taken into consideration in future protein and energy requirement research.

### Introducing well-balanced diets in the aquaculture sector

For obtaining maximum growth response, it is necessary to increase the feed intake which can be improved only by the use of balanced diets (Lovell, 1989; Cho *et al*., 2005). Thus, proper attention is needed about the dietary protein quality and the dietary DP:DE ratios in order to establish well-balanced diets. On the other hand, improving the protein quality and using proper P:E ratios make it possible to formulate well-balanced diets at low cost which are biologically effective and environment-friendly (Catacutan & Coloso, 1995; Martino *et al*., 2002a,b; Okorie *et al*., 2007; NRC, 2011).

Secondly, a high crude protein with low energy usually reduces the protein utilization efficiency and causes unnecessary feed costs coupled with adverse environmental impacts (Catacutan & Coloso, 1995; Tibbetts *et al*., 2000; Alam *et al*., 2008). Thus, to overcome these problems, it is necessary to focus on improving the utilization of protein in order to avoid the supplementation of high levels of this nutrient in fish diets (Moreira *et al*., 2008).

## CONCLUSIONS

The use of high crude protein contents with insufficient energy makes the diets unbalanced, which may result in the determination of improper dietary protein and energy requirements coupled with increased feed costs and aquatic pollution. Future studies should be focused on improving the dietary protein quality, according to the principles of diet-dilution technique and use of proper protein to energy ratios in order to formulate balanced diets. This information may provide an excellent framework to establish bio-economically viable and environment-friendly diets in the aquaculture sector.

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