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Dietary protein requirement for maintenance, growth, and reproduction in fish: A review

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Abstract
In a wide-ranging "Fish Nutrition" concept, protein is a chief and very relevant nutrient for the Fish. As a functional property of a nutrient, the protein will act as a source of energy for Maintenance, Growth, and Reproduction. This distribution will not remain constant throughout the fish life. As in earlier stage protein is mainly used for growth and maintenance, and after attaining sexual maturity, it is utilized for reproduction contributing to lesser growth. The optimum requirement for any species can be obtained mainly from dose-response curves in response to graded increments of dietary protein in the diet. However, the Protein requirement in fish depends on several factors like species, size and age, water temperature, water salinity, stocking density, and dietary Protein/Energy ratio. This article represents brief information on the protein requirement of fish for various activities.

Keywords: Growth, maintenance, protein requirement, reproduction

Introduction
Protein nutrition is the most extensively studied area in fish nutrition [1]. In the early attempts to use compound diets in fish culture, many efforts have been made to define the optimal protein content in the diet [2]. Dietary protein is considered a crucial but most expensive nutrient in fish diets that directly affect feed intake, fish growth, and feed costs [3, 4]. It is widely believed that fishes require more dietary protein than other vertebrates [5, 6]. This high requirement of protein in fishes is mainly because most fish species feed as predators, and many species are confined to a carnivorous feeding habit by the need to meet the protein requirements, thus concluded that fish have adapted over evolutionary time to depend and utilize the protein-rich diet [7]. Protein is the only major nutrient that will reflect the anabolic phenomenon (growth) of the fish body. One of the main characteristics of fish is their use of amino acids as energy substrates. Thus, in fish amino acids, rather than glucose are used preferentially as an energy source [2]. Dietary protein requirement is basically: Protein requirement for maintenance, relative protein concentration required for maximum growth, the efficiency of protein retention in growth, requirement for maturation, and reproduction. As in earlier stage, protein is mainly used for growth and maintenance, and after attaining sexual maturity, it is utilized for the reproduction, only a lesser contribution towards growth. This distribution will not remain constant throughout the fish life span.

Protein requirements are considered to be the sum of the requirements for individual essential amino acids and the requirement for non-essential nitrogen. Theoretically, fish do not have a dietary requirement for protein, but they do require a dietary supply of certain essential (indispensable) amino acids that contribute to protein structure [8, 9]. Dietary protein is the only source of nitrogen for constructing amino acids and proteins in fish. However, the term protein requirement is used here for consistency with the extant literature. Body protein is always in a dynamic equilibrium, and protein requirements vary considerably with age and species. Insufficient intake of protein will result in retardation of growth due to the withdraw of protein from fewer vital tissues to maintain the function of critical parts [10]. Too much supply of protein, however, will be used to synthesize new tissues, and the remainder will be converted to energy [10] or in other words, excess dietary protein or amino acids, which cannot be stored, are catabolized preferentially over carbohydrates and fats and used for energy by some fishes [8, 11]. Requirement study has been obtained mainly from dose-response curves in which graded amounts of high-quality protein were fed in partially defined diets [11].

The response measured is mainly weight gain, and the values are expressed as a percentage of the dry diet. The methods used to determine protein requirements, however, may overestimate the requirement.

Protein requirement varies with different species, within the same species, different life stages (Table 1), physical and physiological activity, sex, and environmental factors. For achieving optimum growth in fish, all essential amino acids and protein should be at the optimum level, which is variable among different species, as shown in table 2 and table 4. Even marginal imbalances or deficiencies may cause antagonistic effects or other substantial adverse effects. Protein requirements, as a proportion of the diet, decrease as fish approach maturity. For example, 25 percent protein was adequate in the diet of channel catfish of 114 to 500 g, but 35 percent protein produced faster gains than did 25 percent protein in 14- to 100-g fish [12]. Somewhat similar results have been obtained with salmonids, common carp, and tilapia [9, 11]. Besides, adequate consideration has not always been given to factors such as the concentration of dietary energy (DE) in the diet, amino acid composition of the dietary protein, and digestibility of the dietary protein [8, 9, 11].

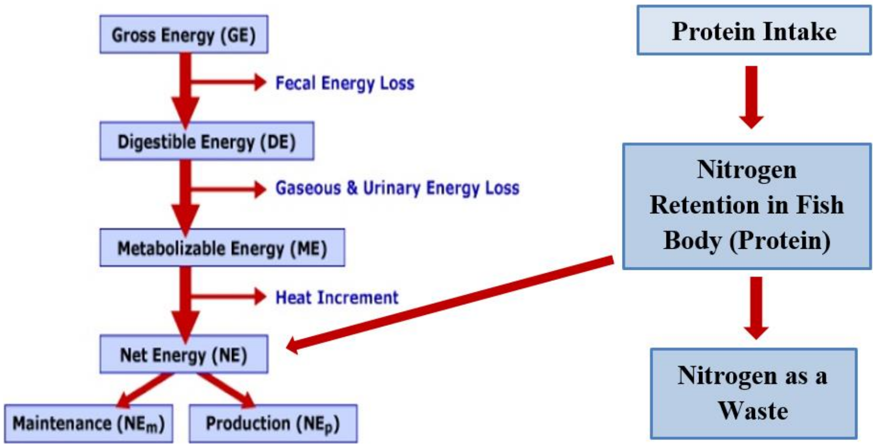

Fig 1: Energy Partition and Protein Utility in Fish Body [13]

The Gross Energy of a fish feed (100%) after its digestion and metabolism, the energy remains as Net Energy that will be utilized for the maintenance and production (Growth and Reproduction). The Retained Protein may contribute to this net energy, and further, it will be utilized for the maintenance and production that can be calculated using different experimental methodologies. Protein utility in terms of growth and reproduction cannot be distinctly mentioned because it depends on the sexual maturity of an animal. When the growth curve reaches the plateau stage, the growth rate will decrease because energy is utilized mainly for reproduction, followed by a very lesser amount for growth. Figures 1 illustrate a considerable allocation of energy where the net energy can be from net protein retention (30-40%), which can be used for maintenance and production [14]. Several studies are conducted on protein requirements for various activities; however, these requirements cannot be mentioned clearly.

Table 1: Protein requirement at a different growth stage in different fishes

Species	Growth stage	Percentage protein requirement
Catla catla	Fry	47
	Fingerling	40
Labeo rohita	Fry	42
	Fingerling	30
Cirrhinus mrigala	Fry	40
	Fingerling	40
Cyprinus carpio	Fry	45
	Fingerlings	35
Ctenopharyngodon Idella	Fry	42
	Fingerling	35
Catfishes	Fry	45
	Fingerling	35

(Renukaradhya KM and Varghese TJ, 1986) [15]

Table 2: The amino acid requirements of several cultivable species

Amino acids	*Catla catla*	*Labeo rohita*	*Cirrhinus mrigala*	*Cyprinus carpio*	Catfishes
Arginine	4.80	5.75	5.25	4.3	4.3
Histidine	2.45	2.25	2.13	2.1	1.5
Isoleucine	2.35	3.00	2.75	2.5	2.6
Leucine	3.70	4.63	4.25	3.3	3.5
Lysine	6.23	5.58	5.88	5.7	5.1
Methionine	3.55	2.88	3.18	3.1	2.3
Phenyl alanine	3.70	4.00	4.00	6.5	5.0
Threonine	4.95	4.28	4.13	3.9	2.0

Tryptophan	0.95	1.13	1.08	0.8	0.5
Valine	3.55	3.75	3.50	3.6	3.0

(Ravi J and Devaraj KV, 1991) [16].

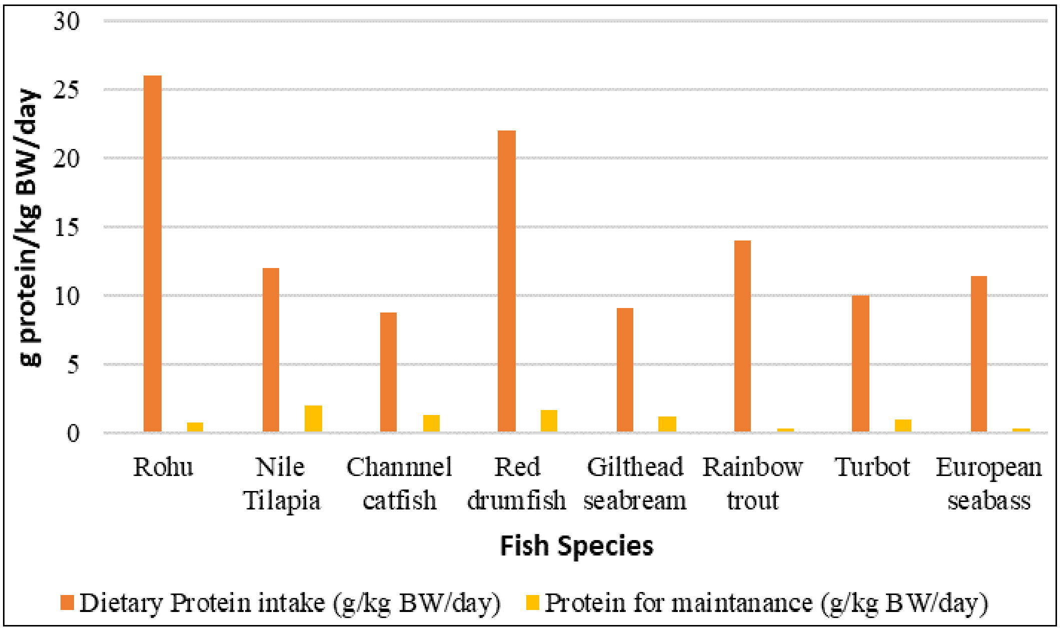

Fig 2: Dietary Protein intake and Protein required for maintenance in various Fish species [17, 18, 19, 20].

The given (figure 2) illustrates the relation between protein intake through diet and its utilization for maintenance. Protein retention efficiency (PRE) is one of the contributing reasons for all fish species showing different protein requirements for maintenance. PRE of some of the commercial fish species are as shown in table 3.

Table 3: Protein Retention Efficiency of some of the commercial Fish species

Fish species	Protein Retention Efficiency	Author
Chanos chanos	21	Lim C *et al.*, 1979 [21]
Cyprinus carpio	30	Takeuchi T *et al.*, 1979 [22]
Cyprinus carpio	40	Ogino C *et al.*, 1976 [23]
Cyprinus carpio	48	Murai T *et al.*, 1986 [24]
Ictalurus punctatus	40	Garling Jr DL and Wilson RP, 1976 [25]
Micropterous dolumieu	27	Anderson RJ *et al.*, 1981[26]
Micropterous salmoides	31	Anderson RJ *et al.*, 1981[26]
Morone saxatilis	25	Mallikin MR, 1983 [27]
Pleuronectes platessa	23	Cowey CB *et al.*, 1972 [28]
Salvenius alpinus	33	Jobling M and A Wandsvik, 1983 [29]
Salmo gairdneri	40	Ogino C *et al.*, 1976 [23]
Sarstherdon mosssambicus	25	Jauncey K, 1982 [30]
Sciaenops ocellatus	32	Daniels WH and Robinson EH, 1986 [31]
Scopthalonus maximus	29	Boorman KN, 1980 [32]
Tiapia nilotica	40	De Silva SS and MK Perera, 1985 [33]
Tiapia nilotica	26	Santiago CB *et al.*, 1982 [34]
Tilapia nilotica × *T. aureus*	31	Viola and Zoha,r 1984 [35]

Bowen SH, 1987 [6]

The amount of Protein bifurcation depends on the fish species, physiological and biological changes, ongoing life span, and protein retention efficiency. Studies conducted on Rohu fingerlings [18] (individual weight 0.55± 0.16g), which were fed 40%CP with different feeding levels for 56days. The experiment results suggested that a 6.5%-7% feeding level is optimum for maximum growth. Protein required for maintenance is 0.8-1.6g/100 g diet/day, and for growth are 2.6-2.8g/100g diet /day. Similar research was conducted [36] on Red Drum (individual weight 3.4-5.5g), where one group fasted, and another was fed with 40% CP with different feeding levels. Data indicated that a protein intake of 20-24 g protein/kg BW/day is optimum for maximum growth, out of which the average protein required for maintenance is 0.5-2.5g protein/kg BW/ day. The most precise data documented experiment on Nile Tilapia [19], were fish (individual weight is 8g) fed with different levels of CP fed for 49 days, showed that 35% of CP feed is optimum for maximum growth. It showed that the daily protein intake for maximum nitrogen gain was 12 g/kg /day, and the maintenance protein requirements were about 2 g/kg/day.

The study conducted on Protein and arginine requirements for maintenance and nitrogen gain in four teleosts [17] reported that the protein requirement for maintenance for Rainbow trout is 38 mg N/Metabolic body weight (MBW)/day, for European seabass is 45mg N/MBW/day, for Gilthead seabream 85 mg N/MBW/day and Turbot 127mgN /MBW/day. Protein required for growth for Turbot is 2.16 g N/g N gain, for Rainbow trout 2.28 g N/g N gain, for European Seabass 2.53 g N/g N gain and Gilthead seabream 2.64 g N/g N gain. A similar study was conducted on Rainbow trout for the estimation of protein required for the maintenance [37]. Individual fish weighed around 147g, were fed with 50.9% CP feed for 28 days. The data from the experiment revealed that the protein required for maintenance is 2.6g protein /kg BW/day. Also, the study conducted on Channel catfish [38] (individual weight 8.3-10g), which, when fed with gradient protein level as 25% CP and 35% CP with different feeding levels represent different traits. In both groups, the feeding level showed a protein intake of 8.75g Protein /kg BW/day is optimum for the maximum growth of fish, out of which protein intake of 1.32g protein/kg, BW/day is used for maintenance. Optimum feeding level for maximum growth was found at a 3% feeding rate. The result suggested that the protein required for maintenance is 0.86 g /BW $(kg)^{0.70}$ /day of DCP (0.72g / Fish/day).

Requirement studies on carps

Few studies revealed the similarity in the requirements of protein and amino acids among major carps. Growth or weight gain percentage of both Catla and Rohu fingerlings were more or less similar when fed with a diet containing 30 – 40% protein [15]. A study conducted [16] indicated that the general pattern of requirements of essential amino acids by Catla fry was almost similar from that of Common carp, although some exceptions are found within the individual amino acid requirement. Several studies suggested that adequate levels of non-protein energy sources such as carbohydrates and lipids provided the dietary protein at the sub-optimum level in the diet can minimize the catabolism of protein in these fishes [39]. Even though it is mentioned that 40% dietary protein level is optimum for growth in *C. mrigala* fry [40] specified the similar weight gain percentage and FCR in the same species fed with 30% protein and 40% dextrin as the carbohydrate source. This result indicates the protein-sparing effect observed in *C. mrigala* fry.

Requirement studies on catfish

Many studies have been conducted to determine the optimum protein required for maximum growth in different species of catfishes. As channel catfish (*Ictalurus punctatus*) is the most widely cultured food fish in many countries, it grabbed the primary focus on requirement studies. Protein requirement for maintenance and higher growth of small channel catfish when fed with purified diets was studied[38][41]. The dietary crude protein requirement for various catfish species are mentioned in table 4.

Table 4: The dietary crude protein requirement for various catfish species

Species	Protein percentage	Reference
Ictalurus punctatus	32	NRC, 1993 [42]
Clarias anguillaris	40	Madu CT and TT Tsumba, 1989 [43]
C. batrachus	39.5	Mollah MRA and MA Hussain, 1990 [44]
C. batrachus (fry)	30	Chuapoehuk W, 1987 [45]
C. isheriensis	37	Fagbenro OA, 1992 [46]
Mystus nemurus	42	Khan MD *et al*., 1993 [47]
Pangasus sutchi (fry)	25	Chuapoehuk W and Pothissong T, 1985 [48]
Heteropneustes fossilis (fry)	28 – 35	Akand AM *et al*., 1989 [49]
Clarius magur (larvae)	55	Mir IN *et al*., 2019 [50]

Protein requirement for maintenance

This requirement implies protein for maintaining the life-sustaining process like respiration, blood circulation, excretion, osmoregulation, and digestion movement, with all other nutrients having been provided in adequate amounts. The maintenance requirement is defined as the amount of an amino acid (AA) to be ingested by the fish to maintain its N equilibrium, which means that no net synthesis or net breakdown of body proteins takes place. Determining the maintenance requirement provides a better understanding of the underlying metabolic needs of the organism. Under conditions allowing no growth (no weight change), all animals have a relatively low, yet absolute requirement for dietary protein to replace proteins lost in maintenance and metabolism [51]. Data on protein requirements for maintenance are available for a few freshwater and marine species. The efficiency of utilization of nutrients for maintenance in fishes may be determined using the method of Requirement by Ration Level (RRL) technique; in this design, incremental rations are fed, with different species of fish [52]. In the RRL technique and the graded supplementation, animals are fed diets with graded levels of deficient amino acids and are combined and a composite regression between intake of amino acid and maintenance is established [52]. This requirement can also be estimated experimentally by the nitrogen excreted by a fasting fish endogenous nitrogen excretion (ENE). ENE estimates for fish is expressed in units of milligram of nitrogen excreted per gram of fish per day [13]. Estimates expressed in these units are influenced by the weight of fish. Estimates of ENE for fishes average 51.0 mg N excreted $kg^{-0.75}$/fish weight/day.

Two types of methods can be used to estimate or determine the protein requirement for maintenance. The first or direct method involves measuring amino acids and proteins. This involves measuring the endogenous nitrogen excretion as the combined fecal, urinary, and branchial losses [1]. The fish are either maintained without food, fed a protein-free diet, or fed a low-protein diet. The protein requirement for maintenance is then calculated based on the endogenous nitrogen excretion data by taking into account the digestibility and biological value of the test protein. The second or indirect method is much simpler and the most convenient method to use for fish. In this case, nitrogen retention can be measured by the difference between nitrogen consumed and nitrogen retained by the fish at the end of the experimental period. These data can also be combined with growth data obtained by feeding an increasing ration size and obtaining the nitrogen or protein intake, which results in zero growth [1]. Dietary protein intake and protein required for maintenance in various fish species are as depicted in figure 3.

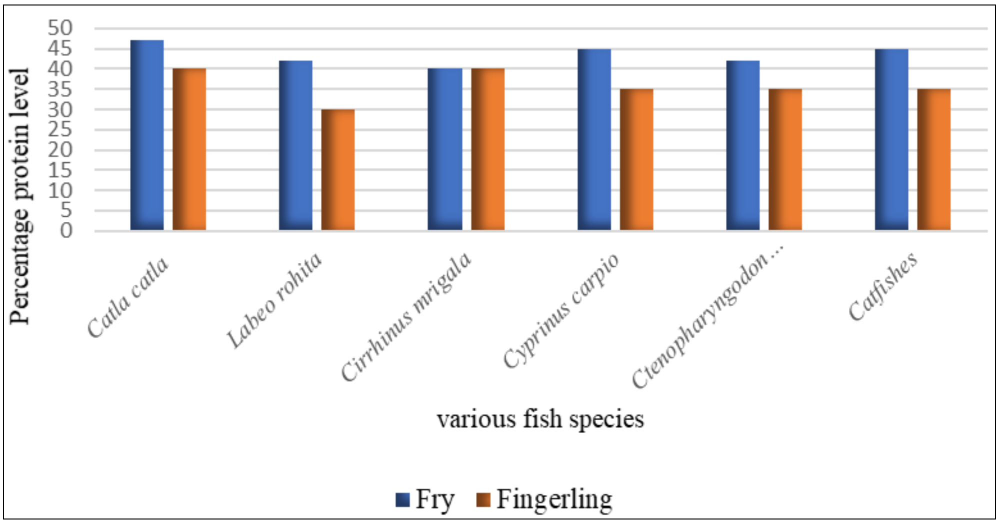

Fig 3: Dietary Protein intake and Protein required for maintenance in various fish species (Bowen SH, 1987) [6].

Only a limited number of studies have been reported on the maintenance requirements of protein in fish. The maintenance requirement for common carp fed casein as the sole source of protein is found to be 0.95 g protein/kg body weight/day is [53]. The maintenance requirement for channel catfish was to found to be 1.3 g protein/kg body weight/day based on growth rates of fish fed increasing rations from 0 to 5% of the body-weight/day of diets containing either 25 or 35% crude protein from casein–gelatin mixture [38]. Somewhat higher values, 1.5 to 2.5, and 2.6 g digestible protein/kg body weight/day have been reported for red drum [36] and rainbow trout [37], respectively.

Protein requirement for growth

Protein requirement for growth can be expressed as a percentage of diet dry weight, the concentration of protein in the diet that produces maximum growth is the most commonly used measure of protein required for growth of fish [7]. The relative protein concentration required by fish may be higher because fish need more protein for growth (numerator relatively greater), fishes require less of some other diet component (denominator relatively smaller). Juvenile shrimp offered 32%protein diets indicated a requirement of about 46.4g DP/kgBWd. Shrimp offered with 48% of protein diet indicated a protein requirement for maximum growth is 43.4g DP/kgBWd. Nonlinear regression analysis on the growth curve of shrimp fed with a 32% diet showed that the protein requirement for maximum growth was 23.5g DP/kgBWd [54].

Protein requirement for reproduction

It is well established that reproductive performance as well as egg quality in fish is affected by several factors such as nutritional status, stress, egg over-ripening, genetics, age of broodstock, water quality, and environmental factors [55]. Gonadal development and fecundity are influenced by dietary nutrient intake especially in synchronized spawning fish such as *P.hypophthalmus* and those with short vitellogenic periods. The dietary nutrients of the female can affect ovarian development, rate of spawning, gametogenesis, quantity, and quality of the eggs [55]. The different levels of dietary protein influence the viability of offspring, the fertilization rates of the eggs, and the percentage of deformed larvae [56]. Components of the energy cost of reproduction include gamete production involving eggs and egg envelopes sperms and seminal fluid, pre-mating processes, migrations, secondary sexual characters differentiation, courtship, individual and territorial competition, parental care, lactation, and learning. In early maturation, fat seems to be used in preference to protein [57, 58].

Later in the maturation period, muscle protein appears to provide the major energy source. This shift from fat to protein may arise simply because very little fat remains or perhaps protein is required for reproductive development during this period. The depletion of muscle protein was mostly matched in females by an increase in ovary protein and, in males, by rapid secondary sexual development. This infers that the final month before maturity is characterized by the transfer of protein from muscle to ovaries and by the use of protein in constructing secondary sexual characters. Protein is particularly crucial for accomplishing morphological changes because secondary sexual characters are composed mainly of cartilage, which cannot be synthesized from fat. The energy requirements of secondary sexual development have been largely ignored despite evidence that they constitute a substantial fraction of the reproductive energy budget. For salmon that migrate exceptionally long distances, protein may also be used to fuel in the final stages of migration. Reproductive development at maturity can be compared with active energy stores in individuals before maturity. A first and indirect estimate of the energy allocation to gonads and other somatic organs and tissues can be obtained from gonadal-somatic-index, which is the gonad weight/body weight ratio.

Factors affecting the protein requirements in finfish and shellfish

Size of the fish

Smaller fishes will require more protein in their diet compared to larger fishes because of their larger growth rate [59]. The total protein requirement will be higher for the larger fishes but the protein requirement per unit weight of the fish body is higher for juveniles. Few examples are shown in table 5.

Table 5: Nutritional requirements of cultivable carps

Sl. No	Nutrients	Fry and fingerlings	Juveniles and growers	Brood fish
1	Protein	40-45	35-40	30-35
2	Carbohydrate	22-26	30-35	35-40
3	Fats	6.8	5	5
4	Vitamins	1	1	1
5	Minerals	1	1	1
6	Digestible energy	310	280	280

(NRC, 1993) [42]

Water temperature
Little convincing evidence exists to show that protein requirement, expressed as a percentage of dry matter, is affected by water temperature. In general, all feeding and growth functions increase in parallel as the water temperature rises, although growth rate may increase more rapidly because of an increased feed conversion efficiency coupled with a higher intake per meal [60]. Temperature controls feed intake, metabolic rate, growth rate, and even the feed conversion efficiency of fishes [60]. The feeding rate of fishes increases with an increase in temperature up to a specific limit and later falls. The metabolic rate also follows a similar trend. The temperature at which maximum growth occurs is a little less than the temperature at which the maximum feed intake occurs, and this is because of a higher metabolic rate, which increases faster gastric evacuation. The temperature at which the maximum feed conversion occurs is also a little less than the temperature at which maximum growth occurs.

Presence of natural food organisms
The abundance of natural food such as phytoplankton and zooplankton in the culture minimizes the protein requirements of fishes in ponds because of their higher availability of protein [61].

Feeding rate
Fishes culturing in intensive or ultra-intensive farms should be fed on a restricted diet of pellets or other feeds only, as they will have a higher protein requirement [62, 63]. Moreover, those culturing in extensive systems utilize the natural live food organisms present in the pond, so the feeding rate in such cases can be kept low, indicating lower protein requirements.

Stocking density
The stocking density of the farms will affect the protein requirements of the shellfish or finfish. Pond with higher stocking density requires greater protein in the diet [64], which is observed due to various high stocking density-dependent behavioral characteristics of species [65, 66, 67, 68, 69] and difficulty in spreading the feed uniformly. Whereas the requirement will be low in low stocking ponds as they are known to have broader access towards natural feeds, and also sometimes provided with other supplementary feeds.

Protein/energy (P/E) ratio
The dietary energy level in the diet affects the dietary protein requirements of fish. If non-protein energy level (lipids and carbohydrates) is low in the diet, fish will utilize protein to meet metabolic energy needs at the cost of muscle growth. It leads to higher excretion of ammonia in water, directly causing stress to fish. A larger amount of such non-protein energy-yielding substances is also challenging as it suppresses food/ feed intake, and fish will not consume enough protein to match the requirement. So, feed should contain optimum amounts of non-protein energy-yielding substances, and the P/E ratio should be maintained at the optimum [25]. Factors affecting maintenance metabolism include water salinity, pH, photoperiodism, carbon dioxide concentration, oxygen concentration, ammonia concentration, physiological state of fish, stage of gonadal development. When considered from the point of fish farming, fish weight (larger the fish the more energy required for maintenance), temperature (higher the temp higher is the rate of metabolic processes), and activity are some of the major factors [60].

Conclusion
Nutritional requirement studies, especially regarding protein bifurcation towards maintenance, growth, and reproduction, can help in efficiently managing protein requirement and feed utilization capacity among fish species. Many studies are conducted to find out the protein requirement that is essential for growth and maintenance based on broken line analysis, which has made insight into the amount of protein required for growth. Protein retained in the body is also a part of net energy to spend. That contribution of retained protein mainly depends on the protein retention efficiency and other biotic and abiotic factors.

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