

ABSTRACT

Copper Mahseer or *N. hexagonolepis* is a predominantly found coldwater fish in riverine system of Darjeeling hills. Its culture is not getting momentum due to unavailability of suitable feeds. In formulating diets for fishes, it is necessary to meet the entire nutrient requirement for their optimal growth. There have been no studies on the protein requirement of *N. hexagonolepis*. Inadequate protein in fish diet results in rapid reduction of growth due to the withdrawing of protein from less vital tissues to maintain the functions of more vital ones. Where all other nutrients are not provided in their appropriate levels, protein in the diet becomes an alternative source of energy. Protein is responsible for a large part of artificial fish feed cost. The protein fraction of diet should therefore be optimally utilized for growth rather than for maintenance of fish. NRC (1983) shows that diets formulated for fish are done considering the minimum protein requirement for optimal growth. The combination of carbohydrates and lipid with protein in the diet therefore spares protein for growth. The carbohydrates in fish diets limit the catabolism of other dietary nutrients for energy, as far as possible. These are the least expensive forms of dietary energy for animals but their utilisation by fish varies and is lower than domestic animals. Lipids are crucial for absorption of fat-soluble vitamins and exhibit many hormonal activities. Knowledge of the optimal level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates can be used effectively in reducing feed costs. A high level of total energy may produce fatty fish and decrease their food consumption. Also, the optimum protein/digestible energy ratio is economically important for fish producers to produce maximum amounts of fish at minimal cost. Therefore, this study reports on the optimum protein requirement, ratios of protein/lipid and protein/carbohydrate and protein/energy in the formulation of *N. hexagonolepis* diet that spares protein for growth as well as standardizes some of the feeding strategies like feeding habit of fingerlings, temperature tolerance range, stocking density, feeding frequency and feeding rate. The preliminary study of this investigation involves selection of appropriate size of fingerlings and standardization of feeding strategies and management prior to its culture with different semi-purified diet to Optimize Protein, Carbohydrates, Lipid and Energy. Based on parameters like Length-weight relationship and Relative Gut Length (RGL) of fingerlings, size group of

more than 5cm (5.4 ± 0.047) was found appropriate for culture and rearing with artificial diets rather than lesser size groups (4.46 ± 0.048) having relatively smaller RGL value indicating its feeding habit less omnivore than bigger size group.

It better withstands lower temperature than higher as its CT_{min} values were not lower than 8.21°C and its CT_{max} values were over 42°C. The results obtained through the present study showed that the *N. hexagonolepis* does adapt completely to the temperatures prevalent in hill regions during winter (less than 10°C are commonly recorded). Further, *N. hexagonolepis* can thus be better adapted to warmer regions, where average annual temperature is around 20-30°C, a finding coherent with the average temperature of the plain region of Darjeeling district with other tropical species, under mild temperatures. The stocking density of *N. hexagonolepis* for aquaculture based on Specific Growth Rate (SGR) obtained was 0.4fish/L. Further studies are needed to check the influence of increasing both fish population size and tank size while keeping a fixed rearing density for *N. hexagonolepis*. Therefore, optimum stocking densities need still to be determined for juvenile *N. hexagonolepis* and this must be done under a variety of operation conditions such as oxygen supply, flow rates, feeding schemes etc. in order to determine the best biomass gain under the lowest possible cost. A feeding frequency of two times a day compared to other experimental groups in this study seemed sufficient for effective growth and nutrient utilization. It is concluded that a feeding frequency of two meal a day till satiation is proper for the improvement of weight gain in juvenile *N. hexagonolepis*. Feeding more than two meal a day results in increased body lipid content without improving growth of fish. In the present study, it appears that the daily feed application rate of 3% body weight was near to optimum based on Specific Growth Rate, Feed Conversion Ratio. The study on Optimum Protein Requirement based on comparative Specific Growth Rate (SGR) study fed on graded level of Crude Protein (25 to 50%) indicated that the optimum dietary protein level for the growth of *N. hexagonolepis* fingerlings was around 40% ($y = -0.002x^2 + 0.158x$; $R^2 = 0.915$; CP-39.50%). This finding is less than *Tor putitora* corresponding to 45%. Therefore, 40% dietary protein gave the best growth in terms of Specific Growth Rate Feed Conversion Ratio and Feed Conversion Efficiency of *N. hexagonolepis* fingerlings. Based on the application of Second-degree Polynomial Regression Analysis relationships of Specific Growth Rate (SGR) to varying levels of dietary carbohydrate provided an estimate

of 25.33% when dietary protein is around 40% (CP 40%) for maximum growth of *N. hexagonolepis* fingerlings ($y = -0.003x^2 + 0.152x + 1.232$; $R^2 = 0.924$). Application of Second-degree Polynomial Regression Analysis relationships of Specific Growth Rate (SGR) to varying levels of dietary lipid provided an estimate of 7.125% when dietary protein is around 40% (CP 40%) for maximum growth of *N. hexagonolepis* fingerlings ($y = -0.012x^2 + 0.171x + 2.878$; $R^2 = 0.876$). Further, the effect of increasing dietary energy on Specific Growth Rate was quadratic. The Regression Equation was ($y = -3E-05x^2 + 0.025x - 1.331$; $R^2 = 0.926$), and the optimal dietary energy concentration estimated for maximum growth was 416.66Kcal/100g. In general active production and secretion of enzymes like protease, lipase and amylase are indispensable for the digestion of macromolecules – protein, carbohydrates, and lipids/fats. The digestive enzyme activity in freshwater teleosts is affected by their feeding behaviour and biochemical composition of the food. In this study, high-protein diets result in a high concentration of proteases, whereas high carbohydrate and fat diets are reflected in high levels of amylase and lipase respectively. Amylase is a widely distributed enzyme in all the dietary trials for optimum protein, carbohydrates, lipid and energy level. High amylase activities in the gut of fingerling of these species suggest extensive amylase synthesis. Therefore, the digestibility of carbohydrate is affected not only by the source and nature of carbohydrate but also by the level of its incorporation. Aside from high nutrient content of the diet, increased food intake of fish fed with diet may have accounted for the strong enzymatic activities. (Kumar and Chakrabarti, 1998). The water quality parameters like water temperature, pH, Total dissolved solids, dissolved oxygen, total alkalinity and total hardness, chloride, Ammonium-N, Nitrite-N, Nitrate-N and Phosphate shows marked variation. However, overall values remain within the suitable range under the influence of different dietary inclusion of macromolecules. Therefore, the present investigation provided the first report on optimum protein requirement of *N. hexagonolepis*, a mahseer species with wide aquaculture potential. The findings, therefore, provided prerequisite information for feed formulation with locally available ingredients at lowest possible cost as well as formulating further strategies for their artificial propagation, while conserving the natural stock.