INTRODUCTION

1.1 Background

Mahseers are popular among coldwater hill stream fishes for angling and tourism, and is considered a cultural icon in 11 Asian countries for economic, recreational and conservation perspective (Nautiyal, 2006). Out of the 47 mahseer species found worldwide, about 15 mahseer species have their natural habitats in India (Nautiyal *et al.,* 2013). Among these, the natural population of Copper Mahseer (*N. hexagonolepis*, McClleland, 1839) in the water bodies of the major rivers and streams of upland of the Northeast Himalaya has markedly decreased due to over-exploitation and habitat loss in most of the regions, along with other species (Dash *et al.,* 2021). As such, it is classified as a 'Near Threatened' fish according to the International Union for Conservation of Nature (IUCN) status (Arunachalam, 2012). However, this represents one of the dominant fish species along the Teesta River and its tributaries in the Darjeeling Himalayas that is why the major stretch of the river is designated as 'Barilius-Mahseer-Snow Trout stream (Acharjee and Barat 2013)**.** Additionally, fishes have sports as well as food value locally due to the highly desirable taste of flesh, abundant amounts of n-3 (omega-3) polyunsaturated fatty acids (n3/n6 PUFA 2.21) and essential amino acids (Sarma *et al.,* 2013). However, the rising concern regarding the decreasing population has led to the need for rehabilitation programmes through artificial propagation, ranching, and building sanctuaries (Mahapatra and Vinod, 2011; Mandal *et al.,* 2012; Laskar *et al.,* 2013; Jyrwa and Bhuyan, 2017). Further, it is considered as a potential candidate species for culture in Jhora pond system of Darjeeling Himalaya (Ayyappan *et al.,* 2001; Laskar *et al.,* 2009; Nur *et al.,* 2010; Laskar *et al.,* 2013, Dash *et al.,* 2021). But, its aquaculture is not getting momentum because of non-availability of suitable feeds that are readily consumed, efficiently digested and utilized for growth and survival. The nutritional requirements of this fish have not been studied for any of the life stages which are essential for formulating feeds for its optimum growth at the lowest possible cost.

1.2 Key areas identified for research

The dietary protein requirement of a species is of prime importance in aquaculture, because feed protein influences growth of the fish and determines the cost of feeding. The quantity as well as the quality of dietary protein is the determinant of the level of

protein utilization by the fish. Therefore, studies on protein requirement are usually one of the first nutrient requirement experiments conducted when a new fish species is introduced into aquaculture (Giri *et al.,* 2011).

Studies on nutritional requirements of cultured species is followed by studies on the nutritional quality of feed ingredients and the prepared diets, which are assessed in two steps process involving chemical and digestibility studies (De Silva and Anderson, 2009)**.** The chemical characteristics looked for in feed ingredients are the Crude protein, Crude lipid, Total Ash, Crude Fibre and Nitrogen Free Extract and Gross Energy content and the most commonly used methodology is that of the American Association of Official Analytical Chemists (AOAC, 1995). The digestibility of each of the ingredients of the diet, as well as that of final product which actually determines the nutrient quality of feed and its ingredients depend on presence and availability of digestive enzymes constituting an important and integral part of diet formulation and preparation (De Silva and Anderson, 2009)**.** Thus, *in vitro* measurement of the digestive enzymatic activity and their characterization can be used as surrogate parameters to predict the ability of fish to use different nutrients which may allow adapting artificial feeding to the nutritional needs of fish (Furne *et al.,* 2005). The analysis of digestive enzyme activities, therefore, is an easy and reliable methodology that can be used as an indicator of digestive process and nutritional condition (Ueberschar, 1988) and can provide relevant tools for the optimization of the relative percentage of their dietary macronutrients as well as for elaboration of feeding protocols to satisfy their nutritional requirements. Hence, knowledge of digestive enzymes of fish has important practical implication for their nutrition (Caruso *et al.,* 2009).

Diet formulation represents translation of nutrient and energy requirement of a given species for a given response into an acceptable diet using a balanced mixture of ingredients which is economically sustainable (Kaushik, 2000). Great part of current research in fish nutrition and feeding is devoted to the study of nutrient requirements, digestive enzymes and digestibility and development of artificial diets for the cultivated fin fish (Oseni, 1996).

The success of feeding operations on fish is reflected in overall productivity and cost effectiveness. Survival rate, growth rate, and feed conversion rate are the main parameters that are routinely determined. They are used to assess performance and plan future feeding operations. Effective feed management should be based on the evaluation and interpretation of data collected by routine fish sampling. During sampling, a

representative group (sub-sample) of animals from the population is weighed or measured. This data can be used to determine the total biomass present and any changes in weight or length that have occurred since the last sampling date.

Further, water quality parameters are also important for the growth and development of fish. Feeding fishes in culture systems in turn leads to the release of potentially harmful organic and inorganic material into the water. Excretory products, mainly nitrogenous wastes resulting from normal metabolic activities are produced more or less continuously in actively feeding fish. Also, faecal material released into the water rapidly break up, contributing to both the suspended and settleable solids loading of the water system. This disturbs the physico-chemical parameters of water in aquatic system.

1.3 Objectives

Based on the above background, this study therefore, is designed to investigate optimum protein requirement and digestive enzymes pattern of fingerlings of Katli, *N. hexagonolepis* (McClelland), with the following objectives:

Objectives of the present investigation

i. To determine optimum protein requirement for fingerlings of *Neolissocheilus hexagonolepis* (McClleland).

ii. To study growth performance (live weight gain, specific growth, protein efficiency ratio, feed conversion ratio, etc) of the Mahseer fingerlings *N. hexagonolepis, in* response to experimental diets.

iii. To analyze the pattern of digestive enzymes (protease, lipase and amylase) in the Mahseer fingerlings *Neolissocheilus hexagonolepis,* during culture operation.

iv. To investigate the physico-chemical parameters in the water during culture operation of *Neolissocheilus hexagonolepis.*