Chapter I

INTRODUCTION

"Hatchery-grown free swimming fries are exclusively dependent on artificial feed for their survival and growth."

—Bardach et al. (1972)

1.1 Rainbow trout

Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (ITIS, 2017) is an important food and game fish (Khanna, 1980). It is an important food fish not only due to taste, delicacy, and flavour but also due to high food qualities (Bista *et al*., 2008). It is much preferred in eating as it does not contain intramuscular bones (Y-bones), a hazardous part in fish eating. Its edible part constitutes 78 to 80% of the total fish. Its flesh contains eicosa-pentaenoic acid (EPA) and docosa-hexaenoic acid (DHA), the poly-unsaturated fatty acid (PUFA) containing (Ω-3), that is very effective in making human skin glistening and lowering human blood cholesterol, thus saving them from coronary heart disease (Birol *et al*., 2015; Ural *et al*., 2016). The Ω-3 also plays key role in memory and cognitive function. Its Y-boneless edible part is highly palatable and easily digestible, with high quality protein containing eighteen amino acids, of which eight are essential, two semi-essential and ten non-essential (Martyshev, 1983). Although climate, altitude, clean water, and several other factors may add flavour to rainbow trout due to several reasons in high hills but one may speculate that some special minerals of the hills may add special flavour to the rainbow trout (Moktan, 2008). Due to the reasons mentioned above, it has become too costly fish.

Rainbow trout is an exotic fish to Nepal, India, and neighbouring countries. Among the exotic coldwater species, rainbow trout is widely cultivated salmonid throughout the temperate world (Bardach *et al*., 1972a) because of its delicacy, nutrient value, and sport purpose. As rainbow trout is a carnivorous fish, it feeds on natural feed like crustaceans, aquatic insects and their larvae, molluscs and small-sized fishes

(Shrestha, 1994; Swar, 2008) which are abundant in hill streams of Nepal. Since it has sportive value so, its ranching was done earlier in 1990 in Nepal because it can thrive well in the hill streams of high altitude (1000 masl or more) rich in natural feed as given above. As its flesh is delicious and has high quality nutrients as mentioned earlier therefore, its commercial culture was done as early as 1990 because hills and mountains of Nepal meet all the requirements regarding its farming.

Rainbow trout prefers cold (below 20 $^{\circ}$C), clear and clean (no silt), hard (100 mg L^{-1}), and high oxygen-containing water (6 mg L^{-1}) moving on stony-bottom shaded by 16 to 17 hours of darkness in fast flowing (1.00 to 1.33 m sec^{-1}) hill streams (Basnet *et al*., 2008; Rai *et al*., 2008) for its better survival and growth. Hence, it has to be cultivated in flowing water therefore, its cultivation can be done in raceway. A raceway or raceway pond, also called flow-through system (Westers, 2000), is artificially constructed cemented body having rectangular-shape but with more length than breadth (15 m × 3 m) and a depth of 1 m maintained up to the thickness of 0.8 m or 0.9 m with running water supplied from a permanent, dependable, reliable and perennial water resource like fountain, rivulet or stream originating either from glacier, lake, or spring.

Rainbow trout, during development, exhibits endogenous feeding period depending on quantity of yolk inside its yolk-sac and after the onset of feeding, shows exogenous feeding period depending on feed – natural feed in the natural habitat and artificial feed in the raceway. The transform from an endogenous to an exogenous feed supply, which marks the onset of larval stage, is one of the most critical phages of the life cycle of rainbow trout and it is this period when much of the motility of hatchery-reared stock occur (De Silva and Anderson, 1995).

1.2 Background of the study

Nepal, the Himalayan country, has three physiographic regions: terai, hills and mountains bordered by the great Himalayas in the north which highly influence the climate of Nepal. The climate, according to altitude, from south to north has been divided into five climatic zones – tropical in terai, sub-tropical in inner terai and lower hills, temperate in upper hills, alpine in higher mountains and tundra in highest or greatest mountains. Since, Nepal is rich in diversity due to its geography (both physiographic and climatic), therefore, it is endowed with many water resources – both lentic (pond, pool, ditch, reservoir, daha, nullah, swamp and so on) and lotic (spring, fountain, rivulet, stream, river and river-system), all of which constitute approximately 4000 square kilometres, that is, 5,760,110 ha (2.8 percent) of the total area and the terrestrial habitat of mountain, hill and lowland terai comprise 143,181 square kilometres (97.2 percent). Among the aquatic habitats, the rivers and their system constitute about 49 percent of the total area of the water resources (CBS, 2017).

Nepal is blessed by the nature with vast array of network of water bodies however, marine water is completely lacking because it is a landlocked country. It has many placid lakes, torrential hill streams and snow-fed rivers. The condition of these lakes, hill streams and rivers while flowing on the hills and mountains and again running in the plain terai region is quite different due to altitudinal variations. The former has coldwater but the later possesses warm water. The physico-chemical and biological conditions of the water of these lakes, torrential streams and rivers play an important role on fish fauna inhabiting in them. The water current, also called water velocity of 1.0 to 3.3 m sec^{-1} due to fast flowing of water or water discharge, low temperature due to rapid and constant motion of the water, plenty of oxygen, quite good shelter of stones and crevices, rich food in the form of algae and macro-biota, and shallow, clean and clear water are quite good initiatives for fish production leading towards cultivation of coldwater fishes in coldwater habitats and warm water fishes in warm water belongings.

Fish farming in Nepal is confined to inland waters including pond, lake, reservoir, stream and rivers. It is mainly confined to terai region where fish species used are warm water carps, tilapia, catfish, live fish, ornamental fish and prawn, both indigenous and exotic. The government and farmers of Nepal, not only produce table fish but also hatchlings, fries and fingerlings of the above mentioned warm-water species in terai region. However, mid hills and mountains of Nepal very much suitable for coldwater fisheries are virtually untouched so far.

Coldwater of lake, spring, fountain, rivulet, stream and rivers of the hilly and mountainous regions of Nepal extending southwards from the Himalayas offer an excellent coldwater habitats to 76 native (Rajbanshi, 2002) and would be habitats to three exotic coldwater species (brown trout, *Salmo trutta fario*; amago, *Oncorhynchus rhodurus*; and rainbow trout, *Oncorhynchus mykiss*). Not a single native coldwater species was introduced in cultivation because of the lacking of their research. Among the native coldwater fish species, hill trout (*Tor tor* and *Tor putitora*) and snow trout (*Schizothorax richardsonii*, *Schizothorax plagiostomus* and *Neoleissocheilus hexagonolepis*) need more focus and attention for their adoption in coldwater aquaculture because they are still on the way to research. Although, native coldwater fishes and their resources offer vast scope for the development of coldwater aquaculture; however, coldwater fishes for their aquaculture promotion are still in their infancy, therefore, at present, this sector is predominated by subsistence and recreational fisheries only indicating towards agriculture and tourism. Therefore, there was a need for the selection of some exotic coldwater species that could adjust well in the coldwater of Nepal. In this context, brown trout and amago which were introduced did not give good results however, Rainbow trout (Bhagat, 2002), when introduced in 1988 proved its suitability.

Rainbow trout are well-studied fish for cultivation in coldwater in the temperate world of Europe and America. Although, Nepal falls under sub-tropical region, its mountains and upper hills constitute what is called temperate climate suitable for rainbow trout where the fish can be cultivated. The production of rainbow trout in Nepal is a recent initiative. However, it is produced in quite a good scale around Kathmandu, Trishuli and Rasuwa road corridor. Because, various researches in several aspects of this species since 1988 are conducted to formulate a combined and common technological package for farmers, rainbow trout has become the suitable exotic fish species in mid-hills and high mountains of Nepal. Further, rainbow trout is the suitable fish species for intensive fish culture and sport purpose in cold waters emphasizing its role in ecotourism and income enhancement. Not only this, rainbow trout farming has become potential for commercial enterprises as it is considered highly renouncing cultivable fish for commercial farming.

As rainbow trout will not get required quantity of natural feed from its artificial habitat, that is, raceway pond, thus, it is totally dependent on artificial feed for its development and growth and ultimately for its production. Rainbow trout culture depends upon adequate supply of nutrients, both in terms of quality and quantity, irrespective of the culture system (extensive-, semi-intensive- or intensive-) in which they are grown. Rainbow trout depends on quality and quantity of artificial feed (Rai *et al*., 2008) that convert 0.07 to 0.10 g free swimming fries into 200 to 300 g table fish in 16 to 18 months in the raceway ponds of Kathmandu, Nepal (ATC, 2004). It means a high production of rainbow trout is achieved when its intensive farming is done but then it requires more flowing water and artificial feed. Being water-intensive and feedlot-based culture system, rainbow trout farming requires better governance and major improvement in the management practices (Prasad *et al*., 2008). Joshi *et al*. (2008) suggested growth of rainbow trout depended on the condition of habitat and quality and quantity of artificial feed. So, when cost and return of rainbow trout production, which is mainly based on artificial feed, was calculated then it was found productive at the level of both government and private farms (Sapkota and Joshi, 2007) in Nepal.

Artificial feed of rainbow trout comprises protein (animal and plant), lipid, carbohydrate, mineral and vitamins (Roy, 2006). The artificial feed should contain protein (high quantity animal and less quantity plant) 40 to 50% (Robinson and Li, 1996), lipid 10 to 15% (Robinson and Li, 1996), carbohydrate 15 to 25% (Hasan, 2001), mineral 1% (Hasan, 2001) and vitamin 1% (Hasan, 2001). In the artificial feed, protein is the main important component. Generally, 35% proteinous artificial feed is suggested for brood and 45% proteinous artificial feed for free swimming fries, fries and fingerlings however, its diet formulation totally depends on age and size of the fish. Rainbow trout, which is highly costly fish (NRs 1500 kg^{-1}) is due to its high production cost. Basically its culture depend upon water quality parameters, seed supply and artificial feed. The artificial feed is the single largest operating cost in rainbow trout culture in the world including Nepal.

1.3 Statement of the problem

Rainbow trout is too costly fish because of its high production cost due to highly costly artificial feed. The feed alone is 76% of the total variable cost and 40% of the total production cost of rainbow trout farming and is one of the major constraints after seed supply to limit expansion of rainbow trout cultivation in Nepal (Nepal *et al.*, 2002). The production cost of rainbow trout is high as artificial feed contains high quantity of highly costly protein diet (Rai *et al.*, 2008). Therefore, rainbow trout culture, an emerging industry in Nepal, is a semi-intensive or intensive-type of farming-system and thus, requires more input resources compared to other fish species for survival, growth and production. Here, input resources mainly comprise well oxygenated cold water and high protein containing artificial feed with high quantity animal protein (Swar, 2008). The protein provides major basis for growth, development, and reproduction (Steffens, 1989; Kaushik, 1995). Therefore, it should biologically be available for rainbow trout through artificial feed and chemically to convert them in required form (Bekibele *et al.*, 2013). The protein component of rainbow trout is the single most expensive portion and important dietary nutrient. Fishmeal and shrimp meals, which are animal source of proteins and soybean and oil cake (mustard oil cake, ground nut cake or cotton seed cake), which are plant source of proteins, are used in the artificial feed of rainbow trout in Nepal. Among animal and plant proteins, animal protein is the main dietary component used in the formulated diet of rainbow trout, since its introduction to Nepal (Roy *et al.*, 1999) as it contains essential amino acids. The animal protein, which is discussed below, is the most costly item which when decreases in cost will sustain rainbow trout farming.

1.3.1 Animal protein

Dried trash fish, although very costly, has become one of the sources of animal protein in the artificial feed of rainbow trout in Nepal because of the presence of required essential amino acids but, it’s bad smell and poor milling quality limits its use (Roy *et al.*, 1999). The presence of fishmeal in a complete rainbow trout diet will supplement any deficiency of amino acids in vegetable protein such as soybean, mustard oilcake, groundnut cake and cottonseed cake. Although it is very much costly, the fishmeal has become one of the main animal protein supplements in the rainbow trout feed in Nepal due to its high nutrient density (20 to 35% protein) and digestibility (Nepal *et al*., 2002). Similarly, the presence of shrimp meal in a complete rainbow trout diet will supplement any deficiency of amino acids in vegetable protein such as soybean, mustard oilcake, groundnut cake and cottonseed cake. Although it is highly costly, the shrimp meal has become another main animal protein supplements in the rainbow trout feed in Nepal due to its high nutrient density (20 to 30% protein) and digestibility (Nepal *et al*., 2002). The fishmeal, shrimp meals, and dried trash fish contain high level of protein and appreciable quantities of fat and minerals. Their protein has high biological value because of their richness in essential amino acids especially lysine and sulphur-containing methionine and cysteine. The fishmeal and shrimp meals, although important feed ingredients of rainbow trout feed in Nepal, their escalating cost, less quantity availability (quite less than demand), and uncertainty in availability have necessitated the use of substitute and alternative animal and plant protein sources to reduce the feed cost without compromising survival and growth. Besides high cost and their unavailability, the quality of fishmeal and shrimp meals, are quite uncertain due to the use of different fish and shrimp species of unknown nutritional value. In addition, fishmeal and shrimp meals are often contaminated with other ingredients such as sand, sawdust and mud as they are stored in unhygienic condition and then supplied. The fishmeal and shrimp meals increase production cost of rainbow trout farming (ATC, 2004; Roy *et al*., 1999; Pradhan, 1999).

The dietary animal protein requirement for rainbow trout ranges from 30 to 35%. Hence, there is need of finding out substitute and alternative animal protein sources of good quality, which are less expensive and readily available either as substitute or alternative or both for the expensive fishmeal and shrimp meals component in the practical diets and also the identification and utilization of easily available plant protein feed resources while formulating artificial feed for rainbow trout. So, there will be reduction in feed cost and increase in profitability without compromising performance. Therefore, most of the studies conducted in Nepal have been focused on finding out natural, nonconventional, cost-effective, substitute animal proteins or alternative to animal proteins or both with required amino acids for the preparation of rainbow trout feed which could be locally available at relatively cheaper rate without affecting survival, growth, production, and quality. The substitute animal proteins and alternative to animal proteins have been explained below:

1.3.2 Substitute animal protein

Rainbow trout nutrition and feed formulation researches have received significant attention on the use of plant and animal byproducts as fishmeal and shrimp meals substitutes in Nepal (Pradhan, 1999; Igarashi and Roy, 1999) which should be natural, unconventional, and cheap protein feed source because fishmeal and shrimp meals are natural, conventional, traditional, and very much costly and highly costly protein feed source respectively. The major emphasis of these studies was on optimizing growth of the trout, feed efficiency of the diet, and general health condition of the fish. So far, very limited numbers of feed ingredients are available to choose for the formulation of balanced diet of the trout in Nepal. Further, investigations into the use of plant feedstuffs in rainbow trout feed have indicated that it is possible to utilize processed soybean meal at high level (up to 60%) without impairing survival, growth, and environment (Bista *et al*., 2008). Again, the mixture of different levels of defatted soybean meal, corn gluten meal, and meat meal could replace up to 90% of the fishmeal, if combination of these ingredients produce the same profile of amino acids comparable to fishmeal (Juadee and Watanabe, 1993). Grain and byproducts are insufficient as these can't fulfill whole requirement of rainbow trout feed. One of the promising substitutes to the fishmeal (FML) and shrimp meals (SML) is silkworm pupae, a waste product of silk industry.

As compared to dried shrimp meal and fishmeal, silkworm pupae of the silkworm moth, *Bombyx mori*, a waste product of silkworm industry, is a cheaper ingredient and rich in both protein and lipid (Bhuiyan *et al*., 1989). The silkworm pupae contains 48.25% of protein, 8.25% of crude fibre, 21 to 38% lipid, and 8.34% crude ash and its amino acid profile is more favourable and comparable to that of fishmeal (Solomon and Yusufu, 2005). Studies on the usefulness of silkworm pupae as artificial feed for Indian major carp and common carp fingerlings have shown that it is more suitable than mustard oilcake and rice bran as feed (Chakrabarthy *et al*., 1973). In an earlier study conducted by Nandeesha *et al*. (1990), it was shown that feeding common carp with artificial diets containing up to 30% silkworm pupae resulted in progressive increase in growth with the increasing level of pupae as compared to a fishmeal based 30% on protein diet, and the highest weight was recorded at 30% of pupae incorporation. Therefore, silkworm pupae (SWP) could be used as a top class nonconventional protein and energy feed for rainbow trout after proper processing at reasonable cost.

Insects constitute 80% among animal species and their population is also quite dense. Insects contain protein content ranging from 20 to 30%. Such a high-class unconventional source of protein and energy feed for rainbow trout could be obtained in very low cost and be processed at a very reasonable cost forming insect powder meal. Compared to fishmeal and shrimp meals, insects are cheapest ingredients but equally rich in both protein and lipid. In this context, the silkworm moths which die after spawning could also be used as another nonconventional, substitute, and cheapest protein and energy feed called silkworm moths (SWM) meal for rainbow trout after proper processing at a quite reasonable cost.

1.3.3 Alternative to animal protein

The dietary crude protein could be reduced from 41.26 to 35.52% in the artificial feed of Pacific white shrimp, *Litopenaeus vannamei* as long as synthetic amino acids are supplemented (Huai *et al*., 2008). Rainbow trout fed fishmeal-based artificial feed containing 37% crude protein supplemented with amino acids such as lysine, methionine, threonine, and tryptophan grow as fast as those fed 42% crude protein. Further, total ammonia nitrogen and soluble phosphorus discharged to the environment is also reduced (Cheng *et al*., 2003). It has been shown that reducing 2.7% (from 27.0% to 24.3%) of dietary digestible crude protein with essential amino acids has no negative impact on growth performance of Nile tilapia (Botaro *et al*., 2007). Therefore, synthetic amino acids (SAA), although less costly, could further be supplemented in the rainbow trout diet as still another unconventional and alternative to animal proteins like fishmeal and shrimp meals.

1.4 Aims and Objectives

Animal protein supplement of the artificial feed of rainbow trout is fishmeal or shrimp meal, which is very much costly increasing the cost of production. So, there is still need of some low cost, non-conventional, effective, easily available, substitute and/or alternative to fishmeal or shrimp meals but required amino acids-containing animal or synthetic protein feed respectively for rainbow trout. As explained above, this may be supplemented through animal proteins of silkworm pupae and silkworm moths and synthetic protein of synthetic amino acids. Hence, silkworm pupae and silkworm moths were taken as nonconventional, low cost, easily available, and substitute animal proteins and synthetic amino acids was taken as unconventional, less costly, less available, and alternative to animal proteins in replacement to conventional, highly costly, available, and traditional shrimp meals for the preparation of three formulated diets of rainbow trout so as to evaluate these with the control diet of shrimp meals.

As shown, on the basis of above mentioned explanation along with background of the study and statement of the problem, the aims of the present research work would be to compare the three formulated diets of cheaper animal protein of silkworm pupae, cheapest animal protein of silkworm moths and less costly synthetic proteins of synthetic amino acids in replacement to highly costly animal protein diet of shrimp meals acting as control when all the four diets (three formulated and one control) would be fed to the free swimming fries, fries, and fingerlings and the impact of all these diets would be evaluated on survival and growth of the above mentioned stages through total feed intake and total protein intake along with feed efficiency indicators of feed efficiency, protein efficiency ratio, absolute growth rate, specific growth rate, relative growth rate, condition factor, feed conversion ratio and protein productive value further including highest growth period and cost analysis. Again, impact of the diets with natural and animal proteins of silkworm pupae, shrimp meals, and silkworm moths respectively would be compared to that of the diet with synthetic proteins of synthetic amino acids on survival and growth of the free swimming fries, fries, and fingerlings of rainbow trout. Further, impact of the water quality parameters of the raceways would be investigated. Furthermore, different stages (age of the broods, and size of the broods, eggs, sac fries, and free swimming fries) of rainbow trout on survival and growth of the free swimming fries, fries, and fingerlings would be studied. Finally, what level of impact did physic-chemical parameters, different stages (age of the broods, and size of the broods, eggs, sac fries, and free swimming fries), and artificial feed could put on survival and growth of the free swimming fries, fries, and fingerlings of rainbow trout would also be mentioned. Hence, to investigate survival and growth of the free swimming fries, fries, and fingerlings of rainbow trout during exogenous feeding in the raceways, the present work entitled **"Impact of artificial feed on survival and growth of rainbow trout, *Oncorhynchus mykiss* (Walbaum) during exogenous feeding in raceways of Kathmandu, Nepal"** has been taken as the research assignment along with the following objectives:

- To assess the utility of **silkworm pupae, insects, synthetic amino acids** as cheap animal protein source.
- To compare shrimp meal/fishmeal (costly animal protein source) with that of **silkworm pupae, insects, synthetic amino acids** (cheap animal protein source) so far **survival** and **growth** of **rainbow trout** is concerned.
- To know some of the **physico-chemical parameters** of **raceways** to compare with survival and growth of rainbow trout.
- To **formulate** low cost **artificial feed** for **rainbow trout** by providing cheap animal protein source.