# Chapter II

# REVIEW OF LITERATRUE

*"The protein provides major basis for growth, development, and reproduction"*.

***—Steffens (1989)*** *and* ***Kaushik (1995)***

## 2.1 Rainbow trout and history of its culture

Rainbow trout is so called, as it appears rainbow colour in glacier water because the sides have rainbow iridescence. It has stream-lined body, modified mouth and lips for rasping particles from rocks, stones, pebbles, and so on; narrow gill opening and gills, thus, adapted to highly oxygenated water and very low temperature; and great power of locomotion and clinging and the burrowing habit, whenever required. It has small mouth, positioning of adipose fin above the anal fin and emarginated caudal fin. The head and dorsal sides are steel blue in colour with reddish along its sides. Its belly is light-coloured. The dorsal fin and caudal fin are marked with dark spots but other fins are slightly pinkish. It doesn't have red spots but the body surface is covered with small-sized star-shaped black spots above lateral line (Page and Burr, 1991). The male adult of rainbow trout has distinctive rose coloured iridescent band on their flanks which is more reflective at the time of reproduction.

Rainbow trout is the native of coldwater of northern hemisphere. Actually, it is the native of natural habitats of the Pacific coasts of North America from Alaska to Mexico inhabiting glacier, lake, spring, stream and rivers. Its commercial culture began as early as in 1853 in the United States of America and even little earlier in Europe (Bardach *et al.*, 1972a). It has been successfully cultured in many countries for hundreds of years. It was first introduced to California of North America Pacific and then to Alaska. Later on, it was introduced to Asia (in Japan in 1877) and Europe (in 1880) during 19$^{th}$ century (Swar, 2008). In Japan, rainbow trout established itself well and now became third highest freshwater fishery product (YMCL, 1991). Therefore, being an important fish species, rainbow trout was distributed to most part of the world for farming and sport (Stickney, 1991). Because cultivation of rainbow trout is an art (Bardach *et al*, 1972b), hence, its culture has become a well-established industry throughout the world for both commercial production and recreational fishing purpose (Barrington, 1983). As a result, it is present in all the continents of the world except Antarctica (Martyshev, 1993) for recreational angling and aquaculture purposes. Trout fisheries are maintained, or culture practiced, in the upland catchments of many tropical and sub-tropical countries of Asia, East Africa and South America. It is now introduced in 82 countries of the world. Several local domesticated strains have been developed, while others have arisen through mass selection and cross-breeding for improved cultural qualities. Europe, Chile, Japan, North America and Australia are major production centres of trout in the world. The wide distribution of rainbow trout attests to its ability to adapt itself to a variety of aquatic environments including aquaculture conditions. Rainbow trout can be propagated artificially, grows fast, which makes it important as fish food production. The fish can be fed artificial feed and can withstand temperatures of up to 26.6 °C for short periods. It also tolerates low dissolved oxygen content of water and is resistant to some of the fish diseases. That is why rainbow trout is considered an important coldwater fish in India (Santhanam *et al*., 1999) and will be established as an important coldwater fish in Nepal.

Rainbow trout, which were introduced in India by Europeans in 1918, are now found in the coldwater of Nilgiris, Kodai hills (Tamil Nadu), Travancore (Kerala), Kashmir and Himachal Pradesh. Trout was introduced to Bhowali hatchery Uttar Pradesh but did not survive. However, it established well in Bhutan. There are several trout hatcheries in India located at Avlanche in the Nilgiris, at Rajmally in Kerala, at Achaibal, Laribal and Harwan in Kashmir, and at Katara and Barot in Himachal

Pradesh (Khanna, 1980). Rainbow trout was first introduced to Nilgiris and high ranges of Kerala, India. Now, they inhabit mainly in rivers, streams, brooks, lakes and ponds of upland areas like Kashmir, Himachal Pradesh, Uttar Pradesh, Nilgiris, Kodai hills, Mannar high range (Sathanam *et al*., 1999). Although, rainbow trout are well-studied fish for cultivation in cold waters (Bardach *et al*., 1972b; Huet, 1975; Shetty *et al*., 1989), however, researches in cultural aspects were meagre in India.

Rainbow trout was introduced in Nepal from the United Kingdom in 1960, from Japan in the late 1965, and from India in the early 1970 (Gurung and Basnet, 2003; Swar, 2008). Later on, 50,000 eyed-eggs of rainbow trout were brought from Miyazaki Prefecture of Japan in 1988 and introduced to Fisheries Research Division, Godawari, Lalitpur where more than 80 percent of the eyed-eggs hatched successfully into sac fries (Gurung and Basnet, 2003). Those sac fries were cultivated successfully till table fish. Some of the fingerlings of rainbow trout were transferred from Fisheries Research Division, Godawari, Lalitpur to Fisheries Research Centre, Trishuli, Nuwakot (Rai *et al*., 2002) for further research. Now, the same stock of rainbow trout has been maintained in research and private sector. First breeding of rainbow trout from the same stock was done in 1990 (Basnet *et al*., 2008). The ranching of rainbow trout in Nepal from the same stock was done in limited area for the limited time and then postponed without any conclusion drawn from the previous one. However, researches in cultural aspects of rainbow trout are continuous in Nepal so as to establish complete technology package for physico-chemical parameters, breeding, and artificial feed applicable at the farmers' raceways, especially small scale farmers. Among these, most of the researches were focused on artificial feed as the production cost of rainbow trout was very high due to the artificial feed.

## 2.2 Site of rainbow trout farm

The selection of feasible and suitable site for rainbow trout culture is one of the essential parts of successful trout farming. The most important factor to consider when selecting a site for a raceway farm is water supply. The best site for rainbow trout farm establishment should have preferably hilly and mountain area with an altitude of 700 to 2000 masl and a slope of 1 to 3% so as to permit adequate water supply (Rai *et al.*, 2005) carried either from glacier-fed, lake-fed and spring-fed torrential hill stream with suitable water discharge carrying required physico-chemical parameters along with nutrients for rainbow trout.

So, when selecting the production site, it is important to check quality and quantity (volume) of available water, as well as suitability of the site where new fish farm is planned to be constructed. A rule of thumb is that about 10 L $sec^{-1}$ (600 L $min^{-1}$) of water source should be calculated for each tonne of rainbow trout produced (Edwards, 1989 and 1990). The daily (day and night) fluctuation in temperature may be 2 to 4 $^{\circ}C$ while the seasonal (summer and winter) changes of water temperature may be as much as 5 to 15 $^{\circ}C$. The temperature of spring-fed water has no daily fluctuation and the differences between winter and summer are minimal if any (Woynarovich *et al.*, 2011). Further, easy access to road, availability of electricity, safety and security, no poaching and getting rid of floods are other important factors that need to be considered during site selection (Rai *et al.*, 2008). The selection of the site of rainbow trout farm indicates success or failure of its culture.

## 2.3 Raceway ponds for rainbow trout culture

Concrete circular tanks ranging from 4 to 6 m in diameter and an average depth of 0.75 m have been utilized in the culture of rainbow trout in overseas to improve productivity but then such tanks are less suited in automated handling, grading and harvesting operation (Bromage and Shepherd, 1990), hence, these tanks can be superseded by earthen raceway ponds. Earthen raceway ponds are not exactly suitable for rainbow trout culture but these ponds can be used in the cultivation of grow outs if abundant year round coldwater is available. If not, earthen raceway ponds are completely replaced by concrete raceway ponds.

Concrete raceway ponds of various shape and sizes are used generally for rainbow trout culture with effectiveness in production (Westers, 2000). If water resource is dependable, permanent and reliable, only then raceway ponds are constructed. Again, if water from the water resource is unlimited then adequate number of stable raceway ponds can be constructed in parallel fashion but if water from the water resource is limited then linear raceway ponds for holding rainbow trout may be constructed but then such linear ponds need filter chamber to clean used and polluted water before sending it to other ponds. Stocking raceway ponds (for the stocking of free swimming fry, fry, fingerling, grow out, yearling and table fish) should be rectangular with the ratio of 1:5 width and length respectively (Basnet *et al*., 2008) and the depth of 1 m. In rectangular raceway ponds, water exchange increases by six times within 24 hours than ponds with other shapes. The suitable size of the raceway ponds are 50 to 150 $m^2$ and the depth of 1 m with water thickness volume of 0.8 or 0.9 m (Rai *et al*., 2008). In this way, raceway ponds constructed in Nepal are 10 m × 2 m, 15 m × 3 m, 20 m × 4 m or 25 m × 5 m with the above mentioned depth of 1 m.

## 2.4 Hatchery for artificial propagation of rainbow trout

The selection of suitable hatchery for artificial breeding of rainbow trout is another essential part of successful trout farming. The best hatchery for artificial breeding of rainbow trout at the small farmers' level should have preferably three to five incubation raceways cum tanks that can adjust required number of atkins and cages for the incubation of fertilized eggs up to the hatching of sac fries and incubation of sac fries up to the emergence of free swimming fries respectively whatever and whenever required during the artificial breeding (TFS, 2010).

## 2.5 Rainbow trout: the requirement for its culture and production

Adequate volume of cold water below 20 $^{\circ}$C throughout the year is the prerequisite for rainbow trout cultivation. Rainbow trout culture site must have year round supply of water with water temperature ranging from 10 to 18 $^{\circ}$C (Yamazaki, 1991). The production of rainbow trout depends on suitable water temperature and dissolved oxygen in the raceway water (Swar, 2008). The trout requires cold, clean, and highly oxygenated water for ripening of the gonads of broods required for successful breeding and hatching. The pH value of 6.5 to 8.5 and dissolved oxygen above 8 mg $L^{-1}$ are considered suitable for rainbow trout culture (Huet, 1975). The preferable range of pH for rainbow trout culture is in between 6.5 to 8.0 with optimum range between 7.0 to 7.5 because at higher pH, even low level of ammonia can be dangerous and toxic (Bromage and Shephard, 1990; Sedgewick, 1985). Moreover, calcareous water, that is, hard water, is preferable (Leitritz, 1963). The nitrogen should be less than 0.4 mg $L^{-1}$. Abowei (2010) reported ideal level of pH for biological activity being higher than 7 and lower than 8.5. The total alkalinity between 30 to 500 mg $L^{-1}$ favours fish and shrimp production (Boyd, 1982).

Gauchan *et al*. (2008) reported that rainbow trout production was tied with agro-climatic condition like fresh cold water (completely clean and clear), low temperature (9 to 14 $^{\circ}$C for spawning and 14 to 18 $^{\circ}$C for fingerling and table fish production), completely unpolluted water with water discharge of 1 L $sec^{-1}$. Deformed sac fries are reported due to high water temperature during spawning (Basnet and Silwal, 1996). Rai *et al.* (2008) revealed that rainbow trout is suitable for intensive culture and sport purpose because of the physiography and climate of the hills of Nepal. Thapa *et al.* (2007) stated that an altitude of 610 to 1,750 masl is suitable for rainbow trout culture. The water in raceways supplied from glacier contains 4 to 5 months of turbid water harmful to rainbow trout cultivation while water from spring or lake is clear and clean useful for the rainbow trout culture.

The stocking density can be kept 50 to 100 trout $m^{-2}$ depending upon quality, quantity and water discharge of the water carried from water resource. The stocking density of rainbow trout is kept 10 to 15 kg $m^{-3}$ at water discharge of 2.08 to 3.13 L $sec^{-1}$; but if water flow rate is 4 L $sec^{-1}$ then stocking density will be kept 20 kg $m^{-3}$; and in case of sufficient water discharge of more than 5 L $sec^{-1}$, stocking density will be kept 35 kg $m^{-3}$.

At present, in the country, the stocking density in both government and private sector has been maintained up to 50 trout $m^{-2}$ only. In such stocking rate, the marketable size of 200 to 300 g reaches at 14 to 16 months of cultivation. Both female and male future rainbow trout brood are segregated (by external appearance and weight) and separated 1 to 3 months prior to breeding and stocked at the rate of 5 to 10 kg $m^{-2}$ in raceway pond with a water discharge of 1.0 to 1.5 L $sec^{-1}$ and 7 mg $L^{-1}$ of dissolved oxygen, however, in case of intensive farming stocking rate is 15 to 30 kg $m^{-2}$. In both (intensive and semi-intensive) farming system, pH should range 7.5 to 8.0 and 7.0 to 7.5 respectively (Basnet *et al.*, 2008; Pradhan *et al.*, 2008b; Rai *et al.*, 2008).

## 2.6 Survival and growth of rainbow trout during exogenous feeding period

The survival and growth of rainbow trout during exogenous feeding period depend partially on age of the broods, and size of the broods, eggs, sac fries and free swimming fries; mainly on water quality variables; and exclusively on artificial feed fed to the free swimming fries, fries, and fingerlings of rainbow trout (Rai *et al*., 2008).

### 2.6.1 Physico-chemical parameters and its role on survival and growth of rainbow trout

Rainbow trout is a cold water fish that always requires crystal-clear but running water with low water temperature, high dissolved oxygen, low free carbon dioxide, required water velocity, and balanced water discharge. In addition, it requires the water having optimum pH, electrical conductivity, turbidity, total alkalinity and total hardness. Besides all these, it also requires suitable nutrients like nitrate, ammonium and phosphate. Furthermore, its culture is to be supported by some climatic factors like relative humidity and rainfall and geographical factors like altitude and water resource. Such condition of ever running water along with all the above mentioned parameters could be met with concrete structures of rectangular size, situated at high altitude, constructed in a series or parallel, and supplied with a channel of perennial but dependable water resource like spring-fed torrential stream situated nearby. These narrow and shallow bodies are called raceways.

Rainbow trout is a clean, cold and high dissolved oxygen requiring fish and is thus, directly related to physico-chemical parameters. Knowledge of water quality parameters is one of the essential components of sustainable rainbow trout farming as these parameters are pre-requisites to start the cultivation of trout. Carp culture in earthen ponds partly depends on water quality parameters and partly on natural process as water is stagnant but rainbow trout culture in raceway ponds highly depends on water quality variables and slightly on natural process because water is flowing, that is, coming in and passing out continuously. These properties for fish culture refer to all physical, chemical and biological parameters that influence the beneficial use of water (Boyd and Lichtkoppler, 1979). These variables are highly influenced by climatic factors, geography, seasons, environment of their origin and occurrence (Schmitz, 1996).

Rainbow trout culture has become popular among fish farmers in the hilly regions of Nepal because of its feasibility and also due to its local consumption, foreign export, more demand and high cost. Even the Government of Nepal has prioritized the rainbow trout culture in the hilly regions. The purpose of understanding water quality in rainbow trout culture is the management of water to maximize fish production without impairing aquatic environment. Water is an essential requirement for fish farming so any properly prepared plan for aquaculture like rainbow trout culture must describe the quality and quantity of water available for this purpose (Summerfelt, 2000).

Trout depend on water quality parameters like water temperature, turbidity, dissolved oxygen, free carbon dioxide, pH, nitrate-N, total alkalinity and total hardness (Manon and Hossain, 2011). Because water temperature, dissolved oxygen, free carbon dioxide, pH and total hardness can cause severe stress hence, water quality is suitability of water for survival and growth of fish (Boyd, 1992). Some of these parameters are important water quality variables that rainbow trout farmers should concentrate on, and attempt to control to some extent by management techniques. Spatio-temporal changes in physico-chemical parameters of water which is used for rainbow trout culture by raceways present essential information for best management options at all time. To great extent, physico-chemical parameters of the raceway ponds determine the success or failure of rainbow trout culture (Piper *et al*., 1982).

The feasibility and suitability of the site for rainbow trout culture farm as well as the survival and growth of rainbow trout are mainly dependent upon water quality parameters of the water of the raceway ponds. So, physico-chemical analyses of the factors of water of the raceway ponds indicate success or failure of the rainbow trout culture as well as survival and growth of rainbow trout respectively. Klontz (1991) studied physico-chemical properties of the raceways where rainbow trout was cultivated. Akbulut *et al*. (2002) studied water quality variables like water temperature, dissolved oxygen, salinity and pH of the Turkish Black Sea Coast and found seasonal variations of the parameters. Clark (2003) compared water quality parameters like dissolved oxygen, total settle-able solids and total gas pressure along with carbon dioxide, nitrite-N, alkalinity, pH and total atmospheric nitrogen (TAN) in aerated and oxygenated raceway ponds.

A preliminary work on water quality parameters like water temperature, water discharge, dissolved oxygen, free carbon dioxide, pH, nitrite-N + nitrate-N, total ammonia, total phosphorus, alkalinity and total hardness of the raceways of Godawari, Kathmandu, Nepal was done by Pradhan *et al*. (2008b) for the introduction of rainbow trout there. Moogouei *et al*. (2010) conducted a study to evaluate the correlation between selected physico-chemical parameters of water and their effects on growth of rainbow trout in raceway system in the area of Sarab Gerdu, Iran. Subba and Gubhaju (2011) investigated physico-chemical parameters of water temperature, pH and dissolved oxygen in the raceways of the Fisheries Research Division, Godawari. Noroozarajabi *et al*. (2013) studied the impact of rainbow trout farm effluents on water physicochemical properties of Daryasar Stream on water temperature, pH (potential of hydrogen ion concentration), electrical conductivity (EC), total dissolved solids (TDS), turbidity, dissolved oxygen (DO), nitrate-N ($NO_3$), nitrite-N ($NO_2$), total atmospheric nitrogen (TAN) and phosphate-P ($PO_4$). Bhagat and Barat (2015a, 2016c and 2017b) reported eighteen physico-chemical parameters of the raceway ponds of the Kakani, Kathmandu, Nepal of the two consecutive years.

The commercial scale of rainbow trout culture requires suitable water quality including appropriate water temperature and water discharge volume. Hence, the water quality is one of the basic pre-requisite to begin the cultivation of rainbow trout. As the trout species completely depends and performs all the functions in water as its living medium, it is essential not to impair their environment. Farmers living in hilly and mountainous regions of Nepal require information on water quality to begin and sustain their rainbow trout farming practices. The parameters further refer to physical (colour, odour, transparency, taste, air temperature, water temperature, water velocity, water discharge, turbidity, relative humidity, rainfall, water resource and altitude) and chemical parameters (pH, electrical conductivity, dissolved oxygen, free carbon dioxide, total alkalinity, total hardness, phosphate-P, ammonium-N and nitrate-N) affecting biological parameters (stocking density, hatchability, mortality, survival, growth and feed).

### 2.6.1.1 Colour

Light scattering by suspended matter is required in order that the blue light produced by water's absorption can return to the surface and be observed. Such scattering can also shift the spectrum of the emerging photons toward the green, a color often seen when water laden with suspended particles is observed. Therefore, the colour of the natural water in the raceway pond is due to light scattering upward from the water and being absorbed en route after passing through it (Bhatnagar and Devi, 2013).

### 2.6.1.2 Transparency

Transparency of water relates to the depth that light will penetrate water. The transmission of light into a body of water is extremely important since the sun is the primary source of energy for all biological phenomena. Light is necessary for photosynthesis, a process that produces oxygen and food for the aquatic biota (Boyd and Lichtkoppler, 1979).

### 2.6.1.3 Taste, flavour and odour

Natural water in its pure form is odourless. However, when some inorganic and organic chemical compounds are dissolved in it, they will impart taste, flavour or odour (APHA, 2005).

### 2.6.1.4 Air temperature

The air temperature is the combined effect of some meteorological factors like solar radiation, relative humidity, wind velocity and precipitation. Besides these, it is also affected by latitude, longitude and altitude of the location (Wetzel, 2001). McGregor and Neuwolt (1998) obtained approximately 0.65 $^{\circ}C$ 100 $m^{-1}$ of altitude in the tropics as being the mean normal lapse rate, that is, the rate of decrease in air temperature with increase in altitude. The air temperature controls physiological behaviour of water temperature APHA (2005) and Arain *et al*. (2009). It directly affects water temperature and is always higher than it. Rawat *et al*. (1993), Wetzel (2001) and Ayoade *et al*. (2009) found significant positive correlation of air temperature with water temperature.

### 2.6.1.5 Water temperature

Water temperature is the temperature of water in the raceways and is the most important water quality variable. It is a limiting factor in aquatic environment (Boyd, 1979). It controls the rate of all chemical reactions; governs metabolic activities of all aquatic biota; and affects feeding, maturity, reproduction, survival and growth of rainbow trout. It affects solubility of gases in water; gaseous solubility increases with decreased water temperature and decreases with vice-versa. Rainbow trout is sensitive to water temperature change as it changes daily, monthly, and seasonally. It is depended on air temperature (Basnet *et al*., 2008; Manon and Hossain, 2011) and altitude (Jacobsen, 2008). Acherjee and Barat (2011) investigated 0.6 °C decrease in water temperature 100 $m^{-1}$ increase in altitude. So, it is directly affected by the air temperature and altitude and is always lower than it.

### 2.6.1.6 Water velocity

Water velocity, also called water current is the current of water in raceways and is an important water quality parameter (Westers, 2000). It helps in mixing of nutrients (Woyanarovich, 1975). Very fast running water is not desirable. If water velocity is too swift, energy might be used up for swimming instead of growth (Huet, 1975). As a rule of thumb, water current should be sufficient enough to provide at least one complete change of water per hour (YMCL, 1991). Water velocity depends on slope of the raceways. Raceways with slanting slope have more water velocity in comparison to raceways with no slope. Potential raceways for commercial rainbow trout production should have year-round supply of adequate running water available from perennial resource like glacier, lake or spring-fed torrential stream. Therefore, it also depends on water resource whether it comes from glacier-, lake- or spring-fed torrential streams. Rainfall increases water velocity (Bhagat, 1999). It is fluctuated according to water discharge. It is also fluctuated due to seasons.

### 2.6.1.7 Water discharge

Water discharge, also known as water flow is the volume of water running in raceways and is another important physical parameter. Due to the water discharge, water remains no more stagnant. If water is stagnant, it might result in depletion of dissolved oxygen below the desirable level and accumulation of wastes. The accumulation could lead to poor growth and performance of rainbow trout. It depends on origin of water resource. If water resource is originated from glacier, it has high water discharge; if it is originated from lake, it has low water discharge; and if it is originated from spring-fed torrential stream, then it has moderate water discharge. Water discharge also depends on slope of the water resource (Wetzel, 2001). If there is no slope, water discharge will be less but if there is steep slope, then water discharge will be more. It is fluctuated according to water velocity (Lampert and Sommer, 2007). If water velocity is high, water discharge may be high but if water discharge is low then vice-versa. Water discharge directly helps in maintaining water velocity and bringing fresh oxygenated water. It also fluctuates due to seasons. During monsoon season, rainfall increases water discharge. It also depended on contour and size of the raceways (breadth and depth) and size (breadth and depth) of the feeder channel that supplies water from the water resource to the raceways.

#### 2.6.1.8 Turbidity

Turbidity, also known as total suspended solids, is the suspension of solid particles in water – either living or non-living and is still another important physical parameter. It comprises silt or clay particles, planktons, organic compounds, inorganic compounds or other microorganisms if turbidity is in between 20 to 25 NTU (Khanna, 1980). It affects transparency and light penetration in water. It is influenced by changes in pH which can cause some of the solutes to precipitate or may affect the solubility of suspended matter. Turbidity depends on origin of water resource and water discharge (Wedemeyer, 1996). It is fluctuated by water velocity (Khanna, 1980) and seasons. Rainfall increases turbidity (Lawson, 2011). Suspended solids have been found higher during monsoon season if water is originated from glacier but if it is originated from spring or lake then it is found lower. It has adverse effects on dissolved oxygen and free carbon dioxide.

#### 2.6.1.9 Relative humidity

Relative humidity is such a physical parameter which is totally dependent on seasons. It directly affects rainfall. Rainfall further affects air temperature which furthermore affects water temperature (Grant, 2017).

#### 2.6.1.10 Rainfall

Rainfall is an important physical parameter. It aids dissolved oxygen, free carbon dioxide, $Ca^{2+}$, $PO_4$ and $NO_3$ in the water resource directly and through surface run offs from where raceways obtain them. It is solely dependent on altitude and monsoon air. It is fluctuated by season (Grant, 2017).

#### 2.6.1.11 Altitude

Altitude imposes directly or indirectly on so many parameters mentioned above. It directly effects on air temperature and water temperature. Altitude less than 1000 masl is not suitable for rainbow trout culture because in monsoon months, the water temperature may exceed 25 $^{\circ}$C but if water comes from glacier, the culture is suitable even at an altitude of 700 masl as water temperature does not exceed 22 $^{\circ}$C (Pradhan *et al*., 2008b).

#### 2.6.1.12 Water resource

Water resource affects directly air temperature, water temperature, dissolved oxygen (Singh *et al*., 1991) and turbidity. It also affects other parameters. It is fluctuated according to the origin of water resource (for the supply of water in raceways). Water from glacier is colder than water from spring or lake. Water from spring is colder than lake. Water temperature of spring water does not exceed 22 °C even in monsoon season. It is also fluctuated due to seasons.

### 2.6.1.13 pH

pH is the measurement of acid/base activity in water. It is one of the vital chemical parameter deciding survival, metabolism, physiology and growth of aquatic biota. Ramanathan *et al* (2005) recommended its optimum range of 6.8 to 8.7 for maximum growth and production of shrimp and carp. It is influenced by acidity of the bottom and biological activities. In unpolluted water, it is governed by the exchange of carbon dioxide with the atmosphere. It is most important in determining the corrosive nature of water. Lower its level higher will be the corrosive nature of water. It is positively correlated with electrical conductivity and total alkalinity (Gupta *et al*, 2009). Various parameters bring about its change. Reduced rate of photosynthesis and assimilation of free carbon dioxide and bicarbonates are ultimately responsible for its increase when dissolved oxygen becomes low and water temperature increases during summer months. Its higher level suggests that free carbon dioxide and carbonate-bicarbonate equilibrium is affected due to change in physico-chemical condition (Karanth, 1987). At the level of 8.3, presence of carbonate is indicated but below this carbonates are converted into equivalent amount of bicarbonates. Abowei (2010) reported its ideal level for biological activity being higher than 7 and lower than 8.5, however, below 4, it is detrimental to aquatic life. It is affected by total alkalinity and acidity, surface run off from surrounding rocks and water discharge. It depends on total alkalinity. It is fluctuated according to electrical conductivity and seasons. It increases with increase in free carbon dioxide.

### 2.6.1.14 Electrical conductivity

Electrical conductivity, also called specific conductivity, salinity or total dissolved solids in natural water is its capacity to conduct electric current. It refers to salinity. It is also the measurement of total dissolved solids. It is influenced by dissolved salts such as sodium chloride and potassium chloride. Raceways producing rainbow trout range between 0.001 to 0.1 S $m^{-1}$ of EC, however, at 0.3 S $m^{-1}$ it is harmful to rainbow trout. It shows significant correlation with water temperature, pH, total alkalinity and total hardness (Patil *et al*, 2012). Electrical conductivity depends on salinity and pH. It is fluctuated by $PO_4$, $NH_4$, and $NO_3$. It increases with decrease in free carbon dioxide.

### 2.6.1.15 Dissolved oxygen

Dissolved oxygen refers to the amount of oxygen that is dissolved in raceways' water due to solubility of atmospheric oxygen by the help of atmospheric pressure and by the photosynthetic activity of microscopic and macroscopic plants. It is an essential water quality variable for rainbow trout culture. Its correlation with raceways gives direct and indirect information, e.g. photosynthesis, solubility and availability of nutrients, bacterial activity and stratification (Premlata, 2009). It is soluble in water and the amount that is dissolved in water equilibrates the amount in atmosphere. During summer, it decreases due to increase in water temperature and also due to increased microbial activity (Moss, 1972; Morissette, 1978; Kataria, 1996). Its low level can result in damages to oxidation state of substances from the oxidized to the reduced form thereby increasing the levels of toxic metabolites. Dissolved oxygen depends on both air temperature and water temperature. During winter, it increases due to decrease in water temperature and also due to decreased microbial activity. It is 15 mg $L^{-1}$ at 0 $^{\circ}$C and 8 mg $L^{-1}$ at 25 $^{\circ}$C. It is close to saturation of 10 mg $L^{-1}$ in unpolluted fresh water. The suitable dissolved oxygen for rainbow trout culture in raceways is 8 mg $L^{-1}$ (Huet, 1975). Dissolved oxygen depends on water temperature. It is fluctuated according to pH, electrical conductivity and seasons. It increases with decrease in free carbon dioxide.

### 2.6.1.16 Free carbon dioxide

Free carbon dioxide is the end product of organic carbon degradation in almost all aquatic environments and its variation is often a measure of net ecosystem metabolism (Smith and Hollibaugh, 1997; Hopkinson, 1985). Free carbon dioxide is another essential water quality variable in rainbow trout culture. Hence, in aquatic biochemical studies, it is desirable to measure parameters that define the free carbon dioxide system. It is also the most important greenhouse gas. Its fluxes across the air-water or sediment-water interface are among the most important concerns in global change and are often a measure of the net ecosystem production metabolism of the aquatic system. It is an essential parameter in primary production and phytoplankton biomass. Its high rate is detrimental to survival, physiology and metabolic activity of aquatic animals including rainbow trout. Free carbon dioxide depends on water temperature. It is fluctuated according to pH, electrical conductivity and seasons. It increases with decreased dissolved oxygen.

### 2.6.1.17 Total alkalinity

Total alkalinity of natural water is a measure of its capacity to neutralize acids to a designated pH (APHA, 2005; Edokpayi, 2005). It is an indirect measure of the concentration of anions in water which come from bicarbonates, carbonates, hydroxides and phosphates being derived from dissolved rocks, salts and bottom sediments. Thus, it is controlled by the concentration of carbonates, bicarbonates, hydroxides and phosphates. It acts as a stabilizer of pH. It along with pH and total hardness affects toxicity of many substances in water. Its range between 20 to 50 mg

$L^{-1}$ permits plankton production for fish culture (Boyd, 1982); between 30 to 500 mg $L^{-1}$ favours fish and shrimp production (Boyd, 1982); and more than this level results in physiological stress on aquatic biota and may lead to the loss of biodiversity.

### 2.6.1.18 Total hardness

The total hardness of water is used to denote the quality of water (Wetzel, 2001). Total hardness of water is governed by calcium and magnesium salts in combination with carbonates and bicarbonates along with chlorides and sulphates. Principal cations responsible for hardness are calcium and magnesium whereas other cations are iron and manganese. Again, anions imparting hardness are carbonates and bicarbonates, chlorides, sulphates and nitrates. The hardness is of two types – temporary and permanent. The temporary hardness is due to cations of carbonates and bicarbonates. The permanent hardness is caused by metals of chlorides and sulphates. Calcareous water is more preferable by rainbow trout in raceways (Leitritz, 1963).

### 2.6.1.19 Nitrate-N

Total nitrogen is the sum of nitrite, nitrate, and total ammonia (Bhatnagar and Devi, 2013). Its desirable range is 2 to 6 mg $L^{-1}$. It is important in terms of productivity. Nitrite-N ($NO_2$) is formed due to metabolic activity of aquatic biota including rainbow trout but soon oxidized into the $NO_3$ after entering into aerobic regime. Nitrate-N ($NO_3$) is an important chemical parameter in terms of productivity. Its level over 5 mg $L^{-1}$ indicates pollution. It becomes toxic at a level of 30 mg $L^{-1}$. Acting as cation, it helps to govern salinity and specific conductivity.

### 2.6.1.20 Ammonium-N

Total ammonia which is also indicated by total ammonia nitrogen (TAN) is the sum of ammonia (unionized) and ammonium (ionized). Ammonia-N ($NH_3$) which is unionized ($NH_3$) is the metabolic product of aquatic organisms (Bhatnagar and Devi,

2013) including rainbow trout. It is highly toxic to the organism including the trout. Ammonium-N ($NH_4$) which is ionized ($NH_4^{++}$) is an important chemical parameter. Acting as cation, it helps to govern salinity and electrical conductivity.

### 2.6.1.21 Phosphate-P

Phosphate-P ($PO_4$) is also an essential chemical parameter (Bhatnagar and Devi, 2013). Acting as cation, it helps to govern salinity and electrical conductivity. It helps in maintaining productivity of the aquatic ecosystem.

### 2.6.1.22 Survival of free swimming fry, fry and fingerling of rainbow trout due to water quality parameters

Rainbow trout prefers clean, cold, and high oxygen-containing water for its survival, which is abundant in the hills and mountain areas of Nepal (Bista *et al*., 2008). The trout requires clean, cold water with high dissolved oxygen for its maturity, survival and behaviour. It can survive at a temperature range of 0 to 25 $^{\circ}$C (Rai *et al*., 2008 and Swar, 2008).

The suitable water temperature for breeding of rainbow trout spawners, their spawning, incubation of zygote and hatching of eggs is 9 to 14 $^{\circ}$C and for that of survival 16 to 18 $^{\circ}$C with large volume of constantly flowing water (Rai *et al*., 2002, 2005 and 2008) advisable rate for which is 50 L $min^{-1}$ (Santhanam *et al*., 1999), that is, 0.83 L $sec^{-1}$.

Rainbow trout can be cultivated at water temperature range of 0 to 25 $^{\circ}$C but normal feeding and survival occurs between 14 to 18 $^{\circ}$C with 6 mg $L^{-1}$ of dissolved oxygen and 6.5 to 8.0 pH. Thapa *et al*. (2007) stated that an altitude of 610 to 1,750 masl is suitable for rainbow trout culture. The health, reproduction and survival of this species are affected by silting of water coming from water resource which decreases transparency, increases turbidity and causes oxygen depletion (Basnet *et al*., 2008; Pradhan *et al*., 2008b and Rai *et al*., 2008).

### 2.6.1.23 Growth of free swimming fry, fry and fingerling of rainbow trout due to physico-chemical properties

Rainbow trout needs clean and cold water for its growth (Rai *et al*., 2008). The water temperature seems to play an important role in growing period of rainbow trout (Basnet *et al*., 2008). The trout grows well in water temperature of 16 to 18 °C (Rai *et al*., 2008). According to Yamazaki (1991) the growth is highest (1.5 g $day^{-1}$) in water temperature between 15 to 18 °C in cemented raceway, moderate (0.79 g $day^{-1}$) in cage, and least (0.69 g $day^{-1}$) in earthen bottom raceway. The rainbow trout prefers clean, cold, and high oxygen containing water for its growth, which is abundant in the hills and mountain areas of Nepal (Bista *et al*., 2008). Its growth depends on water temperature, dissolved oxygen, and quality and quantity of feed attaining commercial size of 200 to 300 g table fish in 13 to 15 months (Swar, 2008). Rainbow trout prefers clean and cold water with high dissolved oxygen for its maturity, growth and production. The trout mostly requires glacier water or clean cold spring water for its successful breeding and commercial growth. If the temperature stays very near the limits of suitable range for more than 6 months, the growth rate of rainbow trout will be so poor that the production farm will not be commercially viable (YMCL, 1991).

With regards to water quality, the best guidelines are that water should be clear, and not turbid because its growth trend is affected by the presence of silt and humus in water (Basnet *et al*., 2008). The growth of rainbow trout occurs in fast flowing raceway water in the temperature range of 0 to 25 °C (Swar, 2008). In case, if water discharge is not regulated, then negative correlation between stocking density and growth may occur (Bekiroglu *et al*., 1995). Rainbow trout grow well between water temperatures of 10 to 20 °C (Yamazaki, 1991 and Swar, 2008) but, here in Nepal, the trout grows best at water temperature of 16 to 18 °C (ATC, 2001a). The consumption of artificial feed decreases when water temperature increases above 20 °C resulting into slow growth and eventually death, if same temperature prevails for a longer period of cultivation.

Rainbow trout cultivation requires dissolved oxygen more than 7 mg $L^{-1}$; a slope of 1 to 3% for permitting adequate water discharge, hence, hill and mountain sloppy regions are well suited for commercial purpose in which by the feeding of artificial feed, the rainbow trout grows to commercial or marketable size of 200 to 300 g during the second year (Marcel, 1995). If 5 to 10 g sized fingerlings are stocked, the marketable size of 200 to 300 g can be achieved in 10 months. Rainbow trout grows to commercial size of 200 to 300 g during the second year (Joshi *et al*., 2008). The marketable size of 200 to 300 g from sac-fry stage was obtained in 12 to 17 months (Rai *et al*., 2008). The fingerlings of 9 to 10 months and weighing 100 g are even sold in November from private farms, however, marketable size of 200 to 300 g reaches within culture period of 13 to 15 months in Godawari and 15 to 17 months in Trishuli (Basnet *et al*., 2008) and 14 to 20 months in the government trout farms (Swar, 2008).

Rainbow trout require 7 mg $L^{-1}$ of dissolved oxygen for breeding and proper growth. The growth can be retarded or rainbow trout may die if dissolved oxygen level remains below 7 mg $L^{-1}$ (GLT, 1998). The growth rate of rainbow trout at farmer's field was higher (0.65 g $day^{-1}$) than Fisheries Research Centre, Trishuli (FRCT) which was lower (0.42 g $day^{-1}$) due to better water quality in farmer's field than in FRCT, which had high silt. Therefore, the fry reached 4.25 g after five months at FRCT; hence, the growth was 0.03 g $day^{-1}$ after 150 days of culture period.

### 2.6.2 Artificial breeding and effect of brood, egg, sac fry and free swimming fry on survival and growth of rainbow trout

The free swimming fry of rainbow trout can be obtained by the breeding performance of the brood passing through the egg and sac-fry. The breeding performance and success of rainbow trout culture highly depend on disease surveillance; selection, management, age, and maturation of brood; physico-chemical parameters; and artificial feed. Good selection of brood is one of the important aspects to increase the rate of hatchability and decrease the rate of mortality of offspring (Basnet *et al*., 2008). The breeding performance of rainbow trout including age of the brood, spawning, fecundity, stripping, eggs, milt, incubation, sac fry and free swimming fry and so on was done by Hoisty *et al*. (2012). Okumus (2002) focused his study on the brood stock management and seed production of the rainbow trout in Turkey. Cakir (2002) also studied the fry production, feeding and management of the brood stock of the rainbow trout practically for getting profitability.

Rainbow trout brood and replenishing stock are assessed on the basis of general health condition, absence of deformities, good external appearance, rapid growth, proper weight, good colouration, prompt activity and swiftness of reaction to stimuli. Other relevant characteristics attained after sexual maturity are age, spawning time, quantity of spawn, size and colour of the eggs (Basnet *et al*., 2008). The commercial production of rainbow trout can be done in suitable physico-chemical parameters with constant water temperature along with proper water volume in raceway pond hence, water temperature ranging from 9 to 14 $^{\circ}$C is considered suitable for maintaining spawners for breeding and incubation (Rai *et al*., 2008 and Shamspour and Khara, 2016).

### 2.6.2.1 Rainbow trout brood for artificial propagation

The rainbow trout broods are fed 35% crude proteinous pellet feed throughout the year at the rate of 2 to 3% of their live weight depending on water temperature before they spawn, i.e., before first week of November, twice daily – once during morning and the next during evening. Later on, broods are fed 1 to 2% of feed just before spawning, i.e., before first week of November two times daily – once during morning and next during evening. The normal feeding occurs at a temperature range of 10 to 15 °C. However, the feeding decreases when water temperature becomes 20 °C or more. Generally, first week of November is the commencement week of breeding during which feeding rate and feeding frequency should be decreased from twice daily to 3 to 4 times week$^{-1}$. The state of maturity should be confirmed and the ripeness of gonads should be examined twice weekly so as to propagate the brood artificially by stripping method (Basnet *et al*., 2008 and Rai *et al*., 2008). The survival rate of the future brood was found to be 95% however the survival rate of brood in brood raceway was 97% when fed at the rate of above recommended dose (Bista *et al*., 2008).

After egg laying and milting, the spent up broods, that is, first and second spawners (both female and male) should be bathed in 3% sodium chloride solution and then stocked in the future brood raceway for future use (as second or third spawners) at the rate of 5 to 10 kg m$^{-2}$ generally in 3 to 5 m$^3$ sized raceway with water discharge of 2.08 L sec$^{-1}$ feeding them 45% crude protein diet (Basnet *et al*., 2008 and Rai *et al*., 2008).

### 2.6.2.2 Egg and milt collected by dry stripping of rainbow trout brood

The external appearance of the body of female and males changes during spawning when eggs and spermatozoa ripen respectively. The ripening can be confirmed when with a mild pressure on vent, a female exudes eggs and a male oozes milts. The body colouration of male becomes brilliant, abdomen remains compressed, appear darker, almost black sometimes, and the lower jaw elongates and sometimes curved upward like a hook. In contrast, a female's abdomen remains rounded or swollen, appear creamy, and almost white sometimes. The quality and maturity, in case of egg and milts, play an important role in successful breeding of rainbow trout. The quality and maturity of egg and milt can be obtained by careful selection of brood. Those broods, which are still in the process of ripening, are left for some duration to ripen during which the brood is examined periodically in 3 to 4 days. A healthy egg is confirmed through observation and that of milt first through observation and then by examination under compound microscope. The qualitative and fully matured eggs are spherical, with a diameter of 0.3 to 0.35 cm (in first spawners) and 0.35 to 0.4 cm (in second spawners), translucent and pale yellow to orange colour which is due to the presence of carotenoids-containing artificial feed. Similarly, qualitative milt is examined on the basis of external appearance when it is cream-coloured with dense consistency whereas that of poor quality or less quality milt is bluish tinge in colour with watery consistency. When examined under compound microscope, qualitative milt shows proper motility of the spermatozoa. Selected spawners are brought to the hatchery. In each spawner, whether female or male before stripping, eggs are collected in a tray by gently pressing the vent and then pouring the milts on them (Basnet *et al*., 2008; Rai *et al*., 2008 and Shamspour and Khara, 2016).

### 2.6.2.3 Breeding performance of rainbow trout

The maximum longevity of rainbow trout is 11 years (Morrissy, 1973). Artificial method of propagation is the successful method of breeding of rainbow trout. The trout can breed at the age of one year however it breeds well at the age of 2 to 3 years. According to Santhanam *et al*. (1999), a female trout spawns during September to February in India. The trout can be artificially bred twice a year, once from November to December and the next from February to March in Nepal however, rate of hatching (of the fertilized eggs) will be less and mortality rate (of the incubated eggs) will be high in comparison to the first chance and that there will be great chance of dying of the broods. The fecundity of rainbow trout changes with age of female broods (Martyshev, 1983). The older brood generally lays larger-sized and higher number of eggs $kg^{-1}$ body weight of the female and the younger brood vice-versa. The smaller eggs might have low hatchability with the result of production of less number of sac-fries. A female trout spawns best at the age of 4 to 7 years and a male at 3 to 6 years. The 3 to 4 years female trout can spawn 3,000 to 3,500 eggs $kg^{-1}$ body weight (Morrissy, 1973) because breeding performance, quality, and quantity of eggs depend upon brood, quality of water, artificial feed provided and various other management practices (Basnet *et al*., 2008; Rai *et al*., 2008; Bhagat and Barat, 2016b and 2016d).

It is not advisable to use eggs collected from female trout spawning for the first time as the roe is comparatively small and yields higher percentage of waste during the period of egg development. However, in Nepal, female and male trout broods less than two years, that is, $1.0^+$ broods (first spawners) and less than three years, that is, $2.0^+$ broods (second spawners) are generally used by the small farmers due to the constraints in keeping them for such a long time of 3 to 7 years as mentioned above. Whatsoever may be, these rainbow trout broods lay 1,000 to 2,000 mature eggs $kg^{-1}$ body weight (Cakir, 2002).

#### 2.6.2.4 Fertilization and incubation of the eggs of rainbow trout

After the confirmation of broods, eggs and milt are collected by stripping (by gently pressing the vent) and then eggs are mixed with milt at a ratio of 2 : 1 or 3 : 1 or 4 : 1 depending upon quality and quantity of eggs (generally 10 in numbers in 1 g) and milt (generally 15 to 20 million $ml^{-1}$) (Schlenk and Kahmann, 1938). The stripped eggs from female trout are fertilized by the milts obtained from male trout. Generally, one male can supply enough milt for the eggs of two females. The brisk milt after mixing with eggs is stirred with the help of a feather for about one minute and then, in the mixing tray containing eggs and milt, about 10 to 20 ml of 0.9% sodium chloride solution is poured to ensure more fertilization. During this, fertilization takes place. Afterwards, fertilized eggs are washed with freshwater and then transferred into locally made incubation cum hatching tray (33 cm × 34 cm), which with other nine trays (altogether ten trays) containing fertilized eggs are staked and adjusted into an atkin. Two or three atkins are kept together in a hatchery where clean and cold (9 to 14 $^{\circ}$C), oxygenated (6 to 8 mg $L^{-1}$) and continuously flowing water with water discharge of 0.017 L $sec^{-1}$ per 10,000 eggs during incubation period is maintained. The fertilized eggs are incubated at water discharge of 0.017 to 0.05 L $sec^{-1}$ with 6 mg $L^{-1}$ of dissolved oxygen. The fertilized eggs hatch within 27 to 30 days at 9 to 14 $^{\circ}$C (Hoisty *et al*., 2012; Bhagat and Barat, 2015b, 2016e and 2016f).

### 2.6.2.5 Sac fry and free swimming fry of rainbow trout

A sac fry, also called yolk-sac fry, alevin or larva, measures about 1.3 to 1.8 cm in length and 0.05 to 0.08 g in weight. Out of the total weight of sac-fry, yolk-sac constitutes about 50 to 60%. The sac-fries are transferred into hatching cages to thin them out from aggregation where water discharge of 0.017 to 0.05 L $sec^{-1}$ per 10,000 sac-fries is maintained. The yolk of the sac-fry is absorbed within 7 to 18 days depending on water temperature (Bhagat and Barat, 2015c, 2016e and 2016f) after which it starts swimming freely and is converted into free swimming fry. The free swimming fries, each of which are 0.07 to 0.1 g in average weight, are fed starter feed every hour at the rate of 15 to 20% of their live body weight till 3 g size taking 10 weeks during which they first convert into fries and then into fingerlings. The fingerlings which reach 3 g in wt. are fed 10 to 15% of their live body weight at 2 hours interval in daytime till they attain 5 g wt. taking 6 weeks (Basnet *et al*., 2008; Rai *et al*., 2008).

### 2.6.2.6 Survival and growth of free swimming fry, fry and fingerling of rainbow trout due to brood, egg, sac fry and free swimming fry

The older brood of rainbow trout generally lays larger-sized and higher number of eggs (Martyshev, 1983). Similarly, sac-fries having yolk-sac obtained from well-fed three years matured female and male broods show more hatchability, survival, growth, activeness, and more yolk than those not well-fed but of the same age and also to those of two years age. Further, sac fries having yolk-sac obtained from well-fed two years matured female and male broods show more survival, growth, activeness, and more yolk than those not well fed but of three years age (Martyshev, 1983). Similarly, free swimming fries obtained from well-fed three years matured female and male broods show more hatchability, survival, growth, and activeness than those not well-fed but of the same age and also to those of two years age. Furthermore, free swimming fries obtained from well-fed two years matured female and male broods show more survival, growth, and activeness than those not well fed but of three years age (Martyshev, 1983). Shamspour and Khara (2016) compared the six combinations of the age of the male and female rainbow trout broods with their breeding performance.

The sac-fry growing period is from March to April when eggs surrounded by yolk inside yolk-sac nourish the developing egg nucleus (Watson, 1993). To guarantee the success of rainbow trout culture, consistence seed supply mechanism is the most important step. So, rearing free swimming fries successfully is one of the most important parts of the culture, as its sustainability highly depends on survival of free swimming fries (Bardach *et al*., 1972b; Huet, 1975).

### 2.6.3 Artificial feeds and their impact on survival and growth of rainbow trout

Actually, fishes including rainbow trout require a balanced combination of twenty naturally occurring essential and non-essential amino acids that make up proteins. Fishes utilize dietary proteins by digesting them into free amino acids which are absorbed into blood and lymph and distributed to tissues throughout the body where they are then reconstituted into new specific proteins of fish tissues. The proteins in fish tissues are formed of all the twenty types of amino acids. Fishes can synthesize some of these amino acids in their own body and some they cannot and these, therefore, must be consumed. Essential amino acids, which are eight in number and semi-essential amino-acids which are two in number cannot be synthesized by fishes, hence these amino acids must be supplemented through artificial feeds. Essential amino acids requirement by animals including fishes is similar. However, amino acids requirement by animals and fishes are common qualitatively but they are quite different quantitatively. Again, the fishes including rainbow trout simultaneously also require a balanced combination of fatty acids that make up the lipids.

Availability of rainbow trout seed (fry or fingerling), physico-chemical parameters, and quality feed are major constraints hindering the rapid expansion of rainbow trout farming in cold waters of the country. All stages of rainbow trout, from free swimming fry to adult, can be grown by providing suitable water quality parameters and feeding them appropriate artificial feed. However, its cultivation is mostly characterized by semi-intensive farming system everywhere, providing proper-sized seed (fry or fingerling), suitable water quality parameters and using high-cost nutrient input in the form of nutritionally-complete formulated diets. In the formulated diets, the most important and expensive ingredient is fishmeal or shrimp meal that has been incorporated to meet protein requirement along with the requirement of amino acids and lipids.

Rainbow trout thrive well in hill streams of high altitude (1000 masl or more) rich in natural feed like aquatic insects, their larvae, crustaceans, molluscs and small-sized fishes (Shrestha, 1994). They feed chiefly on insects, molluscs, fishes and tadpoles, however, they are cannibalistic at times (Santhanam *et al*., 1999) because they have good fighting ability, which can be noticed by their spectacular leaps when hooked (Basnet *et al*., 2008). However, in raceway ponds, they are dependent upon artificial feed in the form of crumble feed for free swimming fries to fingerlings and pellet feed for fingerlings to table fish explained below.

#### 2.6.3.1 Artificial feed requirement

Artificial feed provides essential nutrition for the day to day maintenance. The nutrition is the process by which organism intake food and assimilates for metabolic activities. It involves ingestion, digestion, absorption, and transport of various nutrients throughout the body where these nutrients in food are converted into energy and body tissues (assimilation). Energy, which is defined as the capacity to do work, is essential to life process during all stages of animal's life. The energy need is always associated with the feeding standard of organisms. In this respect, information on energy need, particularly of fishes, is inadequate in comparison to ruminants. In general, it is argued that energy need in fishes is lower than that of warm-blooded animals because fishes do not have to maintain a constant body temperature hence they expend less energy to maintain their position in space (Rai *et al*., 2005).

The nutrition of rainbow trout is similar to that of other animals. Thus, rainbow trout require almost same nutrients as other animals for normal metabolic function. However, the specified amount of nutrient needed by rainbow trout may differ from other animals. Qualitatively, forty elements or nutrients have been identified as necessary for the normal metabolic function of animals including rainbow trout. The qualitative requirement of nutrients does not vary from animal to animal because they require protein, lipid, carbohydrate, mineral and vitamins. However, quantitative requirement for protein, lipid, carbohydrate, mineral and vitamins vary. In this context, quantitative requirement of the nutrient for rainbow trout are protein (40 to 50%), lipid (10 to 15%), carbohydrate (15 to 25%), mineral (1%) and vitamin (1%) (Hasan, 2001) as described below:

#### 2.6.3.1.1 Protein

A continual supply of protein is needed throughout life for maintenance and growth. Rainbow trout require rather high (40 to 50%) dietary protein (Hasan, 2001). Rainbow trout including catfish require a source of nonspecific nitrogen and indispensable amino acids (Robinson and Li, 1996). Usually, the most economical source of these elements is a mixture of protein in feed stuff. Ingested proteins are hydrolyzed to release amino acids that may be used for synthesis of tissue protein or, if in excess, utilized for energy. Rainbow trout require 11.7% essential amino acids in its dry diet (Hasan, 2001).

#### 2.6.3.1.2 Lipid

Use of protein for energy is expensive, thus lipid is primarily included in formulated diet to maximize their protein sparing. Lipid is a highly digestible source of concentrated energy. Rainbow trout have natural diet rich in triglycerides and can be adapted to high fat diet (Hasan, 2001). Dietary lipid level as low as 10% have been reported for rainbow trout (Tacon *et al*., 1983; Tacon, 1990). But the growth of rainbow trout receiving 18.5% fat was dynamic and faster (Parova and Rehulka, 1997). However, weight gain and feed efficiency (FE) is depressed in aquatic species when given diet contains 15% or more lipid (Robinson and Li, 1996).

Dietary lipid provides essential fatty acid (EFA) in the form of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) that rainbow trout, like all animals, cannot synthesize but require from outside for the maintenance of cellular function. Freshwater (both warm water and cold water) fishes have an exclusive requirement of PUFA like linolenic acid (with $\Omega$-3) in their diet (Hasan, 2001). It is apparent that rainbow trout require a small amount (1% of dry diet) of $\Omega$-3 containing fatty acid. Inclusion of too much dietary lipid, however, may result in excessive fat deposition in the visceral cavity and tissues that may adversely affect yield, product quality and storage of processed product (Robinson and Li, 1996).

#### 2.6.3.1.3 Carbohydrate

Carbohydrates are least expensive form of dietary energy and are frequently used for protein sparing in formulated diet. The utilization of dietary carbohydrates appears to differ depending on the complexity or chemical structure of the carbohydrate source used. The ability of carnivorous fish species to hydrolyze or digest complex carbohydrates (starch) is limited due to weak amylolytic activity in their digestive tract. For rainbow trout, starch digestion decreases as the proportion of dietary starch is increased (Hasan, 2001). However, commercial rainbow trout feed contains 15 to 25% soluble carbohydrate. An additional 1.0 to 2.5% carbohydrate is generally present as crude fibre. The crude fibre is considered to be indigestible by carnivores, thus, it is not desirable in carnivore's feed like rainbow trout because indigestible materials may pollute water (Robinson and Li, 1996). However, there is always some fiber inherent in practical feed ingredients.

#### 2.6.3.1.4 Mineral

Minerals are required for metabolism, skeletal structure and for osmotic balance between body fluids and their environment. Freshwater fishes have greater demand for adequate mineral supplies than marine fish and shrimp (Hasan, 2001). Dietary mineral requirement for rainbow trout is 1%. Since, much of the mineral requirement is supplied by the food, potential mineral supplementation may be sufficient to meet dietary need (Hasan, 2001).

#### 2.6.3.1.5 Vitamin

Vitamins are highly diverse in chemical structure and physiological function. They are generally required in small amount in diet for normal growth, health and reproduction by animals. Pathologies related to vitamin deficiency in fish are well investigated, however, quantitative dietary vitamin requirement are available for few cultured species including rainbow trout (Hasan, 2001). Vitamin deficiency mainly appears in formulated feed-lot based intensive culture system as applied to trout (Hepher, 1990) hence, the addition of sufficient level of several vitamins in trout feed are required. Vitamin C actually helps rainbow trout in the achievement of the sexual maturity and thus, maintaining reproduction (Rai *et al*., 2005).

### 2.6.3.2 Feed formulation of rainbow trout

In spite of clear importance of nutrition influencing survival, growth and development of rainbow trout larvae, however, relatively little is known about the absolute nutrient requirement and feed formulation of those stages of rainbow trout in Nepal. Feed formulation of rainbow trout depends upon age and size of fish (Joshi *et al*., 2008 and Rai *et al*., 2008). Bista *et al*. (2008) tried to formulate rainbow trout feed, both crumble and pellet feed because in those days rainbow trout were fed Japanese starter feed and imported pellet feed which were much costly and were not possible to introduce at the farmer’s level.

Alternatives to the costly Japanese starter feed fed during nursing are egg custard, potential alternative for at least 2 to 3 days (Pradhan, 1999). Other alternatives are 30% frozen raw buff liver mixed with starter feed, 30% fishmeal mixed with local feed, 30% earthworm mixed with local feed or 100% earthworm. However, 30% raw buff liver mixed with starter feed has been proven to be the best starter feed (but only after Japanese starter feed) among others so far health and growth are concerned (Rai *et al*., 2008). Different alternatives of the crumble feed have been discussed below.

**2.6.3.2.1 Shrimp meal versus soya meal**

An experiment was conducted in 1994 for sixty days at Fisheries Research Centre, Trishuli to find out either shrimp meals could be substituted with soybean protein or not. The experiment was carried out in troughs of equal size (0.1463 $m^3$). There were two treatments each with two replicas. Each trough was stocked with 142 rainbow trout fry of approximately forty-five days old (average weight 1.052 g). In treatment 1, rainbow trout fry was fed with soybean-based feed (soybean 72%, shrimp meal 10%, wheat 16%, multivitamin 1% and salt 1%) and in treatment 2, with crumble feed (soybean 40%, shrimp meal 42%, wheat 16%, multivitamin 1% and salt 1%). The growth of rainbow trout fed with soybean-based feed was higher (0.155 g $day^{-1}$) than those fed with crumble feed (0.151 g $day^{-1}$) but there was no significant difference ($P > 0.05$) on growths between the two treatments. This might be due to the presence of abundant natural food (invertebrate population) in water supply. It might be possible that the animal protein deficiency could not be seen in the fry fed with soybean protein, if they had consumed the natural food. In a study, shrimp meal replaced up to 75% by soybean had no adverse effect on overall performance of feed utilization efficiency in rainbow trout (Tacon *et al.,* 1983). Except the protein level, the difference between shrimp meal and soybean is their origin. In farm made feeds, the protein level is about 35%. This level of protein can be derived from soybean. However, the cost of shrimp meal is approximately four to six times higher than soybean.

#### 2.6.3.2.2 Egg custard as initial feed

In absence of suitable dry crumble feed for rainbow trout during larval stage, most suitable starter diet could be the egg yolk or egg custard, followed by buff liver. Since, buff liver contains required protein and several other vitamins and minerals; probably it is the best feed till the development of appropriate dry starter feed in Nepal. Although relative performance is poor, the use of egg custard, buff liver, and several other formulations evaluated have shown their suitability for rearing rainbow trout larvae. Rainbow trout larvae, fed upon egg custard and fresh buff liver, grew steadily at specific growth rate of 4.2% with survivability over 99% (Pradhan, 1999). Encouraging results have also been obtained on survivability (> 70%) and specific growth rate (> 3%) of rainbow trout larvae fed upon shrimp meals and milk powder based feed formulations (ATC, 2000b). Results of these experiments have implications that egg custard and buff liver have been recommended as starter feed for rainbow trout farms in the country as they are locally available at relatively cheaper price.

#### 2.6.3.2.3 Buff liver as initial feed

Frozen buff liver was supplied to the fry as supplementary diet at the rate of 2% of body weight two times per day. A study was carried out in nine circular cemented tanks (each 1.4 $m^3$) for a period of sixty days in 1998 (Pradhan, 1999). Three treatments, each with three replicas, were carried out for feeding early larvae of rainbow trout. The three different types of feed were Japanese starter feed (control), frozen buff liver and boiled egg yolk. All tanks were stocked with 525 rainbow trout larvae of 0.35 g individual average weight. In the tanks, water depths were maintained up to 30 cm. The volume of circular tank up to water level was 0.53 $m^3$. In each replica, water flow was maintained at the rate of 0.42 L $sec^{-1}$. Dissolved oxygen, pH, water temperature and mortality were measured every day. The highest growth (0.096 g $day^{-1}$) occurred for rainbow trout fed on Japanese starter feed followed by buff liver (0.066 g $day^{-1}$) and egg yolk (0.06 g $day^{-1}$). Mean individual body weight and total length at the end of experiment were not significantly different among and within the treatments. The condition factor for a normal rainbow trout was approximately 1, tending towards fatness. Computed values of condition factor for the experimental trout were in normal range.

Results indicated that young rainbow trout could efficiently use the above mentioned feed and can be reared with Japanese starter feed, frozen buff liver and egg yolk for their growth. This finding suggests that buff liver and egg yolk are highly palatable and prescribed to use as initial rainbow trout feed. This result supports the study by Brown (1951) who showed that fresh liver was the most satisfactory food than shrimp meal (two parts dried meal, 1 part Bemax and 1 part Farex) and living bloodworm, *Tubifex*. Based on these results, it can be concluded that fresh liver may be equally good as standard starter feed meeting all the nutritional requirements of young trout.

**2.6.3.2.4 Buff liver as fry feed**

Three different diets based on protein percentage were fed to rainbow trout fry for a month to determine whether the liver can be substituted the high grade feed requirement of trout (Igarashi and Roy, 1999). The tested diets were Japanese starter feed (control), raw frozen buff liver and boiled buff liver. The experiment was carried out in six circular plastic buckets (each 0.02 $m^3$) from 24 January to 23 February 1996, a period of 30 days. Each bucket was stocked with hundred FSFs of the size of

0.0994 g each. Weight gain was higher for rainbow trout fed with Japanese starter feed (0.0173 g $day^{-1}$) and raw buff liver (0.0167 g $day^{-1}$) than for rainbow trout fed with boiled buff liver (0.006 g $day^{-1}$). It is clear that the group provided with first two types of diets exhibited high survival, over 90%, while group fed with boiled buff liver had 70% survival. Total length at the end of the experiment was not significantly different in first two treatments. Comparatively, growth of individual fish in the third treatment was fairly lower than first two treatments.

#### 2.6.3.2.5 Earthworm as fry feed

Several studies reported that vermiculture has potential to supply earthworm as a substitute of shrimp meals for rainbow trout diet because of earthworm meal contains all essential amino acids required for rainbow trout feed (Ismail, 1997). Mitra (1997) reported that availability of methionine and lysine are recorded higher in earthworm than shrimp meals. Considering these facts, a comparative study was carried out on the effect of the growth of rainbow trout fed with diet contained 25% earthworm plus 75% farm feed only. Rainbow trout fed with farm diet showed better growth than rainbow trout fed with diet contained earthworm. The reason might be the earthworm powder was not accepted by rainbow trout due to earthworm's unfavourable smell though it contained high protein.

### 2.6.3.3 Prospect of feed formulation

Solvent extracted soybean meal (SESM) contains 45% protein and could be the predominant protein source in rainbow trout feed. It has best amino acid profile of all common plant protein sources and is highly palatable and digestible to rainbow trout. Rainbow trout is a predatory fish however, it may consume and assimilate plant protein but its intensity of growth on such food is much lower. This is partially explained by the fact that in the protein from plant sources, deficiency of important amino acids such as lysine and methionine usually occurs (Martyshev, 1983). The trout suffer from cataracts when given a diet deficient in methionine (Poston *et al*., 1977). This is likely to occur only when animal protein sources are completely replaced by plant protein (Medale *et al*., 1998) and in such case methionine supplement is necessary. It was reported that protein digestibility reduced in salmonids when diet contains high level of soybean due to its anti-trypsin factor. Anti-nutritional factors such as trypsin inhibitors are destroyed or reduced to insignificant levels ($< 0.32$ mg $g^{-1}$) with heat that is applied during the solvent extraction (Robinson and Li, 1996; Medale *et al*., 1998). The heat treatment also reduces phytic phosphorous contained in soybean and consequently increase the retention of crude phosphorous (Medale *et al*., 1998) and protein availability (Spinnelli *et al*., 1982). Levels of solvent extracted soybean meals up to 48% of the diet have been used for rainbow trout feeding in Nepal. Preliminary studies on rainbow trout feed development with plant protein sources conducted in the country (Roy *et al*., 1999; Yamada *et al*., 1999; ATC, 2003) and other rainbow trout farming countries (Tacon *et al*., 1983; Kausihk *et al.,* 1995; Medale *et al*., 1998) revealed that high level (up to 75%) inclusion of SESM is possible without imparting the growing environment and growth of rainbow trout.

Refinement of buff liver into micro-encapsulated feed and incorporation of nutrient supplement in egg custard is needed to improve the quality of these formulations as feeding in raw form, they would increase water turbidity (Pradhan, 1999) and bent caudal fin of rainbow trout (Igarashi and Roy, 1999), respectively. Poultry byproduct can be made up of ground, rendered or clean parts of the carcass (head, foot, underdeveloped egg and visceral organs) of slaughtered poultry, since growing poultry industry scattered throughout the country could be a major source of protein in rainbow trout feed in future. At present, it is seldom used in rainbow trout feeds because it is not available on a regular basis at a reasonable cost per unit of protein (Rai *et al*., 2005).

### 2.6.3.4 Guiding rules of feed formulation

All fish, including rainbow trout, require protein, lipid, carbohydrate, vitamin and mineral for normal growth and physiological function. Because nutrient contribution from natural food organism is considered to be minimal in rainbow trout farming, nutrient and energy are provided primarily by prepared feed. Feed formulation is a process in which feed ingredients and various vitamin and mineral supplements are blended to produce a diet with required quantities of essential nutrients. Rainbow trout have a definite qualitative and quantitative need for various nutrients, some of them highly interrelated. These can be supplied by a large number of natural feedstuffs in a compounded ratio however; no single feed ingredient consists of all nutrients that are needed in the correct proportion.

In order to increase the feed efficiency of rainbow trout production, both nutrition and feed cost must be taken into consideration. Supplying adequate nutrition for the trout involves the formulation of diet containing 40 essential nutrients and proper management of numerous factors relating to diet quality and intake. The bioavailability of nutrients, diet palatability, feed manufacture, storage, methods and chemical contamination may have propounded effects on fish performance (Lall, 1991).

Feed stuffs are of varied composition. Generally, the values given in composition tables are averages reflecting the concentration of nutrient most likely to be present. The protein content of grains may vary from batch to batch due to differences in soil fertility, time of harvest, genetic constitution, moisture content and several other factors. An inverse relationship between protein content and concentration of some essential amino acids occurs in cereal grains (Lall, 1991). Animal protein sources are also subject to variation as a result of nature of raw material from which they are processed. Severe heating during drying will lower digestibility and cause some loss of essential amino acids. Proximate analysis of major fish feed stuffs collected from all over the country revealed considerable variation in major nutrients. Hence, it is desirable to have each batch of feed ingredient analyzed for actual content prior to feed formulation (Roy, 2006).

Although nutritional consideration is of prime importance, non-nutritional factors often influence the composition of the final product. The logistics of procuring and storing feedstuffs and feed additives are primarily non-nutritional consideration. In general, feed ingredients must be available on a consistent basis, be easily handled in the manufacturing process, and be economical. These characteristics are the primary reason that soybean meal, wheat flour and rice bran have been the main feedstuffs, except fishmeal or shrimp meal, typically used in rainbow trout feeds in Nepal. Linseed and cottonseed meals are often priced economically and could be used in trout feed, but their use is limited not only because of nutritional deficiencies but also because they are not available in required quantity consistently (Rai *et al*., 2005).

#### 2.6.3.5 Ingredient of feed formulation

In order to develop efficient and economic feed formulation for rainbow trout, basic information is required on nutrient requirement of the rainbow trout cultivated and the chemical composition and organoleptic properties of feed ingredients in relation to their acceptability and the ability of the trout to digest and utilize nutrients from various sources. Although, knowledge on nutrient requirement for trout has been expanding in recent years (Lall, 1991 and Hasan, 2001), quantitative and qualitative information on practical feed stuff to supply required nutrients availability for rainbow trout should be listed. Amino acid profile and mineral composition of major fish feed ingredients (soybean, fishmeal and shrimp meal) should be given (Roy, 2006).

Rainbow trout require high protein containing diet which is obtained through fishmeal, shrimp meal, and soybean (ATC, 1999a). Other feed ingredients are wheat, rice bran, oilcake, mineral and vitamins (Rai *et al*., 2008). All these ingredients are included as protein supplements, energy supplements, and mineral and vitamins supplements.

#### 2.6.3.5.1 Protein supplement

Feedstuffs containing 20% crude protein (CP) or more are considered protein supplements. The protein supplements may be classified as animal and plant proteins. Full fat containing but roasted soybean (whole) and soybean meal constitute major component of protein supplement (20 to 45%) in rainbow trout feed in Nepal (Rai *et al*, 2002; ATC, 2002; ATC, 2003). The national production of soybean was 19820 metric tons in 2010 and most of the production came from hilly area of the country (CBS, 2017). Solvent extracted and extruded soybean called soybean meal (SESM) is particularly important for trout feed because of its high protein and low fat content. However, soybean processing plants are mostly located in Terai region with a production capacity of 750 metric tons of solvent extracted soybean per year (Roy, 2006).

##### 2.6.3.5.1.1 Plant source of protein for rainbow trout feed in Nepal

Several types of cake produced from oilseeds can be used in rainbow trout feed to substitute soybean meal. These include mustard oilcake, peanut cake, sunflower cake, sesame cake and linseed cake. The level of oilcake used in rainbow trout feeds is restricted to 5 to 10% because of amino acid deficiency, high level of fibre present and anti-nutritional factors they contain. Mustard oilcake is formed by compressing leftover after removing oil from mustard (whole). It contains harmful stearic acid as its principal fatty acid. The production of mustard oilcake from several extraction mills in the country estimated to be 175 metric tons year$^{-1}$ (Roy, 2006).

Peanut cake is obtained by removing oil either mechanically or by solvent extraction from de-hulled peanuts. Solvent extracted cake contains 48% protein and the mechanically extracted product contains 45% protein and crude fat below 1%. Peanut cake contains no known anti-nutritional factors but deficient in lysine. However, peanut cake is seldom used in trout feeds because of its sporadic availability. Sunflower cake, sesame cake and linseed cake are formed by extraction of oil from dry seeds. Production of these cakes are deficient in lysine and methionine (Robinson and Li, 1996), hence in their use in trout feed requires supplemental lysine and methionine.

Solvent-extracted cottonseed meal is obtained by grinding the cake remaining after the oil has been solvent extracted. The product generally contains 41% protein but must not contain less than 36% protein. It is deficient in lysine, and contains free gossypol and cylco-propenoic acids, which can be toxic. However, levels of these chemicals in commonly available cottonseed meals are generally well below toxic levels (Robinson and Li, 1996). The production of cotton seed in the country is limited to western terai and the quantity is insignificant to use in trout feed.

#### 2.6.3.5.1.2 Animal source of protein for rainbow trout feed in Nepal

Animal source of protein such as shrimp meals and fishmeal contribute in significant proportion (20 to 50%) to supply protein in trout feed (Gurung *et al*., 1994; ATC, 2001a; ATC, 2001b; ATC, 2002). Shrimp meals and fishmeal are prepared by cooking and drying of under-composed whole shrimp or fish or cutting parts of fish. These products are collected for several other purposes including human consumption in various forms. Nutritional values of these feed stuffs may not always consistent as the feed stuffs are comprised of varying size of shrimp and different species of trash fish (Roy, 2006).

Bone meal collected from slaughter houses or meat shop and later processed in several processing plants contains protein ranging from 24 to 29% (Roy, 2006). Its protein quality is inferior to white meat, i.e., fishmeal because it contains less lysine and the quality of product may vary considerably (Robinson and Li, 1996). It could be a good source of minerals and inorganic phosphorous. However, its high ash content (5.6 to 5.9%) may limit its use because of possible mineral imbalance. Blood meal is prepared by heating and grinding clotted animal blood (buffalo, goat and so on). It contains 80 to 86% crude protein and is an excellent source of lysine but deficient in methionine (Robinson and Li, 1996). However, its availability in quantity is limited to urban areas of the country only.

Buff liver is one of the most important sources of animal protein. Despite of low protein content and limited availability, moist or dry buff liver has proven to be good source of digestible protein for early stages of trout grow out (Pradhan, 1999). Silkworm pupae and silkworm moths are available in good quantity but their performance has not yet been evaluated in rainbow trout feed in Nepal. Similarly, synthetic amino acids are available, although less, but their performance also has not been evaluated in trout feed in the country.

#### 2.6.3.5.2 Energy supplement

Energy supplements are feedstuffs that contain less than 20% crude protein. These include grain, byproducts of grain and animal fat or vegetable oil. Wheat flour and rice bran are major sources of energy and have been used in rainbow trout feed up to 40% of the feed without adverse effects. The availability of these ingredients and other sources of carbohydrates such as corn and millets are abundant and locally available in rainbow trout growing areas at comparatively cheaper rate (Rai *et al.*, 2005).

Wheat is a good source of energy for rainbow trout. Depending on cost, wheat flour are used to levels up to about 25% in trout feed. Wheat is produced all over the country and the national production in the year 2010 was 1.557 million metric tons (CBS, 2017). Flour mills in terai region of the country have a capacity of 4,000 metric tons of wheat flour to process annually (Roy, 2006). In humid areas, using levels greater than 25% may cause the feed to become sticky resulting in clumping of feed pellets and handling problems. Low levels (< 15%) are often used to improve pellet binding. Wheat flour has digestible energy value of about 1588 Kcal $kg^{-1}$ for rainbow trout (ATC, 1997).

Corn grain and corn screening can be used interchangeably in rainbow trout feed as a relatively inexpensive source of energy. Corn grain (whole corn) is grounded prior to use. Corn screenings is obtained in the cleaning of corn and includes light and broken corn grains. Cooking improves energy digestibility of corn for the trout. Corn grain is produced all over the country and the national production in the year 2010 was 1.855 million metric tons. Quality Protein Maize, rich in lysine and tryptophan, recently introduced in Nepal for farming (ATC, 1999b) could be a good source of carbohydrate and amino acids for rainbow trout feed in future.

Rice bran is the bran layer and germ of rice grain with hulls or broken rice at the low level only that is unavoidable in milling rice grain. Defatted rice bran pellet is also produced into cake in terai by extracting fat from raw rice bran with organic solvent.

Although national rice production (4.81992 million metric tons in 2010) favours the use of rice bran, high fat and fibre content limits its use in the rainbow trout feed. However, rice bran has been used in the trout feed at levels of 15 to 25% in the trout farms of the country (Roy *et al*., 1999).

Animal and plant fats and oil are highly concentrated sources of energy as well as source of essential fatty acids. The use of soybean oil in the rainbow trout feed at level 5 to 10% has been tested on experimental basis in the country (Yamada *et al*., 1999). Animal fats such a buff fat and poultry fat available in the country could be used in the trout feed; however, warrants research on extent of their utilization in the trout feed. However, rainbow trout growth with 18.5% fat in feed was faster and dynamic (Parova and Rehulka, 1997).

#### 2.6.3.5.3 Vitamin and mineral supplement

Vitamin and minerals are added from outside and hence, called additives. Vitamin and mineral premixes (1 to 2% of dry feed) are generally added to rainbow trout feed. Generally, mineral premixes include only macronutrients and vitamin premixes supplied are under requirement (ATC, 2002; ATC, 2003). Usually, vitamin and mineral premixes are commercial formulation to meet the requirement like that of poultry industry. In the rainbow trout feed in Nepal, mineral premixes added are Technovet-M, vitamin premixes added are Technovet-F and vitamin-C added is Technovet-F.

### 2.6.3.6 Feed processing for rainbow trout

Rainbow trout feed processing involves grinding, mixing, agglomerating, heating, drying, screening, etc. Thus, the value of certain feedstuffs or feed additives may be lowered during feed processing. The variation in nutrient composition between formulated feed and the processed final product is not uncommon in the trout farms of the country and rainbow trout fed upon these gave erroneous result (Roy, 2006). However, the overall process should result in a final product of proper form that meets nutrient specification. The manufacturing process may also improve digestibility, inactivate certain undesirable substances present in feedstuffs, reduce the occurrence of moulds and bacteria, and improve palatability. Nutritional and non-nutritional factors must be considered in manufacturing trout feeds as well as feed manufacturing practices and quality control. Several forms of dry and moist trout feeds are produced locally, for small scale farm use in Nepal.

### 2.6.3.7 Feed preparation and manufacturing

Rainbow trout feed preparation and manufacturing involve crumble and pellet making. When formulating trout feeds, the feed manufacturing process must be considered because there is an interrelationship between feed formulation and feed manufacturing. For example, at least 25% of the feed be composed of grains or grains milling byproducts for proper gelatinization and expansion necessary for good pellet stability and float. This is generally not a problem, but the type and amount of grain or grain milling byproducts that are used may be affected by humidity in the air (Robison and Li, 1996). High fat feedstuffs, such as rice bran, should generally be limited to 10 to 15 % of the feed because high levels of fat make the feed more difficult to pellet.

Another consideration during rainbow trout feed manufacture is that the condition of high temperature, pressure, and moisture encountered during pellet making destroy certain nutrients and improve the availability of others. Vitamins are particularly sensitive to destruction; thus feeds are normally over fortified with vitamins to account for losses during feed manufacture. Highly fibrous feedstuffs must be limited to rather low levels because high levels of fibre reduce pellet quality (Rai *et al*., 2005).

## 2.6.3.8 Artificial feed supply

When supplied with artificial feed containing below mentioned crude proteins percentage and based on percentage of their body weight, different stages of rainbow trout fingerlings with different weights can show the following results under following feeding rate and feeding frequency:

### 2.6.3.8.1 Fingerling (above 5 g)

Rainbow trout fingerlings with a size below 10 g, 30 g and above 30 g grow well when supplied with 45% crude proteinous diet respectively 7 to 8 times, 3 to 4 times and 2 to 3 times daily. The artificial feed is provided 5 to 6% of the bodyweight of fingerlings below 30 g and 1.5 to 2.0% of bodyweight of fingerlings above 30 g (ATC, 2008). If 5 to 10 g size fingerlings are stocked, the marketable size of 200 to 300 g can be achieved in 10 months (ATC, 2000b). Fingerlings below 50 g are fed 35% crude animal protein at the rate of 1.5 to 2.0% 2 to 3 times daily (Basnet *et al*., 2008).

### 2.6.3.8.2 Fingerling (above 50 g)

Fingerlings more than 50 g are fed 30% animal protein at the rate of 1.5 to 2.0% of their body weight 2 to 3 times daily (Basnet *et al*., 2008). Fingerlings, each of which were 3 months old, 12.5 cm long and 50 to 70 g weight, were stocked at the rate of 2500 fingerlings $m^{-2}$; fed 40% proteinous pellet feed (dried shrimp 35%, soybean 12%, bone meal 5%, wheat flour 14%, skimmed milk 7%, rice bran 6%, corn 6%, oil cake 2%, yeast 10%, minerals 1%, vitamins 1% and supplementary vitamin C extra 0.01%) at the rate of 5% of their live bodyweight 7 to 8 times daily initially, 5 to 6 times $day^{-1}$ later on and 3 to 4 times $day^{-1}$ during last; at water temperature 14 to 15

°C, dissolved oxygen 7 to 8 mg $L^{-1}$, pH 7.0 to 9.0, solar illumination 4 to 5 hours and water discharge 0.42 L $sec^{-1}$; revealed 85% survival. The growth was 0.85 g $day^{-1}$. Cannibalism was seen for times. Parasites were not noticed but diseases like fin-rot and tail-rot was noticed. After five months of rearing, above mentioned fingerlings were converted into fingerlings (below 150 g) and were ready to be transferred into raceway for table fish production (Basnet *et al*., 2008).

#### 2.6.3.8.3 Grow out (above 150 g)

Grow outs, each of which were 8 months old, 16.0 to 17.5 cm long and 100 to 150 g weight, were stocked at the rate of 1500 $m^{-2}$; fed 35% proteinous pellet feed (dried shrimp 30%, soybean 35%, wheat flour 17%, rice bran 10%, oil cake 6%, minerals 1%, vitamins 1% and supplementary Vitamin C extra 0.01%) at the rate of 2 to 3% of their live bodyweight, 1 to 2 times daily; at water temperature 10 to 20 °C, dissolved oxygen 7 to 8 mg $L^{-1}$, pH 7.5 to 9.5, solar illumination 4 to 5 hours and water discharge 0.5 L $sec^{-1}$; revealed 90% survival. The growth was 0.95 g $day^{-1}$. Cannibalism was seen at times. Parasites like *Dactylogyrus* sp. and diseases like fin-rot and tail-rot were seen simply. After seven months of intensive management, above mentioned fingerlings were converted into table fish (below 300 g) and were ready to be transferred into marketing raceway (Basnet *et al*., 2008).

#### 2.6.3.8.4 Table fish (above 300 g)

Table fish, each of which were 15 months old, 30 to 35 cm long and 300 to 500 g weight, were stocked at the rate of 200 to 250 $m^{-2}$; not fed (to check compensatory growth); at water temperature 8 to 9 °C, dissolved oxygen 8 to 9 mg $L^{-1}$, pH 7.5 to 8.5 and water discharge volume 1 L $sec^{-1}$; revealed 98% survival. Cannibalism was rare. Parasites and diseases were not seen. When these table fishes were kept hungry (some fishes were not kept hungry so as to compare the compensatory growth than that of hungry) for a week and then fed 35% proteinous pellet feed (dried shrimp 20%, soybean 35%, wheat flour 22%, rice bran 12%, oil cake 9%, minerals 1%, vitamins 1% and supplementary vitamin C extra 0.01%) at the rate of 1.0 to 1.5% of their live bodyweight once daily then the growth was found to be 1.25 g $day^{-1}$ in comparison to others (not kept hungry). Under favourable condition, rainbow trout can reach a marketable size of 200 to 300 g after 14 to 15 months, however, if 5 to 10 g size fingerlings are stocked, same marketable size of 200 to 300 g can be achieved in 10 months (ATC, 2000a).

#### 2.6.3.9 Artificial feed in the form of crumble and pellet feed

At present, some problems associated with the development of crumble and pellet feed for commercial rainbow trout production could be delineated as maintenance of quality and quantity feed. Locally available ingredients for artificial feed tend to vary in quality and quantity. Thus, sustainable development of rainbow trout feed might depend on inputs based on local circumstances and balance maximizing profitability with social and environmental costs. Hence, this research may confirm several specific issues in the field of rainbow trout nutrition and feed formulation that are critical for sustainable rainbow trout production in the country. These include nutrient requirements for rainbow trout, availability and cost of feed resources, options to develop cost effective artificial feed in local conditions, and maintenance of environmental quality and sustainability (Rai *et al*., 2005).

In many countries, high quality commercial and branded crumble feed for free swimming fries are available (Hinshaw, 1999). However, in Nepal, commercial diets for rainbow trout are not yet been available. Thus, to initiate sustainable rainbow trout farming methodology, use of local feeds becomes essential. Rainbow trout being carnivorous obtain limited amount of energy from fat and carbohydrates; thus, it needs diet rich in animal protein (Nomura, 1993; Hinshaw, 1999). Besides, the amount of water in raceway ponds and seed supply, the success of rainbow trout farming depends on the types of feed on which they are cultivated (Ghittino, 1972; Maruyama, 1983). Previously, Japanese starter feed was used as initial feed for early fry in Nepal. The dry feed obtained from abroad is not only expensive but always bears risk upon unavailability. In previous years, before the methodologies were available for pellet feed, rainbow trout were usually fed raw feed of animal origin (Bardach *et al*., 1972a; Sedgwick, 1985). Since, these methodologies could be still appropriate for far remote places where modern technologies would not be available.

In Fisheries Research Centre, Trishuli and six private rainbow trout farms at Nuwakot and Rasuwa districts, the free swimming fries were fed with dry starter crumble feed with 45% crude protein (CP). In all the cases, source of animal protein was shrimp meals (47% CP). The feed was supplied till satiation 7 to 8 times $day^{-1}$. However, the preparation of composite artificial feed is a complex process. In previous years, in the absence of appropriate modern technologies for the preparation of dried crumble feeds, several local methods were tried in Nepal. The previous investigations suggest that in the absence of well-balanced dry starter feed, boiled egg yolk and buff liver was the appropriate feed for larval rearing as starter feed (Pradhan *et al*., 2008a). In Fisheries Research Division, Godawari, after yolk sac absorption, the free swimming fries are fed only with boiled egg yolk at the rate of 15% of the bodyweight with feeding density of 12 times at every hour from morning till evening for seven days. After seven days, crumble feed (crude protein 35%) is supplied (Nepal *et al*., 2002; Rai *et al*., 2005).

### 2.6.3.10 Artificial feed and breeding performance of rainbow trout

The artificial feed of rainbow trout influence fecundity because shortage of quality feed without sufficient amount of animal protein (shrimp meals and fish meal) reduces fecundity (Huet, 1975) whereas quality feed by promoting brood management produces quality seed and table fish. It is also advisable that brood maintenance and feeding management are important factors in rainbow trout culture for successful breeding, obtaining quality seed in quantity and ultimately sac fry, free swimming fry, fry, fingerling and table fish production (Basnet *et al*. 2008).

### 2.6.3.11 Artificial feed and disease prevalence in rainbow trout

Rainbow trout is susceptible to diseases that originate from contaminated water and leftover decayed feed. The contaminated water may stimulate bacteria, protozoa, and cytozoic parasites for infection, that is, *Eimeria* sp. while leftover decayed feed may trigger hepatomas and several other diseases. These harmful diseases, if not controlled in time, may cause severe economic losses to farmers so far production is concerned. The low occurrence of disease may be due to low intensity of rainbow trout in raceway ponds (Raymajhi and Dhital, 2008).

Post-hatchlings, each of which were seven days old, 1.2 cm long and 0.05 g weight, were stocked @ 5000 $m^{-2}$ and fed 45% proteinous dough feed (small dough balls with a composition of shrimp meals 40.4%, roasted soybean 40.4%, wheat flour 10%, boiled egg yolk 5%, mustard oil cake 2.2%, minerals 1% and vitamins 1%) at the rate of 10% of their body weight every hour; at water temperature of 10 to 15 $^{\circ}C$, dissolved oxygen 6.5 to 8.5 mg $L^{-1}$, pH 7.5 to 8.5 and water discharge 0.42 L $sec^{-1}$; revealing 80% survivability. The growth was 0.08 g $day^{-1}$. Cannibalism was not noticed. Parasites like *Trichodina* sp. were seen but there was no sign of any disease.

After three months of feeding of artificial feed, free swimming fries were converted into fingerlings (below 5 g) and were ready to be transferred into rearing raceway.

### 2.6.3.12 Survival and growth of free swimming fry, fry and fingerling due to artificial feed

Rainbow trout survival and growth is totally dependent upon quality and quantity of artificial feed fed to them (Swar, 2007 and 2008). The free swimming fries of rainbow trout start exogenous feeding when their yolk-sacs are completely absorbed (Pradhan, 1999; Pradhan *et al.*, 2008a). During exogenous feeding, naturally hatched free swimming fries feed on natural feed in natural habitat. However, hatchery-grown free swimming fries are exclusively dependent on artificial feed from the time they commence first feeding for their survival and growth (Bardach *et al.*, 1972a and 1972b). So, their rearing highly depends on quality feed for development, survival and growth and ultimately for their production. Thus, an increase in rainbow trout production requires corresponding increases in nutrition, feeding, and feedstuffs through artificial feed (Boujard *et al.*, 2002). It grows to a commercial size of 200 to 300 g in 14 to 20 months because its growth depends on quality and quantity of artificial feed (Swar, 2008).

In fishes, generally the growth pattern follows the Cube's law. Growth of a fish is of vital importance for the confirmation of yield (Brody, 1945; Lagler, 1972; Begenal and Tesch, 1978). Therefore, a high production of 200 kg table fish $m^{-2}$ is possible in raceway pond by feeding them artificial feed (Santhanam *et al.*, 1999). 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 (Prasad *et al.*, 2008). 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 *et al.*, 2007) in Nepal.

The requirements of proteins have been studied in rainbow trout for several years. Most of the studies of protein requirements of rainbow trout have been based on weight gain and feed efficiency. Data from these studies indicate that dietary protein requirement of rainbow trout range from 30 to 35%. Recent studies have indicated that protein level as low as 30% may be adequate for grow out when they are fed full artificial feed during growing season. The reason behind these studies is that optimum dietary protein level is driven by economics as much as rate of gain and survivability. Thus, to maximize profit, the optimum dietary protein level should be changed as stages of rainbow trout change, thus changing the prices of artificial feed.

Given that artificial feed is the biggest source of nutrient loading in rainbow trout production from extensive to semi-intensive to intensive system, clear understanding of its impact is essential for sustainable development. This will help reduce negative impacts and improve predictability of environmental effects. With respect to water quality, total substitution of fishmeal or shrimp meals by soybean meals cannot be recommended. When rainbow trout are fed a ration containing soybean concentrate as the sole protein source, then it albeit an increased retention of crude phosphorus but induced an increase in ammonia excretion. However, up to 75% of fishmeal can be replaced by soybean meals in solvent extracted or concentrate form without impairing total feed intake, growth, and artificial feed utilization; and thus should have a positive effect on the environment.

Major protein source at low protein profile is raw buff liver (30 to 50%) for juvenile and shrimp meals (20 to 25%) and soybean meals (18 to 42%) for advanced fry and grow out. Response studies have been conducted on various proportions of shrimp meals and soybean meals in rainbow trout artificial feed for corresponding increase of dietary protein percentage and their effect on survival and growth of different stages of rainbow trout. Significant differences ($P > 0.05$) were not observed among types of artificial feed on survival and growth attributes of fry and fingerling stages of rainbow trout, although an improved survival and growth was evident for such rainbow trout fed upon an artificial feed containing 45% crude protein that is comprised of 35% shrimp meals and 48% soybean meals. Similarly, no correlation existed between survival and growth of grow out fed upon rations comprised of different proportion of shrimp meals and soybean meals with corresponding increase in dietary proteins. Proximate analysis of rainbow trout flesh fed upon these artificial feeds revealed a narrow range of crude protein (19.3 to 20.4%) and crude fat (3.1 to 4.1%) and these differences were not significant ($P > 0.05$). It is apparent that a feed formulation containing 30 to 35% protein supplemented with 20 to 30% shrimp meals and 18 to 32% soybean meals may be appropriate for fingerling and grow out.

The growth of rainbow trout depended on the condition of habitat and quality and quantity of artificial feed because when fed with 35% proteinous feed, the highest growth rate was obtained during July when water temperature was maintained between 17 to 19 $^{\circ}$C. The growth was best (0.96 g day$^{-1}$) due to buff liver, moderate (0.66 g day$^{-1}$) due to buff liver plus fishmeal and least (0.60 g day$^{-1}$) due to egg-custard. The growth rate was calculated maximum (1.8 g day$^{-1}$) in March, minimum (0.68 g day$^{-1}$) in September and October, and average (1.24 g day$^{-1}$) in other months when 5 g weighted rainbow trout reached a weight of 258 g in 180 days.

One of the major challenges in fish nutrition is to decrease the dietary amount of shrimp meals without impairment of production and water quality. One of the most promising alternatives to shrimp meals seems to be soybean which is rich in protein and generally low in phosphorus. Earlier studies with rainbow trout in Nepal led to the conclusion that soybean protein could be a partial alternative to shrimp meals.

There is also evidence that the total replacement of shrimp meals by soybean protein concentrate is without negative effect on growth performance and flesh quality of rainbow trout. However, studies conducted at Fisheries Research Centre, Trishuli have shown that 60% substitution of shrimp meals with soybean is possible because it eventually reduces artificial feed cost by about 40% without affecting growth and feed efficiency ($P > 0.05$) of grow out. Inclusion of shrimp meals below 20% in carbohydrate enriched feed without soybean substitution, albeit low artificial feed cost by 10%, the growth rate day$^{-1}$ is lower than 0.35% to that of artificial feed containing 55% soybean meals.

Results from several experiments (Roy *et al.,* 1999*)* concluded the inclusion of soybean oil in feed enhancing the feed efficiency, survival and growth of rainbow trout. This is more important to supply energy especially when the supply of shrimp meals in feed is decreased (Yamada and Roy, 1997). Low protein and high calorie feed for rainbow trout relying on animal protein has been reported for increased survivability and growth (Takeuchi *et al*., 1978). The use of oil in feed of carnivorous fish could economize 10% of shrimp meals (Yamada *et al*., 1999) and provide concentrated source of energy which may virtually increase the protein sparing effect.

Two feeding trials were conducted at Fisheries Research Division, Godawari (FRDG) in 1993 (Gurung and Tamang, 1993) to examine the effectiveness of various local feeds so as to find out simple formulation, if crumble feed is not available. The fries of 0.92 to 0.11 g in first trial and 0.23 to 0.35 g in second trial were reared with two types of feed for thirty days. The two types of diets were Trishuli farm made crumble feed (crude protein 32.59%, crude fat 6.38%, ash 16.23%, and moisture 6.48%) and boiled egg yolk (crude protein 12 % and crude fat 12%). In both the trials, survivability and growth were higher in group fed with crumble feed than with boiled egg yolk. The growth of group fed with crumble feed was higher (1.86 g $day^{-1}$) than fish fed with egg yolk (1.415 g $day^{-1}$). But there was no significant difference ($P > 0.05$) on survivability and growth between the treatments.

Subba *et al*. (2009) studied length-weight and length-length relationship of a freshwater fish, *Gadusia godanahiae* for the study of its growth in natural feed. Franco *et al*. (2015) included sea cucumber meal in the artificial diet of rainbow trout.

### 2.6.3.13 Feed efficiency indicator

The growth of rainbow trout can be measured through length and weight with the help of total feed intake (TFI) and total protein intake (TPI). The growth measurement is further assessed with the help of feed efficiency indicators also called growth parameters. The feed efficiency indicators are feed efficiency (FE), protein efficiency ratio (PER), absolute growth rate (AGR), specific growth rate (SGR), relative growth rate (RGR), condition factor (*K*), feed conversion ratio (FCR) and protein productive value (PPV).

Different-sized rainbow trout should be graded timely to attain uniform growth and to avoid competition and cannibalism. The size category for separation is 2 to 5 g, 10 to 20 g, 50 to 60 g, 60 to 100 g and >100 g. Such grading management helps improve feed conversion ratio (FCR) thus decreasing production cost. Shrestha *et al*. (2007) collected data of rainbow trout production from nine farmers of Nuwakot and Rasuwa districts where feed conversion ratio was 2 : 1 with newly formulated feed. The feed conversion ratio, in Nepal is 2 (Basnet *et al*., 2008 and Rai *et al*., 2008), that is, FCR, in the country is 2 : 1, which means 2 kg artificial feed is required to produce 1 kg of rainbow trout. Similar results were obtained in rainbow trout farming countries (Bromage and Shepherd, 1990; GLT, 1998).

The cost of rainbow trout production has been analyzed which shows that for 1 kg of marketable size (containing 200 to 300 g sized table fish) about Nepalese Rupees

(NRs) 170 is required (Joshi and Westlund, 1996). The longer cultivation period of rainbow trout after 200 to 300 g results in the increase of production cost (PC) which is calculated from the feed cost (FC) from both of which cost analyses (CA) is done. Hence, by analyzing highest growth period (HGP), the period of cultivation is fixed. Therefore, it is advisable to harvest the table fish after attaining the size of 200 to 300 g to get maximum profit. The specific growth rate (SGR) decreases with increasing size of rainbow trout (Basnet *et al*., 2008; Rai *et al*., 2008).

The feed efficiency (FE) of rainbow trout in Japan ranges from 60-80% (Tasiro *et al*., 1974) but it has been found lower from 43 to 46% in Nepal (ATC, 2000a). This suggests that there is still need for research in feed efficiency improvement of rainbow trout (Nepal *et al*., 2002; Rai *et al*., 2005).

Klontz (1991) studied growth of rainbow trout by the help of weight gain through condition factor (*K*) and feed conversion ratio (FCR). Akbulut *et al*. (2002) compared survival and growth of three weight groups of the rainbow trout through daily feeding rate (DFR) with the help of mortality count and weight gain. The growth was further studied through feed efficiency parameters of specific growth rate (SGR), condition factor (*K*) and feed conversion ratio (FCR). Clark (2003) evaluated survival and growth of rainbow trout in aerated and oxygenated raceway ponds by mortality count and weight gain respectively. The growth was justified through growth parameters like feed conversion ratio (FCR) and carrying capacity. Yildiz (2004) studied growth of rainbow trout feeding Vitamin E by weight gain through specific growth rate (SGR), condition factor (*K*) and feed conversion ratio (FCR). Furuya *et al*. (2004) studied replacement of fishmeal (FM) by soybean meal (SBM) in the diet of tilapia. Bulut *et al*. (2009) compared growth of rainbow trout due to soybean meal (SBM) with that of hazelnut meal (HNM) with the help of weight gain through specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU).

Subba and Gubhaju (2011) compared survival and growth of rainbow trout larvae in four types of formulated diets by the help of mortality count and growth in length and weight. The growth was studied through length and weight correlation as well as feed efficiency indicators of specific growth rate (SGR) and feed conversion ratio (FCR). Gumus and Aydin (2013) compared survival and growth of rainbow trout by fishmeal diet (FM) with that of poultry by-product meal diet (PBM) and soybean meal diet (SBM) through feed intake (FI) with the help of mortality count and growth in weight. The growth was studied through feed efficiency indicators of specific growth rate (SGR), feed conversion ratio (FCR), condition factor (*K*), hepato-somatic index (HSI) and visceral-somatic index (VSI). Wang *et al*. (2015) compared survival and growth of diploid and triploid masu salmon (*Oncorhynchus masou*) through mortality and feed efficiency indicators like AGR, SGR, RGR, *K* and FCR respectively. Dogan and Bircan (2015) studied the growth of rainbow trout with the help of hazelnut meal (HNM) with synthetic amino acids of lysine and methionine through specific growth rate (SGR), feed conversion rate (FCR), protein efficiency ratio (PER) and protein productive value (PPV). Bhagat and Barat (2016a and 2017a) evaluated survival and growth of rainbow trout due to four formulated diets through total feed intake (TFI) and total protein intake (TPI) by the help of mortality count and growth in length and weight. The growth in weight was further justified by feed efficiency parameters of feed efficiency (FE), protein efficiency rate (PER), absolute growth rate (AGR), specific growth rate (SGR), relative growth rate (RGR), condition factor (*K*), feed conversion rate (FCR) and protein productive value (PPV). The cost analyses (CA) was done along with the study of highest growth period (HGP).