TRAINING MANUAL

NFDB SPONSORED TRAINING OF TRAINERS PROGRAMME

ON

BREEDING, SEED PRODUCTION AND HEALTH MANAGEMENT OF RAINBOW TROUT

(*Oncorhynchus mykiss*)

4th March to 8th March 2019

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Sponsored By

National Fisheries Development Board

Ministry of Agriculture & Farmer's Welfare

Department of Animal Husbandry

Dairying & Fisheries, Hyderabad-500 052

Published by

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RAINBOW TROUT: AN INTRODUCTION

About the species

Rainbow trout (*Oncorhynchus mykiss*) is one of the promising cultivable fish species in coldwater and has considerable scope for its expansion. Being a low volume high value commodity, the trout has good potential for domestic consumptions as well as foreign export. In spite of having excellent positive traits, the development and expansion of trout farming has yet to be done on large scale. Trout culture is intensive type of farming which requires more input resources compared to other species for survival and growth. The feasibility of achieving required production naturally depends on a number of factors including seed, feed, health management and environmental consideration. Potential success in trout production requires better governance and significant improvement in the management practices.

Rainbow Trout

Morphology

Certain features such as base of anal fin shorter than its height, moderate sized scales and dark-colored spots on the body distinguish this species from the other salmonids such as salmons and chars. Elongated fusiform body with 60-66 vertebrae, 3-4 dorsal spines,10-12 dorsal soft rays, 3-4 anal spines, 8-12 anal soft rays, 19 caudal rays, presence of adipose fin, absence of nuptial tubercles and body coloration of blue to olive green above a pink band along the lateral line are identifying characteristics of this species.

Distribution

Rainbow trout is native to the Pacific drainages of North America ranging from Alaska to Mexico. However, it is the world's most widely introduced fish species which is cultured over 65 countries including India. Trout farming in India is over a century old and there are several species of trout but rainbow trout is a species of choice. Rainbow trout is relatively easy to and has better growth and maximum cultivable traits. They thrive in water originating from aquifers, springs, and streams as well as in lakes. (Joshi *et al.,* 2005).

Introduction in India

The species was transplanted from Europe by British settlers around the beginning of the last century primarily to meet their needs for sport fishing or recreational angling. Since early-nineties the country has taken-up farming of rainbow trout in big way in and the fish is now available for food and farming. (Pandey and Ali, 2015)

Rainbow trout introduction in India

	State	Year of introduction	Status
1.	J &K	1904, 1986	Established
2.	Himachal Pradesh	1919, 1992	Established
3.	Uttarakhand	1976	Established
4.	Tamil Nadu	1909,1920,1968	Established
5.	Kerala	1941	Established

Trout production in India

Trout farming has progressed steadily during last decade in India and the total trout production in the country elevated from 147 tonnes during 2004-05 to 842 tonnes during 2015 - 2016 which is over five-fold. Jammu & Kashmir, Himachal Pradesh and of late Sikkim are leading states where trout farming is undertaken in both private & public sectors. (Singh *et al.*, 2017)The production in India has increased remarkably from 755.27 (2014 - 15) to 842.23 tonnes (2015 - 16), with a growth rate of 11.51 percent. The trout production of J&K was 265 tonnes; Himachal 417 tonnes; Sikkim 120 tonnes while other states including Uttarakhand and Arunachal Pradesh was 40 tonnes during the current year 2015 – 16. The increase in total production has a significant contribution from the private sector mainly from Himachal Pradesh and Jammu and Kashmir. However, Sikkim, a north-eastern state has shown significant increase in trout production in recent years while other states such as Uttarakhand, Arunachal Pradesh and states of southern India contribute a meagre production. (Singh et al., 2017)

State wise rainbow trout production in India

The trout production infrastructure has also developed steadily in the country in last 30 years and presently around 62 trout farms (under Government sector) with 1241 numbers of raceways are spread over seven states in the western, north-eastern and peninsular region of the country.(Singh et al., 2017) A total of 32 trout hatcheries with an estimated eyed ova production capacity of 13 million are present in the country which have been mostly established by the various state governments. These hatcheries or seed production units cater the need of farmers, private entrepreneurs and also supply to the different government agencies for building their stocks. Moreover, an estimated1181 numbers of trout units in the private sector have been established in the country mostly concentrated in the Himachal Pradesh and Jammu and Kashmir and Sikkim states.(Pandey and Ali, 2015)

Status of trout infrastructure in India

Sr. No.	**State**	**Infrastructure**			
		No. of Govt. farms	**Hatcheries**	**Feed mills**	**Culture status**
1.	Jammu & Kashmir	42	14	2	Govt. & private sector
2.	Himachal Pradesh	5	5	1	Govt. & private sector
3.	Uttarakhand	4	3	1	Govt. & private sector
4.	Sikkim	5	5	Nil	Govt. & private sector
5.	Arunachal Pradesh	3	2	Nil	Govt. farm only
6.	Tamil Nadu	1	1	Nil	Govt. farm only
7.	Kerala	2	2	Nil	Farms (Private sector)

The average fish farmers are not well aware about the technique of trout farming and their present practice is based on the indigenous knowledge and on partially adopted technology. Being different agro-climatic conditions and resources in these states the similar farming technique is not suitable to all hill states. During the past three decades, the ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, in partnership with fisheries departments of different states has made concerted research and development efforts to popularize trout production in hill states and to refine location specific farming practices (Pandey and Ali, 2015).A detailed survey was conducted for the identification of the constraints in the culture of rainbow trout, its breeding and other related intercultural operation. This is a basic data base to understand the constraints in the expansion of trout culture as well as identification of potential areas for the introduction of trout farming. However, there is ample scope for further enhancement of trout production in hill states through participatory approach. Potential success in trout production requires better governance and significant improvement in the management practices through adequate R & D support. In order to achieve the goal of enhancing trout production, it is quite necessary to focus on improving existing technologies or developing new ones for increased and sustained production. (Pandey and Ali, 2015).

Status and Production trend

Coldwater states have an inherent potential for trout culture due to its suitable climatic conditions like cool and well oxygenated water *etc.* Perennial cold water resources are available in these states for local people to adopt trout culture as sustainable livelihood option in hills. (Singh et al., 2017)

North-Western Himalayan Region

North-Western regions of India have progressed in rainbow trout farming due to most suitable climatic condition and topography for trout farming. Jammu and Kashmir and Himachal Pradesh are two important states where rainbow trout farming has progressed on large scale. Since the introduction of rainbow trout in India, Jammu and Kashmir remained the forerunner in trout production. The trout fish farming project with the assistance of European Economic Community (EEC) in the year 1984 at Kokernag in Kashmir, has tremendously helped in developing trout farming in the state. Presently under the state government around 42 trout rearing units have been established in the state, besides 14 hatcheries (8 operational and 6 under construction) for seed production which is spread over three main provinces *viz.* Jammu, Kashmir and Leh & Laddakh. The majority of trout farms (around 28) are situated in the Kashmir division. The state fisheries department has consistently worked for the development of trout farming and production enhancement and also established excellent infrastructure for trout grow-out as well as seed production (Hassan and Pandey, 2012).At Kokernag trout farm a feed mill of 0.5t/hr capacity has been installed to fulfil the feed requirement of the government farms as well as private farmers' field.

Himachal Pradesh is one of the leading states in rainbow trout farming and seed production in India. With the assistance of Norwegian Government during 1989-91, trout infrastructure has been developed in the state for rainbow trout production. Introduction of the improved strain of rainbow trout resulted in increased trout production in the state. The state fisheries department own five trout farms and seed production unit in the state. The largest one is located in Patlikuhal in Kullu district. The rainbow trout production in the state has increased from 17 tonnes (2004-05) to 417.23 tonnes (2015-16). The state fisheries department has also installed a feed mill having 0.3t/day capacity for the preparation of different types of feed which caters the need of government and private farmers. (Pandey and Ali., 2015)

North-Eastern Himalayan Region

North-Eastern Himalayan states such as Sikkim and Arunachal Pradesh also have trout production infrastructure as well as trout ova production units in the state.

Sikkim has considerable water resources suitable for rainbow trout farming. The production of rainbow trout during 2010-11 was around 53.4 ton which has significantly increased up to 120tonnes during 2015-16. State Fisheries Department of Govt. of Sikkim is associated with Directorate of Coldwater Fisheries Research, Bhimtal under the project entitled "Sustainable Utilization of Mountain Fishery Resources: A Partnership Mode. The Directorate has taken all possible initiatives to promote trout culture in the state considering the suitable climatic condition and abundance of water. As desired to the state, programme on broodstock management and seed production have taken on the priority basis since last four years. Broodstocks of the trout were maintained at state trout farm Uttarey and Yuksom with technical guidance of the Directorate.

Although rainbow trout production is very low in the state but recently many farmers and entrepreneur have adopted trout farming and thus contributing to the total trout production. Presently three trout breeding units are functional and producing trout seed for stocking in various government and private farms.

In the state of Arunachal Pradesh trout broodstock and seed production is being done in two main hatcheries situated at Shergaon of West Kameng and Nuranang in Tawang district. Shergaon has ova production capacity of 50,000. However, rainbow trout farming is yet to reach private farmers in the state. Eyed ova of trout were introduced directly in the stream around the Dargiling during 1966. These eggs were brought from Kashmir. This effort miserably remained unsuccessful. In spite of potential in Nagaland, progress is very poor for trout farming. There is only one private farm with a mini hatchery at Dzulakia in Kohima district. Some natural stock of trout was also reported in the Laangriver in Tuensang district.(Pandey and Ali., 2015)

Central Himalayas

In central Himalayas, Uttarakhand is one of the promising states where trout farming has good prospects. In this region, trout culture commenced with the transplantation of eyed-eggs from Kashmir to Talwari and Kaldayani hatcheries.The trout thrived in the hatcheries and some tributaries in the Garhwal but could not survive in Kumaun hills, due to comparatively high summer temperature. The two hatcheries were set up to provide stocking material for the rivers Pinder, Birehi Ganga and Asiganga which were kept in reserve to provide angling pleasure. However, presently only one trout farm and hatchery is functional and is producing around 0.3 million eyed ova and 0.2 million advanced fry annually. There are around 35 raceways under the state fisheries department of Uttarakhand. Recently a few private trout farmers have started farming through the initiative taken by ICAR-DCFR and state fisheries department. The ICAR-DCFR, Bhimtal has a farm facility at Champawat with ova house and raceways for rearing rainbow trout. This experimental farm is situated on the banks of holy stream Gandaki in 3.13 hactare land area (1620 m above MSL) in Kumaun region of Uttrakhand. The eyed-eggs of rainbow trout were procured from the department of fisheries, Govt. of Himachal Pradesh during

February 1999. Since then the stocks have been successfully raised through various stages of alevins, fry, fingerlings and table size fish. The annual water temperature at the farm ranged between a minimum of 4.5°C in January/ February to a maximum of 21.5°C in the months of May/June. Different farm trials indicate that with suitable modifications, it is possible to raise trout at marginally higher temperatures prevalent at lower altitudes. This farm also supply trout seed to different states and local trout growers.(Pandey and Ali., 2015)

Peninsular Plateau

In peninsular India, the very first attempt to introduce trout eggs and fry from New Zealand was made in 1863 by Sir Francis Day in Nilgiri, Tamilnadu and a hatchery was constructed in Avalanche in 1909-1910 (Jhingran and Sehgal ,1978; Gopalakrishnan *et al.*, 1999; Singh *et al.*, 2017)

In Tamil Nadu the main objective of trout fishery development in the Nilgiri water remained to promote recreational fishing such as angling and sports. Fry and fingerlings produced were mainly stocked in streams, lakes and reservoirs. The trout culture is completely under the domain of state fisheries department without any opening to private culturists. Total ova production was around 2 lakh (Pandey and Ali., 2015).

The Fisheries department Tamil Nadu took over the management of hatchery in 1958. In February 1968 fresh consignment of 15000 eyed ova of golden rainbow trout, 15000 of ordinary rainbow trout were imported from Nikko Laboratory Japan for stocking in Avalanche hatchery. In 1969, another batch of *O. nerka* was imported from Canada. Off this new release only golden strain of rainbow trout survived well and established itself as a dominant strain. Another 10000 eyed ova of rainbow trout were brought from Himachal Pradesh during 1999. The Palni Hills Game Association, which till 1965, controlled trout fishing in Kodai hills had stocked the Konalaar and Pollavachi streams with rainbow trout fingerlings brought from Munnar. From 1965 onwards Tamil Nadu Fisheries Department has been stocking the Kodaikanal lake with rainbow fry produced in the Avalanche hatchery. The average weight of rainbow trout in the Southern region ranged between 100-200 g.

In Kerala, effort for the development of trout culture in the Munnar hilly range commenced in the year 1909 with introduction of eyed ova from United Kingdom. A trout farm was established in 1941 Eravicolam followed by another at Rajamallai. The trout hatchery situated in Rajamallai is under the control of TATA Tea company with a capacity of 48000 eyed ova production. Rajamallai hatchery supplies stocking material for stocking in Mudupatty and Kundally reservoirs. Presently no private trout farming is in practice in the southern India. The main objective of trout culture in western ghat is to meet the requirement of sport and recreational fishery. (Pandey and Ali., 2015)

WATER SUPPLY AND WATER QUALITY MANAGEMENT IN RAINBOW TROUT FARMING

Water quality and water management ensure the optimal aquaculture production without impairing environment. To great extent water quality determines the success or failure of trout farming. Water quality is a combination of chemical, physical and biological parameters that affect the growth and prosperity of growing trout fish. The success of trout farming depends on the optimal environment conditions for accelerated growth at the lowest cost of resources. Water quality affects the general condition as it determines the conditions of health and growth of the trout. The water quality is therefore, an essential factor to consider when planning for rainbow trout farming. Although the environment of fish culture is a complex system, consisting of some water quality variables, only few of them play a crucial role. Trout farming is an intensive type of aquaculture practice and critical parameters are temperature, dissolved oxygen, ammonia and pH. However, water temperature is the most important parameter, requiring continuous monitoring in trout farming. The ideal water temperature for production of trout is one that does not rise too high beyond 18^0C nor fall too low (4^0C) in winter. The best possible water supply is one in which the temperature remains in the range of 10-15^0C for as long as possible (Boyd and Tucker, 1998). The temperature of water supply should never exceed 20^0C. There are cumulative effects of synergistic interactions between and among different variables, which influence the growth and survival of growing trout at any moment in rearing practice. For example, increasing water temperature directly affects to trout as well as reduces the dissolved oxygen content in pond water. This synergistic situation results in improper swimming and mortality. (Pandey and Ali. 2017)

Water supply by gravity to a trout farm is an economic method as it saves energy and, consequently, large amounts in terms of production costs. Water can be supplied either in parallel (separately) or in series raceways. In series connected raceways, water should be used first in raceway having younger trout, from where water flows into the raceway having trout of older age. Although arranging raceways in series is rather frequent, construction of parallel raceways with individual water supply is better practice. Construction of a water reservoir at the highest point of the trout farm facilitates easy and efficient water management. The elevated central water reservoir serves as a buffer, where water also settles. The water from the reservoir is channeled to the raceways through open canals or pipes. Recirculation system can also be used if all the important criteria have been met satisfactorily such as optimum water flow rate, dissolved oxygen, turbidity and other physico-chemical parameters. (FAO, 2011)

Trout culture requires a flow-through system. An abundant and continuous water supply is required to sustain this flow-through system. In a conventional flow-through system, the oxygen requirement of the fish is supplied by the inflow water. The quantity of water required in a trout farm depends on the age and actual quantity of the developing fish. The quantity of eggs, fry and growing fish per unit area of raceway is determined by the oxygen content of supplied water. In colder water, the metabolism and respiration remain slow and require comparatively less water quantity, while it remains more at higher temperature. Water supply is expressed by the flow rate, which is the quantity of water needed for 1000 specimens of eggs, fry or fish. It is expressed either in liters per second (LPS) or liters per minute (LPM). Water supply may also be expressed by the exchange rate of water per hour or day. To hold one ton of fish nearly 3-5 L/second (180-300LPM) of water flow is sufficient at an average temperature of 15^{0}C (Nepal et al., 2002). Some of the estimated water requirement for trout culture is 1.0 L/min/kg of trout without aeration or 0.3 L/min/kg of trout with aeration. The water supply in concrete raceways or lined tanks remains high than in earthen ponds, hence the density of fish can also be higher in concrete raceways. In earthen ponds, water can be exchanged a maximum of 4–5 times/day, but typically it is done only 1–2 times/day (FAO, 2011). In general, requirement of rainbow trout for the chemical composition of the water environment is summarized in table.

Water requirements in different life stages of trout

Stages/age in months	**Stocking density nos. per m^3**	**Water flow per m^3**		**Water flow in raceway (30 m^3)**
		at 5°C	**at 20°C**	**at 12-18°C**
Egg incubation/swim up fry	1000	0.5 LPM*	1.0 LPM	-
Fry (1-5g), 0.5-2 month	1000-2500	3-6 LPM	4-8 LPM	110-180 LPM
Fingerlings (5-25g), 2-4 months	100-250	3-8 LPM	5-11 LPM	120-270 LPM
Growing fish (25-250g), 4-10 months	60-100	3-6 LPM	5-8 LPM	120- 210 LPM
Table fish (250-350g) 10-12 months	50-60	2-3 LPM	3-5 LPM	90-120 LPM
Adult fish (>350g) 14 months	30-50	2-3 LPM	3-5 LPM	90-120 LPM

*LPM- Liters per minute

Temperature ranges and incubation period in rainbow trout

Stage	Incubation period (days) against different water Temperature Range in °C	
	$7.0\text{-}11.0^0C$	$11.0\text{-}13.0^0C$
Green egg to eyed ova stage	21-29 days	10-15days
Eyed-egg to alevin	20-27days	8-12days
Alevin to swim-up fry	10-12days	10-12days

Physico-chemical parameters for trout culture

Parameters	Range
Dissolved oxygen	near saturation (≥ 9 mg/l)
Temperature:	7-20 ºC
Transparency	1.5-1.8 m
Free CO_2	<1.5 mg/l
pH	7.0-7.5
Suspended solids	< 10 mg/l
Alkalinity (as CaCO3) Hardness	50-150 mg/l
Ammonia (NH3)	< 0.05 mg/l
Nitrites (NO2-)	< 0.05 mg/l
Nitrates (NO3-)	< 1.0 mg/l
Phosphates (PO4-)	< 0.03 mg/l

ESTIMATION OF IMPORTANT WATER QUALITY PARAMETERS

Water quality is a combination of chemical, physical and biological parameters that affect the growth and prosperity of growing trout fish. To great extent water quality determines the success or failure of trout farming. The success of trout farming depends on the optimal environment conditions for accelerated growth at the lowest cost of resources. The functioning of an aquatic ecosystems and its stability to support life forms depend to a great extent on the physico-chemical characteristics of water. Present chapter is focused on measurement of important water quality parameters for trout culture.

WATER TEMPERATURE

Water temperature is recorded with the help of an ordinary thermometer having range of 0-50°C, with mark up to 0.1°C.

Apparatus required: Thermometer- 0.1° C division.

Procedure:

Temperature measurement is made by taking a portion of the water sample (about 1litre) and immersing the thermometer into it for a sufficient period of time (till the reading stabilizes) and the reading is taken, expressed as °C.

pH

The effect of pH on the chemical and biological properties of liquids makes its determination very important. It is one of the most important parameter in water chemistry and is defined as -log $[H^+]$and measured as intensity of acidity or alkalinity on a scale ranging from 0-14. If free H^+ are more it is expressed acidic (i.e. pH<7), while more OH^- ions is expressed as alkaline (i.e. pH> 7).

In natural waters pH is governed by the equilibrium between carbon dioxide/bicarbonate/carbonate ions and ranges between 4.5 and 8.5 although mostly basic. It tends to increase during day largely due to the photosynthetic activity (consumption of carbon-di-oxide) and decreases during night due to respiratory activity. Waste water and polluted natural waters have pH values lower or higher than 7 based on the nature of the pollutant.

* The colorimetric indicator method can be used only for approximate pH values.

Apparatus required:

pH indicator (BDH) method: BDH Indicator (Universal Indicator) and test tubes.

Electrometric method: Glass electrode, reference electrode (mercury/calomel or silver/silver chloride) and pH meter.

Procedure

Colorimetric method

- ✓ About 10ml of the sample is taken in a wide mouth test tube, 0.2ml of BDH indicator is added, and shaken gently.
- ✓ The colour developed is matched with the chart and the pH noted.

Electrometric method

- ✓ The pH is determined by measuring the Electro Motive Force (E.M.F) of a cell comprising an indicator electrode (an electrode responsive to hydrogen ions such as a glass electrode) immersed in the test solution and the reference electrode (usually a mercury/calomel electrode).
- ✓ Contact between the test solution and the reference electrode is usually got by means of a liquid junction, which forms a part of reference electrode. E.M.F of this cell is measured with pH meter, that is a high impedance voltmeter calibrated in terms of pH.
- ✓ The electrode is allowed to stand for 2 minutes to stabilize before taking reading for reproducible results (at least ±0.1 pH units).

DISSOLVED OXYGEN (DO)

Oxygen dissolved in water is a very important parameter in water analysis as it serves as an indicator of the physical, chemical and biological activities of the water body. The two main sources of dissolved oxygen are diffusion of oxygen from the air and photosynthetic activity. Dissolved oxygen is calculated by many methods.

Winkler's method

Principle

Oxygen present in the sample oxidizes the dispersed divalent manganous hydroxide to the higher valency to precipitate as a brown hydrated oxide after addition of potassium iodide and sodium hydroxide. Upon acidification, manganese reverts to its divalent state and liberates iodine from potassium iodide, equivalent to the original dissolved oxygen content of the sample. The liberated iodine is titrated against N/40 sodium thiosulphate using fresh iodine as an indicator.

Procedure

- ✓ The samples are collected in BOD bottles, to which 1ml of manganous sulphate and 1ml of potassium iodide are added and sealed.
- ✓ This is mixed well and the precipitate allowed to settle down.
- ✓ At this stage 1ml of conc. sulphuric acid is added, and mixed well until all the precipitate dissolves.
- ✓ 50 ml of the sample is measured into the conical flask and titrated against N/40 sodium thiosulphate using starch as an indicator.

- ✓ The end point is the change of colour from blue to colourless.

Calculations:

$$\text{Dissolved oxygen (mg/l)} = \frac{\text{Volume of sodium thiosulphate consumed} \times 1000 \times 8}{\text{Volume of sample} \times 40}$$

FREE CARBON-DI-OXIDE (Free CO_2)

The important source of free carbon-di-oxide in surface water bodies is mainly from respiration and decomposition by aquatic organisms. It reacts with water partly to form calcium bicarbonate and in the absence of bicarbonates gets converted to carbonates releasing carbon-di-oxide.

Principle

Free carbon-di-oxide reacts with sodium carbonate or sodium hydroxide to form sodium bicarbonate. The completion of the reaction is indicated by the development of pink colour, characteristic of phenolphthalein indicator at an equivalent pH of 8.3

Apparatus required: Lab glassware - measuring jar, pipette, conical flask etc.

Reagents:

- ➢ Sodium hydroxide solution (N/44): 1g of sodium hydroxide was dissolved in 100ml of distilled water and made up to 1000ml to give N/44 NaOH.
- ➢ Phenolphthalein indicator

Procedure:

- ✓ A known volume (50ml) of the sample is measured into a conical flask.
- ✓ 2-3 drops of phenolphthalein indicator is added and titrated against N/44 sodium hydroxide till the pink colour persists indicating the end point.
- ✓ Record ml of N/44 NaOH used during the titration.

Calculation:

$$\text{Free Carbon- dioxide} = \frac{\text{Volume of N/44 NaOH consumed} \times 1000 \times 44}{\text{Volume of sample} \times 44}$$

TOTAL ALKALINITY

Alkalinity is assessed titrimetrically using phenolphthalein and methyl orange as indicators **(APHA, 1985).**

a. Phenolphthalein alkalinity

- ✓ 5-6 drops of phenolphthalein indicator solution is added into 50 ml water sample. If the sample remained colourless, phenolphthalein alkalinity is absent.
- ✓ The appearance of pink colour indicated the presence of phenolphthalein alkalinity.

- ✓ The sample is then titrated with 0.02 N H_2SO_4 solution to a colourless end point.

Calculation

Phenolphthalein alkalinity is calculated by using the following expression.

$$\text{Phenolphthalein alkalinity } (CaCO_3 \text{ mg/l}) = \frac{A \times N \times 50{,}000}{\text{ml sample}}$$

Where,

A = ml 0.02 N H_2SO_4 used for titration

N = normality of acid (H_2SO_4)

b. Methyl orange alkalinity

- ✓ Five to six drops of methyl orange indicator solution is added to 50 ml sample.
- ✓ The sample was titrated with standard 0.02 N sulphuric acid till yellow to faint orange colour.

Calculation :

$$\text{Methyl orange alkalinity as mg/l } CaCO_3 = \frac{B \times N \times 50{,}000}{\text{ml of sample}}$$

Where, B= ml titrant used for sample to develop pink colour

N = normality of acid (H_2SO_4)

Total alkalinity (mg/l) = Phenolphthalein alkalinity + Methyl orange alkalinity

BREEDING AND SEED PRODUCTION OF RAINBOW TROUT

Introduction

Among the freshwater salmonids, *Onchorhynchus mykiss* popularly known as rainbow trout is one of the promising cultivable fish species in coldwater and has considerable scope for its expansion in uplands region. Being a low volume high value commodity, the trout has good potential for domestic consumptions as well as foreign export. Inspite of having excellent positive traits, the development and expansion of trout farming has yet to be done on large scale.

Breeding and Hatchery management:

Rainbow trout requires cold, clean and highly oxygenated water for ripening of brooder, successful breeding and hatchery activities. Rainbow trout breed during November to February and attains maturity after completion of 3rd year. The whole process of breeding includes brood stock rearing, stripping of males & females, mixing of eggs and milt, incubation of eggs in trays fitted in the troughs with continuous flowing water, rearing of sac fry and swim up fry in FRP tanks.

Brood stock rearing:

Males and females are segregated prior to 2 months of spawning and reared at density of 5-10kg/m^3. Trout will not spawn naturally in captivity. Generally, two males to one female are deemed a satisfactory sex ratio for brood stock. Brood stock may be reared at density 5-10 kg/m^3. During breeding season, female has round body appearance, bloat and soft belly, and swollen and reddened vent, while male has dark and dull in appearance, large pointed snout with hooked lower jaw and oozing of milt. Feeding condition notably influences the fecundity. The larger the brooder size, larger the egg size, larger the alevin and more resistant young one.

Spawning and egg incubation:

Dry stripping method is applied for spawning. Fertilized eggs remain lemon yellow or light green in colour with the size of 4-5 mm. 1500-1800 mature eggs can be achieved by a mature female trout of 1kg weight. Following are the steps of dry striping operation-

1. Weighing selected brooder
2. Wiped with soft and dry cloth
3. Stripping of female with thumb and index finger of the right hand
4. Fish is then immediately released in the aerated water
5. Check fecundity- nos. of eggs in 5g.
6. Stripping of male for milt
7. Mixing eggs and milt with the help of feather
8. Adding of 100 ml of saline water (0.9 % NaCl) and keep for 5 minutes
9. Washing of eggs with the fresh water

10. Placing of eggs in hatching trays
11. Transfer of trays in trough
12. Fixed 2 liter per minute of water flow for 10,000 eggs
13. Dead eggs are segregated
14. Hatched in 40-60 days at 9-14 ^{0}C

Eggs are incubated undisturbed until the eyed stage in hatchery. Hatchery of the trout with flowing water system is known as Ova house, where incubation and hatching of eggs takes place.

An ova house is comprised of an indoor structure having troughs, trays, nursery tanks and rearing tanks with continuous water flow.

1. Rectangular trough-220X50X40 cm (to hold 10000-15000 fertilized eggs)
2. Trays- 50X30X10 cm (Capacity-2000-3000 eggs)
3. Nursery tanks- 2.0X0.5X0.6cm (for raising 10000 fry)
4. Rearing tanks- 2.0X1.5X0.75 m.

Eggs are placed in meshed trays (mesh size- 1.5 -2.5 mm in dia). A tray can hold a layer of 2000 eggs. Trays are placed 5 cm above the bottom, and water passes through the tray from bottom to top across the tray. Trout have prolonged incubation period extending to several days (40-60 days). Duration of incubation depends on water temperature. There are 4 distinct stages of eggs during incubation-

1. Green egg (fertilized eggs)
2. Eyed egg
3. Alevin or sac fry
4. Swim up fry

Temperature range and incubation period of rainbow trout ova

Species/Stage	**Incubation Period in Days at different temperature range**	
	Temp. 7.0-11.0 ^{0}C	**Temp. 11.0-13.0 ^{0}C**
Green egg to eyed ova stage	0-29 days	0-15 days
Eyed-egg to alevin	29-49 days	15-30 days
Alevin to swim-up fry	49-60 days	30-40 days

As the eggs hatch, the fry drop through the mesh to a bottom trough. Sac fry can remain in trays until swim-up at about 10 to 14 days after hatching. Initially hatchlings of trout feed on reserve yolk material up to 2-4 weeks and called alevin (size 1.5 -1.8 cm, weight 45-50 mg). Hatching of the batch of eggs usually takes 2-3 days, during which time all eggshells are regularly removed, as well as dead and deformed fry. Transportation of eggs can be done at eyed ova stage prior to 5 days of hatching. Sac fry are carefully removed from the trays into the mesh caged arranged into rectangular troughs where running water is maintained @0.3-0.5 liter per minute for 1000 larvae. The sac fry are protected from bright light and remained in the tray until the absorption of the yolk sac and the fry become able to swim. Free swimming fry are fed 10 times a day @5-10% with starter feed. After one week, feeding frequency is reduced to 3-4 times a day and fry are transferred into fry rearing tanks.

Fry and fingerlings rearing:

Fry are traditionally reared in fiberglass or concrete tanks, preferably circular in shape, to maintain a regular water current and uniform distribution of the fry. Fry can be stocked at density of 1000 numbers/m^2 and provided starter feed. Growth is about 8-12 cm (3-5 inches) in length at the end of 3 months of rearing. These 3 months old fingerlings can be stocked in raceways. For the production of 1 lakh fingerlings, 400kg brooder are required, which produced 2 lakh fertilized eggs, 1.5 lakh fry with cumulative survival of 50% from eggs to fingerlings. Two production cycle (60 days each) can be achieved during breeding season.

Brood stock

Trout raceway

Stripping

Egg incubation

Eyed ova

Fry of rainbow trout

Culture of Rainbow trout

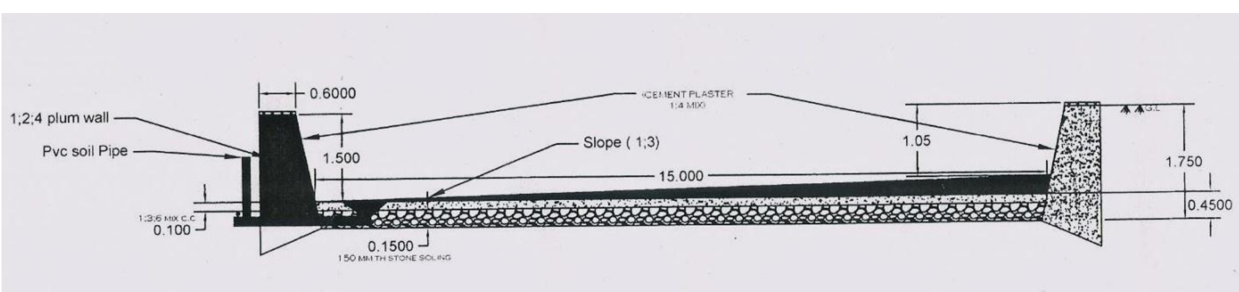
Rainbow trout is one of the best coldwater fish species for the culture in coldwater region of India. It is fast growing, tolerate wide range of environmental conditions and accomplished of occupying different habitats.

Site selection for trout culture:

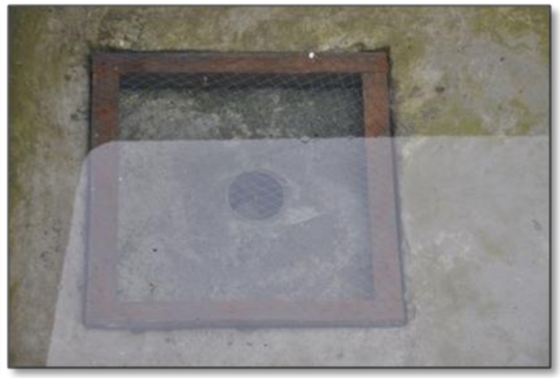
A sloppy terrain with a perennial water source free from heavy metals and silt is suitable for trout farming. Water supply should have higher dissolved Oxygen level of above 7 mg/l, and pH 6.5-8.0. Thermal regime of 13-18^{0}C is suitable for better feeding and growth, however, trout can survive with water temperature range of 0-20^{0}C. Lower thermal regimes (9-14°C) are required for breeding activities. Water temperature above 18^{0}C for longer duration creates environmental stress and mortality in growing stock.

A flow-through system is essential for trout farming with continuous flowing cool, clean and highly oxygenated water. A cement concrete raceway is constructed with an area of 30-45m^2 (15m length, 2m or 3m width) and 1m depth towards inlet and 1.5m depth near the outlet. A minimum 3% bottom slope is required for proper cleaning and to flush out metabolic wastes. One or a battery of parallel raceways may be constructed in trout farm with individual inlet and outlet.

Raceway is a rectangular linear pond (length: width; 10-5:1) to facilitate flowing water current towards the outlet. This species is sensitive to lower dissolved Oxygen, excess ammonia and unhygienic conditions, therefore, adequate water flow from an inlet, regular cleaning and proper outlet design is essential. Bottom pit L shaped pipe or 3 shutters outlet may be constructed in deeper zone of raceway. Raceway should be properly cleaned, washed with 1mg/l potassium permanganate solution and filled with fresh water upto 80cm level before seed stocking. In case of silt and clay in incoming water, a settling tank may be constructed adjacent to raceway along the inlets.



Layout design of a raceway



Sluice gate structure **Bottom pit structure**

Stocking density and growth potential

Quality and quantity of water source and scale of operation determine the stocking density per cubic meter in culture raceways. Generally, rainbow trout takes 12-14 months to attain marketable size (250-260 gm) in J & K, Himachal Pradesh and Garhwal region of Uttarakhand. However, comparatively better growth of 500-600 gm was observed in 12 months at state govt. farm and private farms in West Sikkim. This is due to favourable thermal range of water (14-18^{0}C for 8 months in a year) and availability of sufficient water volume. In central Himalaya, average growth was recorded as 300 g (range 260-400 g) at thermal regime of 5.0-22^{0}C (Vass *et al.* 2010) which indicates good prospects of trout production at marginally higher water temperature range of 10.0-20.0^{0}C in Central Himalayas.

In Nepal marketable size of 200-300 g reach at 14-16 months of culture period with the stocking density of 50 fish/m^3 (Rai *et at*. 2008). Whereas the fish takes approximately 8 months to reach a market size of 300-350 gm in trout farms of Idaho, U.S.A after being stocked in the raceways as a 4 inch long fingerling (Gempesaw *et al.*,1995).

In general, the production level of rainbow trout in Indian conditions is 300-500 kg per raceway of the size of 15mx 3mx1m (45m^3) in 12 months. However, productivity of 1 tonnes or more has been achieved at state govt. farms and at private trout farms of J & K, Kullu valley of Himachal Pradesh and West Sikkim. This trend of production reflects the inherent better potential of productivity having conducive environment and sufficient water volume. In present practice, stocking density of 45-50 fish/m^3 has been adopted by the trout growers, which can be increased up to 100 fish/m^3 with better management practices and sufficient water flow in the raceways. Based on their size attainment in culture facilities, grading of smaller and bigger ones is essential at regular intervals to avoid cannibalism and for achieving uniform growth. The fish is sensitive to lower DO and unhygienic conditions, it is therefore, always advisable to ensure the cleanliness of culture raceways on regular basis. Under suitable water and sufficient supply of good quality of feed, better growth may be achieved in10 months after stocking of 10-20 g sized fingerlings. Cost of rainbow trout production has been analyzed which shows that nearly Rs. 240/-cost is required to produce 1 kg of marketable size (200-300 g each) of trout. (Pandey and Ali., 2015)

Culture management

Trout culture is intensive type of farming which requires more input resources compared to other species for survival and growth. The feasibility of achieving required production naturally depends on a number of factors including seed, feed, health management and environmental consideration.

Water supply and water quality management:

The success of trout culture mainly depends on the water quality and quantity supplied to the farm. Availability of year-round supply of cool, clean, Oxygen rich, pollutant free and suspended matter free water is one of the most important prerequisite for successful trout farming. Optimal or near to optimal conditions of water supply depends on the age of fish and its biomass. The source of water may be an irrigation canal, river, creek, lake or spring which can be supplied via feeder channel, storage tank or pipeline by gravity. Water supply by gravity to a trout farm is an economic method as it saves energy and production cost. This synergistic situation results in improper swimming and mortality. Thus, any abnormal responses by trout may be due to temperature, or dissolved Oxygen levels, or the synergistic response due to both variables, and another, unknown variable.

Physico-chemical parameters for trout culture

Parameters	**Range**
Dissolved Oxygen	near saturation ($\geq$ 9 ppm)
Temperature:	7-18 ºC
Transparency	1.5-1.8 m
Free CO_2	>1.5 mg/l
pH	6.5-8
Suspended solids	< 10 mg/l
Alkalinity	50-150 mg/l
Ammonia (NH_3)	< 0.05 mg/l
Nitrites (NO_2^-)	< 0.05 mg/l
Nitrates (NO_3^-)	< 1.0 mg/l
Phosphates (PO_4^-)	< 0.03 mg/l

The ideal water temperature for production of trout is one that does not rise too high beyond 18^{0}C nor fall too low (7^{0}C) in winter. The best possible water supply is one in which the temperature remains in the range of 13-18^{0}C for as long as possible. The temperature of water supply should never exceed 18^{0}C. There are cumulative effects of synergistic interactions between and among different variables, which influence the growth and survival of growing trout at any moment in rearing practice. For example, increasing water temperature directly affects to trout as well as reduces the dissolved Oxygen content in pond water.

An abundant and continuous water supply is required to sustain the flow-through system. Adequate water flow is required to sustain optimum fish biomass and to get better growth and optimum production. Water flow is expressed by the quantity of water needed for 1000 specimens of eggs, fry or fish. It is expressed either in litre per second (LPS) or litres per minute (LPM). To hold one tone of fish nearly 3-5 LPS (180-300LPM) of water flow is required at an average temperature of 15^{0}C. Some of the estimated water requirement for trout culture is 0.3 LPM for 1 kg of trout with aeration.

Water requirements in different life stages of trout

Stages/age in months	**Stocking density per m^3**	**Water flow per m^3**		**Water flow in raceway (30 m^3)**
		at 5^{0}C	**at 18^{0}C**	**at 12-18^{0}C**
Egg incubation/swim up fry	For 1000 unit	0.2 LPM	0.5 LPM	Not applicable
Fry (1-2g), 0.5-2 month	1000-2500	3-6 LPM	4-8 LPM	110-180 LPM
Fingerlings (2-20g),2-4 months	100-250	3-8 LPM	5-11 LPM	120-270 LPM
Young fish (20-300g), 4-10 months	60-100	3-6 LPM	5-8 LPM	120- 210 LPM
Marketable fish (300-400g) 10-12 months	50-60	2-3 LPM	3-5 LPM	90-120 LPM

LPM- Liters per minute.

Feed and Feeding:

Trout accepts artificial pelleted feed easily and its dietary protein requirement is in the range of 30-50% on dry matter basis. Trout has an exclusive requirement of n-3 or ω-3 PUFA in their diet. 10-14% lipid is included in the trout diet. Sterilized fish meal (60% protein), solvent extracted soybean meal, mustard oil cake, wheat flour, Starch, fish oil, Brewer’s yeast powder, Linseed oil cake and Vitamin and minerals mixture may be used for formulation of trout diet. In general, 50% protein and 14 % lipid for starter feed, 45% protein and 16 % lipid for fingerlings feed and 35% protein and 14 % lipid for grow out feed is required for proper growth. Requirement of Arginine (6.427%) is comparatively higher than the other essential amino acids. Feed alone comprises 76% of total variable cost and 40% of the total production cost of trout farming. A major issue for trout feeding is the high cost of manufactured pelleted feed due to the use of largely imported fish meal. The increasingly scarce supply of fish meal and its high market price had made the trout feeding more expensive. One of the promising alternate seems to be soybean which is rich in plant protein and generally low in phosphorous. Solvent extracted soybean meal (SESM) contain 48% protein and has best amino acid profile and is highly palatable, digestible to trout (Digestion coefficient 80%). It also contains Arginine (3.91% of dry basis) but is not a complete substitute of fish meal. Fish oil is also an expensive feed ingredient of trout feeding. Trout feed is available at state trout farms and in the market in the form of starter feed, fingerling feed, and grow out feed (FCR-1.4) which are used for different life stages of the fish. Due to high content of animal protein and fish oil, trout feed has low shelf life and cannot be stored more than 3 months. In order to avoid the wastage, it is advisable to broadcast the feed or feed dispenser can be used.

Feeding rate for different size of growing trout:

Size of the Fish	Protein Content	% of body weight	Feeding frequency (times in a day)
< 10 gm	40 %	5-10 %	7-8
<50 gm	35 %	5- 6 %	3-4
> 50 gm	35 %	2-3 %	3

Health monitoring:

Regular health monitoring in trout hatchery and raceways is essential to recognize clinical signs of both infectious and non-infectious health problems in order to prevent and control them. Commonly occurring health problems of rainbow trout are eye infection, fungal infestation, dermal head necrosis, mortality of un-striped trout brooders. Most of the health disorders originate from the poor management and stocking of infested seed. Fungal infestations are common in all life stages of trout. Bath treatment in 3% common salt solution for 5 minutes or in 1mg/l solution for 1-2 minutes may be adopted at monthly interval. Regular cleaning of raceway, proper feeding, stocking of disease free seed and disinfection of farm accessories with potassium permanganate solution are prophylactic measures.

Harvesting:

Fish takes approximately 10-12 months to reach a marketable size of 300-400 gm. In general, the production level of rainbow trout in Indian conditions is 500-700 kg per raceway in 12 months. However, productivity upto 1 tonne/raceway has been achieved in Himachal Pradesh by some progressive trout growers. Fish can be partially harvested by drag netting or complete harvesting can be done by complete draining of water from raceway. Before harvesting, fish should be starved for 24- 48 hrs. Harvested fish is degutted, washed with fresh water and packed with ice and salt. Grading of size before packing is also required. Fish can be sold in local markets in fresh condition. Ice packed fish can be sent to fish markets and restaurants of metropolitan cities however the bulk of the produce can be exported.

Constraints-pitfalls; precautions

- Require continuous supply of cool, clean and well oxygenated water.
- Culture is purely based on artificial diet even from first feeding.
- High capital cost and initial investment for raceways construction.
- Intensive production results in the release of organic wastes and soluble inorganic nutrients such as nitrogen and phosphorus.
- Phosphorous present in the discharged water of the trout farm is subject to regulatory restrictions.
- Requires high animal protein content in diet.
- High cost of manufactured pelleted feed due to the use of costly and scare fish meal.
- Non-availability of adequate seed round the year
- Less genetic variability, slow growth.
- Highly perishable, seasonal, bulky, small scale and scattered production.
- Poor market infrastructure and high cost of transportation.
- Dead eggs remain more susceptible to the fungal attack during incubation
- Lack of readily available financial resources to prospective rainbow trout farmers

DOs and DONTs

S.no.	Dos	DONTs
1.	Cleaning of raceway and washing with 1 mg l^{-1} potassium permanganate solution before stocking.	Stocking in unhygienic pond
2.	Adequate water supply into raceway.	Irregular water supply
3.	Stocking of healthy seed of uniform size in appropriate density.	Stocking of undersized seed, over /under stocking of seed
4.	Periodic grading of growing stock.	Improper feed storage for longer duration(not more than 3 months)
5.	Regular feeding at fixed scheduled time with protein rich diet. Limited feed during increased water temperature.	Over feeding and under feeding
6.	Use of pelleted grow out feed.	Use of raw slaughter house waste
7.	Thinning of stock if water temperature is in higher side (> 18^{o} C)	Use of rancid feed
8.	Bath treatment with 10% Common salt solution for 5 minutes at monthly interval	Mishandling the fish
9.	Prophylactic measures for health	Ignorance for health monitoring
10.	Degutting and ice packing after harvesting	Improper packing and unhygienic storage

A. Economics of rainbow trout farming (raceway area-30m^3)

Capital Cost	**water area 30m^3 (Rs.)**
1. Construction of Raceway	2,00,000/-
2. Water channel	50,000/-
Total Capital Cost	2,50,000/-
Running Cost	
1. Cost of Fingerlings (2000 nos.)	20,000/-
2. Cost of Feed 1 tonn @ 100/- per kg.	1,00,000/-
3. Depreciation @ 10% of Capital Costs	25,000/-
4. Labour cost, 40 man- days	12,000/-
5. Farm accessories & miscellaneous expenditure	15,000/-
Total Running Cost	**1,72,000/-**
Total Cost (Capital+ running cost)	4,22,000/-
1. Interest on fixed & working capital @ 12% P.A.	50,600/-
Total Annual Cost (running cost+ interest)	**2,22,600/-**
Sale of fish of 700kg @ Rs. 500/- per kg	3,50,000/-
Net Profit	**1,27,400/-**

Rs. 318/- is required to produce 1 kg of marketable trout. The annual rate of return in trout farming is nearly 57% of the total annual investment.

B. Economics for 1 Lakh fingerlings production of rainbow trout

Capital Cost	**Amount (Rs.)**
1. Construction of ova house (5m X 10m)	10,00,000/-
2. Brooder raceways-2, nursery raceway-1	6,00000/-
3. Water channel & storage tank	2,00,000/-
4. Trough-15, trays-60, FRP tanks-4	6,00,000/-
Total Capital Cost	24,00,000/-
Running Cost	
1. Cost of 400 kg brooder @ Rs. 600/ kg	2,40,000/-
2. Cost of Feed 1 tonn @ 100/- per kg.	1,00,000/-
3. Depreciation @ 10% of Capital Cost	2,40,000/-
4. Labour Cost, 100 man- days	30,000/-
5.Hatchery accessories & miscellaneous expenditure	40,000/-
Total Running Cost	**6,50,000/-**
Total Cost (Capital+ running cost)	30,50,000/-
1. Interest on fixed & working capital @ 12% P.A.	3,66,000/-
Total Annual Cost (running cost+ interest)	**10,16,000/-**
1. Sale of 1 lakh fingerlings @ Rs. 10/ piece	10,00,00/-
2. Sale of spent brood fish 600kg @ Rs. 600/- per kg	3,60,000/-
Net Profit	**3,44,000/-**

NUTRITION AND FEEDING MANAGEMENT

Feed alone comprised 76% of total variable cost and 40% of the total production cost of trout farming. Previous studies reveal that the dietary protein requirement to trout is in the range of 30-45% on dry basis. Adequate nutrition, both in terms of quantity and quality, is of utmost importance to the success of any aquaculture operation. For starter feeding and broodstock, the necessity is even greater (40-45%). It has been recommended that a diet of no less than 35% crude protein be administered at a rate of 2-3% of bodyweight per day for growout stock of rainbow trout. Several amino acids are essential to fish growth and development and, therefore, must be present in the feeds. Proximate composition of carcass of fingerlings of rainbow trout reflected that the requirement of Arginine (6.427%) is comparatively higher than the other essential amino acids. Therefore, better utilization of the protein in the diet of trout should be obtained by adding ingredient having more Arginine (Fontagne-Dicharry *et al.*, 2017).

However, use of protein for energy is expensive, thus lipids are primarily included in formulated diets to maximize their protein sparing. Lipids are a highly digestible source of concentrated energy. Dietary lipid level as low as 10% has been suggested for rainbow trout on the basis of previous studies. Generally, weight gain and feed efficiency depressed when diet contains 15% or more lipids (Kamalam *et al.*, 2017).

Rainbow trout has an exclusive requirement of n-3 or w3 PUFA in their diet. High protein level in the diet is always resulted an increase in unwanted ammonia excretion. It was observed that diet with 45% and 40 % protein level resulted as 0.12 mg/l and 0.08 mg/l ammonia excretion in the drain water, respectively. But, lowest level of ammonia as 0.05 mg/l was noted with 35% protein level in the diet. (Singh *et al.*, 2017; Pandey and Ali., 2015)

A major researchable issue for trout feeding is the high cost of manufactured pelleted feed due to the use of largely imported fish meal. The increasingly scarce supply of fish meal and its high market price had made it necessary to seek a cost effective replacement of fish meal to supply dietary protein in trout feed. For addressing the issue, two cost - effective practical starter feeds were formulated for first feeding rainbow trout fry, based on single or multiple protein sources. The experimental diets were made up of commercial ingredients and were conventionally prepared by steam cooking, pelleting and drying. To evaluate the performance of these two diets as compared to a widely used commercial diet, a five week feeding trial was conducted and found more efficient and significant over the commercially used starter feed. (Singh et al., 2017)

Digestibility is affected by many factors and it is possible for the same ration to have various digestibility. Supplementation of active enzyme (Protease) may enhance the digestibility of the protein. Higher water temperature partially affected the feed intake and dietary protein requirement of the trout, which can be covered by increasing feeding frequency and improving digestibility. The comparative growth performance in experimental trials indicated significant variations, the test fishes fed at 31 % protein diet attained size of 55-100 mm in 300 days while those fed at 35% protein diet recorded a size range of 55-145 mm in 240-280 rearing days but at higher protein level of 48% the size range attained in 280 days was in the range of 60-150 mm indicating that much higher protein levels did not promote better growth in comparison to medium protein level of 35%. (Pandey and Ali 2015)

Feed requirement for different life stages of rainbow trout

Feed type	Approx. Protein content (%)	Approx. Fat content (%)	Pellet size (mm)	Fish size (g)
Starter/fry	60	14	0.5–1.5	0–10
Growing/fingerling	46	23	2.0 -	10–50
On-growing	43	30	3.0–9.0	50–1,000
Brood stock	50	13	9.0	1,000–4,000

Feeding rate for different size of growing trout

Size of the Fish	Protein Content	% of body weight	Feeding frequency
< 10 gm	40 %	5-10 %	7-8
<50 gm	35 %	5- 6 %	3-4
> 50 gm	35 %	2-3 %	2-3

FEED FORMULATION FOR TROUT

Practical feed formulation

The major aim in developing commercial diets for trout is to formulate a diet that satisfies the nutritional requirements of the target trout species at minimum possible cost. This is called as **least-cost formulation.**

Information that is required before feed formulation can begin with-

- A list of available raw materials and information on their compositions and costs.
- Knowledge of nutritional requirements of trout.
- The specification of the diet to be made (desired levels of protein, lipid, amino acids etc.)
- Knowledge on the suitability of available raw materials for the trout.

PROXIMATE ANALYSIS OF FEED

Information on the proximate composition of feed ingredients are useful for the initial choice of potential raw materials for trout feeds. Usually the information in the proximate composition of feed ingredients is given according to six major components. These are Moisture, Crude protein, Ether extract, Nitrogen free extract, Crude fibre and ash.

The 6 components in the feed and substances contained in them are listed in the table shown below

Proximate composition	Substances in respective composition
Moisture	Water, volatile substances
Organic matter	**Crude protein** - Pure protein, amino acids, non-protein compounds
	Crude fat (Ether extract) - Fat, complex lipid, sterols, fatty acids, fat soluble dye
	Crude fiber - Cellulose, hemicellulose, lignin **Nitrogen free extracts** -Soluble carbohydrate hemicellulose, lignin, pectin, organic acids, tannin, water-soluble dyes
Inorganic matter	**Crude ash** - Pure ash, organic residue,soil

Various computer software packages are available nowadays to formulate trout feed. Followings are the methods for trout feed formulation.

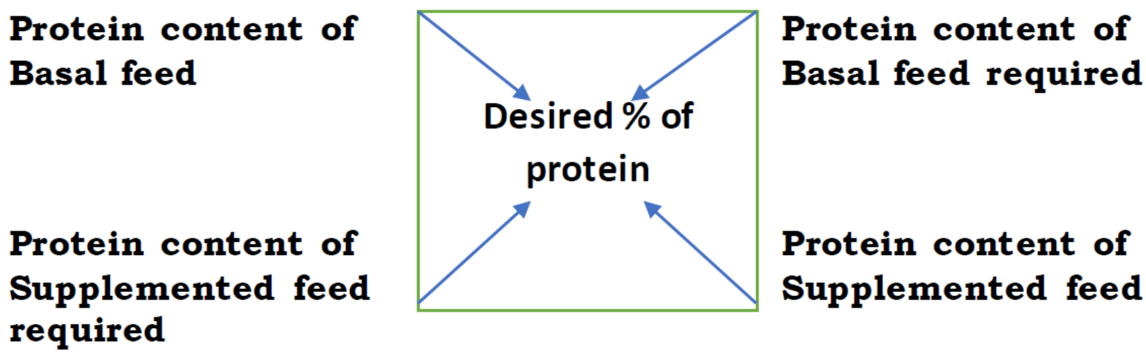
Formulation of diets:

1. Pearson Squares Method

- Simplest method for formation of diets
- Used for two and more ingredients
- It could be balanced one nutrients (make your choice for crude protein or crude fat)

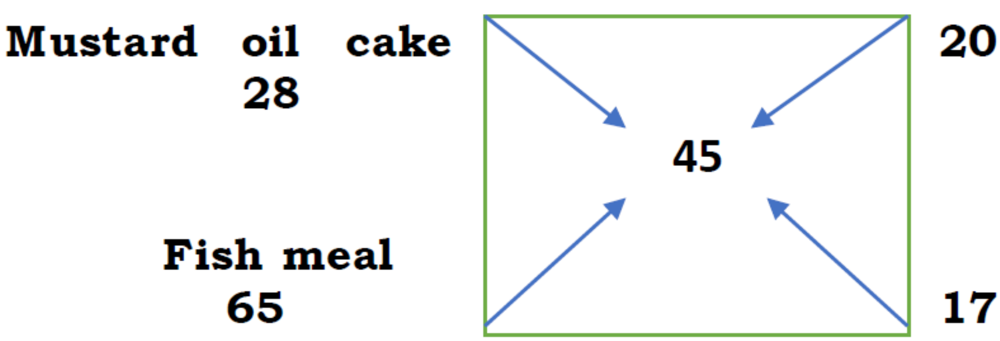
How to use

1. Make a square and place the desired protein in the center
2. Place the percentage of crude protein value of basal feed(less than 20% protein) or a mixture of feed stuffs at upper left corner of square
3. Place the percentage of crude protein value of supplement (more than 20% protein) or a mixture of protein supplements at lower left corner of square.
4. Subtract smaller values from larger diagonally and forget signs
5. Add right values
6. Take out the percentage of ingredient separately



Example 1.

Assume that a diet needs to be formulated with 45% Protein using the Fish meal (65% crude protein) and Mustard oil cake (28% crude protein).We want to make a feed that contain 45% protein.



Now we add 17 + 20 = 37

To make a feed with 45%protein we must mix

Mustard oil cake = $\frac{20}{37} \times 100$ = 54.05%

Fish meal $\frac{17}{37} \times 100$= 45.95%

Example 2.

To prepare a feed with 40% protein by using five ingredients such as Rice bran 13%protein, Mustard oil cake 28%, Fish meal 65%, Tapioca 2% and Soybean oil cake 35%.

Solution: -

Step 1: - Grouped all ingredients into basal feed supplement (less than 20% protein) and supplemented feed (more than 20% protein).

Basal feed: Rice bran 13% Tapioca 2%

Supplemented feed: Mustard oil cake 28%, Fish meal 65%and Soybean oil cake 35%

Step 2: -Averaged the protein content in each group

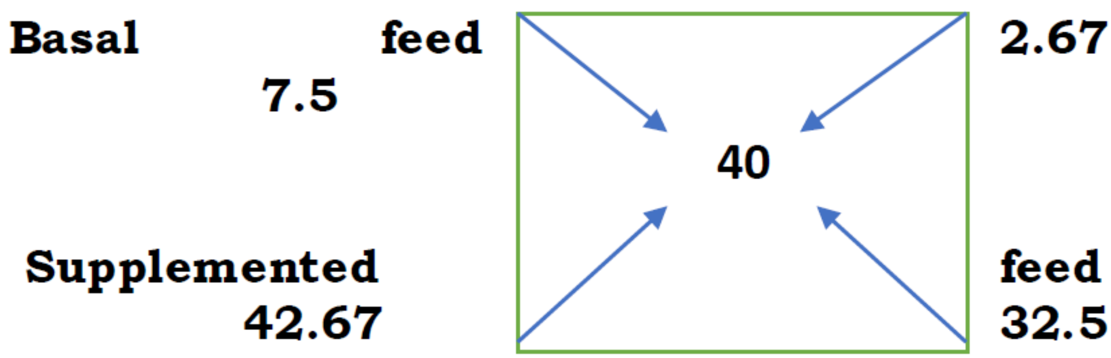
Basal feed: Rice bran 13% Tapioca 2%

Average: 7.5%

Supplemented feed: Mustard oil cake 28%, Fish meal 65%, and Soybean oil cake 35%

Average: 42.67%

Step 3: - Put the values in square



Step 4: - Total = 2.67 + 32.5 =35.17

Step 5: Now the composition will be

Basal feed =$\frac{2.67}{35.17} \times 100$ **= 7.59%**

Supplemented feed = $\frac{32.5}{35.17} \times 100$ **= 92.41%**

Step 6: final feed formulation

Rice bran $= \frac{7.59}{2} =$ **3.8%**

Tapioca $= \frac{7.59}{2} =$ **3.8%**

Mustard oil cake $= \frac{92.41}{3} =$ **30.8%**

Fish meal $= \frac{92.41}{3} =$ **30.8%**

Soybean oil cake $= \frac{92.41}{3} =$ **30.8%**

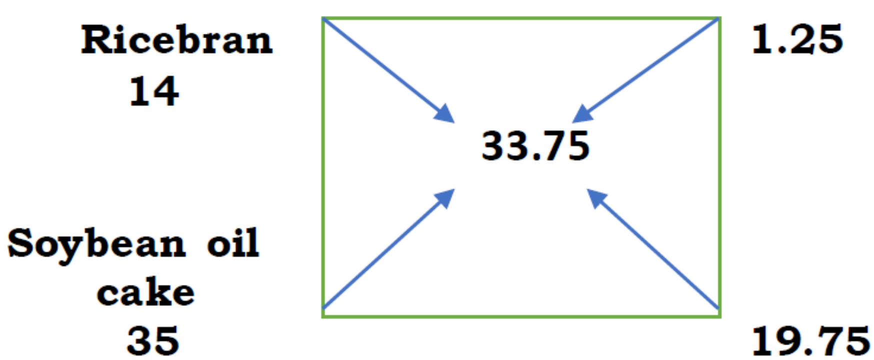
Example 3.

Assume that a diet needs to be formulated with 40% protein using the following ingredients Fish meal 65%, Soybean oil cake 35% and Rice bran 14%. Find the proportions of different ingredients that would initially give a dietary protein level of 40% when mixed with 20% fish meal.

Solution: Fish meal (20%) will contribute 13 protein to the diet

Therefore, the other 80% will have to make up to 27% (40-13) of protein. If this portion of the formulation is treated separately, the non-fish meal portion of the diet must contain 27 ×100/80 = 33.75% protein.

In the order to provide 33.75% protein the amount of each of the ingredients is calculated by Pearson square.



The actual amount of ingredients to be used is:

Rice bran = 1.25/1.25+19.75 × 100 = 5.95%

Soybean oil cake = 19.75/1.25+19.75 × 100 = 94.05%

These ingredients, however should constitute only 80% of the mix. Therefore, the amount of Rice bran and Soybean oil cake to be incorporate in the final mix is 4.76% (5.95 × 0.8) and 75.24% (94.05× 0.8) respectively.

20% Fish meal with 4.76% Rice bran and 75.24% Soybean oil cake provide 40% protein in diet.

2. **Linear programming:**

Another mathematical technique available to nutritionists for selecting the best combination of feed ingredients to formulate diets at the least possible cost is linear programming. Least cost linear programming software for diet formulation is readily available, the price varying with the sophistication required. A commonly used spreadsheet such as Lotus 1-2-3 can also be utilized for formulating feeds, incorporating a smaller number of variables. It should be noted that least cost feed formulation is not always practical for small scale aqua-culturist using on farm feed manufacture facilities where the choice of ingredients available is limited.

COLDWATER FISH DISEASE & HEALTH MANAGEMENT

AN OVERVIEW

Fish disease pathogens are ubiquitous, present in the water, soil, air, or fish. In nature fish are often resistant to these pathogens, and they are able to seek the best living conditions available. Food fish reared under commercial aquaculture conditions are confined to the production unit and are weakened by stress conditions including high stocking & poor water quality, poor nutrition, handling injury. Physiological stress and physical injury may cause poor health or death of fish.

In cold water aquaculture virus (though not detected in India), parasite, bacteria and fungus are potential disease causing pathogen which may adversely affect growth and health status of fish.

A. Parasitic Diseases

Disease	***Causative agent***	***Etiology/Sign/symptom***	***Management/Treatment***
White spot (Ich) Disease	*Ichthyophthirius multifiliis*	Adult parasite is 1mm long round hairy often brown colored parasite, oval with "C" or horse shoe shaped nucleus, moves very slowly.	-Dip treatment in 2-3% salt solution for 1-2 minute -Dip treatment with KMnO4 @ 1ppm -Applying lime @30-50mg/l
Costiasis	*Costia necatrix*	-Skin gets covered with a light gray-blue film -Seriously affected body parts show red patches -Gills becomes brown in color	-30 minute formalin bath in a concentration of 1:4,000 or 15 minutes - 20 minute bath in a table salt solution of the concentration of 10g/liter of water.
Trichodinasis	*Trichodina, Trichodinella and Tripartiella.*	-Reddening of gills, minute red spots over gills, skin, fins and especially anal fins	- Dip treatment in 2-3% salt solution for 2-3 minutes every alternate day for a week - Dip treatment in 1:1,000 Acetic acid for 2-3 minutes

			- Dip treatment with KMnO4 @ 1ppm
Whirling diseases	*Myxobolous cerebralis*	- Fishes show circular or whirling movement due to imbalance and loss of equilibrium, erratic swimming, darkening of tail region, deformity of skeleton and mortality.	-Dip treatment in 2-3% salt solution for 1-2 minute -Dip treatment with KMnO4 @ 1ppm
Trematode Infections	*Dactylogyrosis* *Gyrodactylosis*	- light bluish mucus, changes in body coloration, hemorrhages, light yellow lining in the gill filaments, fading of gill color - small red spots over body, fins and gills, excessive secretion of mucus, emaciation, restlessness in infected fishes, breaking of gills filaments and development of hemorrhagic wounds in gills	Dip treatment in 2-3% salt solution for 2-3 minutes every alternate day followed by dip treatment in 1-4ppm of KMnO4 solution.
Argulosis	*Argulus*	- reddish patches at the base of ventral and pectoral fin	- Butox can be applied @70-75ml/ha m - Dip treatment in 2-3% salt solution for 2-3

			minutes - Use of Chitin synthesis inhibitor (diflubenzuron and Lufenuram) prevent new shell formation after molting

B. Bacterial diseases

Disease	***Causative agent***	***Sign***	***Treatment***
Fin rot and gill rot	*Aeromonas hydrophila* *Psudomonas fluroscence*	Tail and fin erosion	$CuSo_4$ bath treatment 1:2000 for 1-2 minute Tetracyclin
Dropsy	*Aeromonas hydrophila*	Body cavity filled with fluid Scale protrusion	Terramycin
Eye disease	*Aeromonas liquifaciens*	Eye get vascularized or protruded eye	KMnO4 at 0.1 ppm and lime treatment
Lactococcosis	*Lactococcus garvieae*	Unilateral exophthalmia Swollen vent and swollen liver	-
Furunculosis	*Aeromonas salmonicida*	Boil like lesions and blood clot on fins	Tetracyclin

C. Fungal like Diseases

Saprolegniosis

Saprolegniosis disease is characterized by white or grey cotton like patches/hyphae on skin and fins of infected fish. Among several strains of *Saprolegnia*, *Saprolegnia parasitica* and

S. diclina are responsible for significant infections in fish and eggs, particularly in aquaculture facilities. *S. diclina* has been reported to be dominate species (79% of recovered isolates) in Norwegian salmon hatcheries. Several other Saprolegniales ;*S. ferax, S. hypogya* and *Scoliolegnia asterophora* also have noteworthy impact on salmonids and freshwater carp.*Saprolegnia diclina* infections found more common in winter months, whereas *Saprolegnia ferax* occurs predominantly in the spring and autumn.

Sign/symptom:

- Initially the disease appears as white mats over the skin that gradually spreads and invades in deeper tissues causing mortality in acute cases.
- As the infection progresses the fish becomes increasingly lethargic due to overgrown mycelium

Control measures

- Malachite green is considered the most effective chemical for controlling *Saprolegnia*. However, because of concerns about potential carcinogenicity, malachite green is banned in the United States and some other countries.
- Formalin, a solution of 37% formaldehyde, Hydrogen peroxide, and Sodium chloride at high concentrations has also been recommended for the cure of fungal infected fish.
- Currently, the most effective strategy for controlling and preventing *Saprolegnia* infections is a combination of good fish management and husbandry techniques, combined with salt treatment.
- Water flush treatment with 2-3% of common salt, weekly, for 3 weeks can be given to the infected stock.

Fish Health management in coldwater aquaculture

In addition to practicing good fish husbandry, a fish disease control plan includes proactive health management, judicial use of appropriate and approved chemotherapeutics when disease occur or the application of selected chemicals after handling, and the use of vaccines when available.

Management procedures that help reduce stress as it relates to increased disease susceptibility are (1) fish handling and stocking, (2) feed management, (3) water flow and temperature management, (4) aeration management, (5) controlling other environmental problems, and (6) waste management.

Water quality

- Maintenance of proper stocking density not exceeding carrying capacity
- Routine monitoring of water quality parameters
- Prevent the accumulation of organic debris, nitrogenous wastes (ammonia and nitrite)

- Maintenance of appropriate pH, alkalinity, and temperature for the species

Handling and transporting

- While handling try to minimize physical injury and stress.
- Transport and holding tanks should be large enough to allow free movement of fish
- Slowly changing water temperature from one environment into another (tempering) is advisable while transferring fish
- Sodium chloride or potassium permanganate ($KMnO_4$) may be used after holding fish as prophylactic to help prevent secondary bacterial infections

Feed management:

- Accumulated metabolic waste in water creates oxygen demand as it decomposes and serves as a source of nutrients for phytoplankton, which in turn exert an oxygen demand.
- Good quality feed should be provided with optimum frequency

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