Chapter III

MATERIALS AND METHODS

3.1 Field and laboratory work

Based on the background, statement of the problem and aims and objectives of the present investigation entitled **"Impact of artificial feed on survival and growth of rainbow trout, *Oncorhynchus mykiss* (Walbaum) during exogenous feeding in raceways of Kathmandu, Nepal"**, the materials and methods include field and laboratory work mentioned in the experimental design as per protocol.

3.2 Site and period of study

The field work, which was conducted in the raceways and hatchery of a private farm in Kakani, Nuwakot district, Kathmandu Valley, Nepal (Figure 1) is situated at latitude 27 ° 48 ' N, longitude 85 ° 15 ' E (CBS, 2017), altitude 1550 masl (Figure 2). It occupies an area of 10 hectares, a slope of 2.5% and is 30 km north from Kathmandu city. In the field experiments, artificial propagation of broods and exogenous feeding of the free swimming fries, fries and fingerlings of rainbow trout were conducted. The laboratory work required for the field experiments like analyses of the physico-chemical parameters, proximate analyses of the feed ingredients, proximate analyses

Figure 1: Site of present investigation situated at Kakani (as seen in 'insat' in the north of Kathmandu city), Nuwakot District, Kathmandu Valley, Nepal.

Figure 2: Private rainbow trout farm, the site of research work, situated at an altitude of 1550 masl at Kakani, Nuwakot District, Kathmandu Valley, Nepal

of the diets of broods, and proximate analyses of the formulated and control diets of the free swimming fries, fries and fingerlings were conducted in the Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, West Bengal, India. The period of the present study was three years commencing from June 2009 and ending in May 2012.

3.3 Raceway pond for stocking, nursing, feeding and rearing of rainbow trout

The raceway ponds, which were cemented on all sides and bottom, and rectangular in shape were eleven in number with running water being supplied from dependable, perennial, and permanent spring-fed torrential stream (Figure 3) but with limited volume of water supply due to hardness to carry it, that is why, the raceways were constructed in a linear fashion (Figure 4, Figure 5, Figure 6 and Figure 7) and not in parallel arrangement. Thus, being linear in arrangement (Westers, 2000), each raceway was connected to the adjacent one, in both sides – backward and forward except, the first one and the last one.

Figure 3: Dependable, perennial, and permanent spring-fed torrential stream supplying water in the raceways at the site of private farm at Kakani, Nuwakot District, Kathmandu Valley, Nepal.

Figure 4: Linear arrangement of the raceways as seen in aerial view in the private rainbow trout farm of Kakani, Nuwakot District, Kathmandu Valley, Nepal.

The first was backwardly connected to the feeding channel of the water resource and forwardly to the next adjacent raceway. The last one was backwardly attached with the prior raceway and forwardly with the outgoing outlet (Figure 4, Figure 5, Figure 6 and Figure 7). Again, one could note down the water flowing from one raceway pond and entering into another due to linear construction of the raceways (Figure 4, Figure 5, Figure 6 and Figure 7) and the same sequence was seen in all raceways.

The size of the raceways was variable; however, most of them were 15 m × 3 m × 1 m (45 m^3) with water volume of 15 m × 3 m × 0.9. The raceways engaged in stocking of the brood or table fish, nursing of the free swimming fries, feeding of the fries and rearing of the fingerlings were respectively called stocking or brood, nursing, feeding and rearing raceways. Now, one could notify the stocking of the advanced fingerlings in two stocking raceways as shown in Figure 6 for the production of table fish. Further, some of the raceways could accommodate two rows of sixteen nursing cum feeding cum rearing cages (one row of eight cages each) for nursing, feeding, and rearing of free swimming fries, fries, and fingerlings as required in the present investigation. So, raceways were sufficient in number and spacious in size to complete the experimental work.

Figure 5: Raceways as seen in closer view in the private rainbow trout farm at Kakani, Nuwakot District, Kathmandu Valley, Nepal.

Figure 6: Stocking raceways as seen in closest view with the stocking of advanced fingerlings for table fish production in private rainbow trout farm of Kakani, Nuwakot District, Kathmandu Valley, Nepal.

3.4 Hatchery for incubation and hatching of fertilized egg into sac fry and free swimming fry

In the Figure 7, one could mark the shaded construction of hatchery opened from the front side but closed behind and above which attached to the road, there is a double-storied living apartment cum store house cum watch tower. Further, one could also notice in its (hatchery) side and in between these two (apartment and hatchery), there is one narrow feeding channel carrying running water for both hatchery and raceways from the nearby water resource of spring-fed torrential stream.

The hatchery was provided with five raceways cum tanks inside the shaded construction. Each raceway cum tank could accommodate five atkins at a time, and each atkins could be stacked with ten locally-made incubation cum hatching trays for the incubation of fertilized eggs and their hatching into sac fries.

Figure 7: Hatchery which is connected backwards with feeding channel of the water resource and downwards with other raceways in linear fashion is beset with raceways cum tanks for the incubation of fertilized eggs and sac fries.

Again, each raceway cum tank could accommodate two incubations cum hatching cages at one time, and each cage wasmeant for the incubation of sac fries till endogenous feeding period and ultimately their hatching into free swimming fries.

3.5 Rainbow trout: the study animal

Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), earlier known as *Salmo gairdnerii* Richardson, 1836 has recently been classified (ITIS, 2017) as:

Kingdom – Animalia
Subkingdom – Bilataria
Infra-kingdom – Deuterostomia
Phylum – Chordata
Sub-phylum – Vertebrata
Infra-phylum –Gnathostomata
Super-class – Actinopterygii
Class – Teleostei
Super-order – Protacanthopterygii
Order – Salmoniformes
Family – Salmonidae
Sub-family – Salmoninae
Genus – *Oncorhynchus*
Species – *Oncorhynchus mykiss* (Walbaum, 1792)

The strain of rainbow trout (Figure 8 and Figure 9) studied in the present research work was from Miyazaki Prefecture, Japan and was brought from there as a gift of Japan to Nepal in 1990 by the initiation of Japan International Cooperation Agency (JICA) in the form of eyed-eggs (pro-larvae). The eyed-eggs were acclimatized, nurtured, fed and reared in the raceways of Fisheries Research Division, Godawari, Lalitpur, Kathmandu, Nepal to establish them as broods and were, later on, disseminated in both government and the private farms of Nepal.

Figure 8: Fully grown and well matured rainbow trout (male)

Figure 9: Fully grown and well matured rainbow trout (female)

3.6 Rainbow trout culture: semi-intensive farming

Semi-intensive farming method of rainbow trout culture, which was based on dissolved oxygen content of cold water, level of management and stocking density of rainbow trout, and which could be governed by water discharge volume, rate of feeding and feeding frequency of different stages of rainbow trout, was adopted in the present research in Nepal as the dissolved oxygen concentration reported so far, for the raceways till those date was in between 7.2 to 10.5 mg L^{-1}. Again, because the water in the raceways was carried from the spring-fed torrential stream that contained comparatively less dissolved oxygen in water than the water brought from the lake-fed (containing comparatively more dissolved oxygen than spring-fed streams) or glacier-fed stream (containing comparatively more dissolved oxygen than lake-fed streams), semi-intensive culture of the rainbow trout was the only option left for the rainbow trout culture (Figure 10).

Figure 10: Semi-intensive farming of rainbow trout in the raceways.

3.7 Experimental design of the present work

For the fulfillment of the aims and objectives put forward for the "Impact of artificial feed on survival and growth of rainbow trout, *Oncorhynchus mykiss* (Walbaum) during exogenous feeding in raceways of Kathmandu, Nepal", the research work was conducted in three different phases – first phase of initial work, second phase of experimental work and third phase of experimental work:

3.7.1 First phase of initial work

This phase was deputed for one year starting from June 2009 to May 2010. No monthly recordings of the water quality variables were done except the measurement of water temperature, pH, dissolved oxygen and carbon dioxide which were required for the brood management. To start up with the research experiment, fingerlings were stocked in a stocking cum experimental raceway of the size 10 m × 2 m × 1 m (20 m^2)

designated as Raceway 1 (**R 1)** as shown in Figure 11. Fingerlings were fed 35 to 45% crude protein containing artificial feed (crumble feed initially and pellet feed later on) at the rate of 2-3% to 8-10% of their live body weight in feeding frequency of 2-3 to 8-10 times day^{-1} based on their size and was monitored every three months. Data collection of the maturity of gonads, mortality/survival, growth, health and behaviour were done at 9:00 a. m. Fingerlings were obtained as experimental broods in May 2010.

Experimental design of first phase

Raceway 1

- Fingerlings stocking (number) – 200 to 250
- Fingerlings stocking (rate) – 200 to 300 g m^{-2} (25 to 50 fingerlings m^{-2})
- Water temperature, pH, dissolved oxygen and free carbon dioxide recorded
- Water discharge maintained – 0.083 to 0.3 L sec^{-1}

Figure 11: Stocking cum experimental brood raceway designated as Raceway-1 (R-1)

3.7.2 Second phase of experimental work

This phase was assigned for the period of one year commencing form June 2010 and ending in May 2011. Month-wise assessments of physico-chemical properties of the raceway water and the surroundings were done for the whole year. Broods were fed as per protocol given inside the raceway-box later on. The maturity of the gonads, mortality/survival, growth, health and behaviour of the broods were studied. Experimental broods of the first phase were treated as **future broods** – half number of which were left in the Raceway 1 (**R 1)** and rest half were stocked in Raceway 2 (**R 2)** (Figure 12) for two months (June and July). Future broods became **broods** when males developed rough upper surface on pectoral fins and females swollen belly and future brood raceways became **brood raceways** after two months. Broods were kept in the **R 1** and **R 2** (Figure 13) for one month (August). Broods became **segregated**

broods when males were bright and brilliant in colour with compressed abdomen and elongated lower jaw, sometimes curved upwards like a hook and some appearing darker in colour, almost black and females comparatively light-coloured than males but with swollen and enlarged abdomen containing slightly reddish vent and brood raceways became **segregated brood raceways** after one month. Broods which were segregated sex-wise were called segregated broods and were put into two separate segregated brood raceways: male in **R 1** and female **in R 2** (Figure 14) for two months (September and October). Segregated broods became **current broods** when segregated males oozed milt on pressing their abdomen and females eggs and segregated brood raceways became **current brood raceways** after two months. Current broods were left in two current brood raceways **R 1** and **R 2** (Figure 15) during November. Current broods became **gravid broods** (1.0^{+} broods or first spawners) when males and females developed complete sign of readiness for breeding and current brood raceways became **gravid brood raceways** before breeding and were left in gravid brood raceways **R 1** and **R 2** (Figure 16) during 1^{st} and 2^{nd} week of November 2010 and 2011 respectively. One hundred gravid broods were collected out of which six gravid males and twelve gravid females were selected for the artificial propagation and put into happas, **Happa 1** and **Happa 2** (Figure 17).

Experimental design of second phase

Raceway 1

- Future brood stocking (number) – 100
- Future brood stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2})
- Fed 35% CP containing artificial pellet feed @ 2-3% of their body weight twice daily
- Water discharge maintained – 0.042 L sec^{-1}

Raceway 2

- Future brood stocking (number) – 100
- Future brood stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2})
- Fed 35% CP containing artificial pellet feed @ 2-3% of their body weight twice daily
- Water discharge maintained – 0.042 L sec^{-1}

Figure 12: Two future brood raceways – Raceway 1 (R1) on the left side and Raceway 2 (R2) on the right side. R1 was the one in which fingerlings were stocked in the first phase of the initial work and the R2 was newly arranged as per requirement.

Experimental design of second phase (contd.)

Raceway 1	Raceway 2
➢ Brood stocking (number) – 100 ➢ Brood stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 2-3% of their body weight twice daily ➢ Water discharge maintained – 0.052 L sec^{-1}	➢ Brood stocking (number) – 100 ➢ Brood stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 2-3% of their body weight twice daily ➢ Water discharge maintained – 0.052 L sec^{-1}

Figure 13: Two brood raceways. Future brood raceways R 1 and R 2 that became brood raceways R 1 and R 2 respectively

Raceway 1	Raceway 2
➢ Segregated males stocking (number) – 100 ➢ Segregated males stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 1-2% of their body weight twice daily ➢ Water discharge maintained – 0.063 L sec^{-1}	➢ Segregated females stocking (number) – 100 ➢ Segregated females stocked (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 1-2% of their body weight twice daily ➢ Water discharge maintained – 0.063 L sec^{-1}

Figure 14: Two segregated brood raceways – R 1 for males and R 2 for females

Raceway 1	Raceway 2
➢ Current broods (males) stocking (number) – 100 ➢ Current broods (males) stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 1% of their body weight 4-5 times weekly ➢ Water discharge maintained – 0.063 L sec^{-1}	➢ Current broods (females) stocking (number) – 100 ➢ Current broods (females) stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 1% of their body weight 4-5 times weekly ➢ Water discharge maintained – 0.063 L sec^{-1}

Figure 15: Two current brood raceways. Segregated brood raceways R 1 and R 2 that became current brood raceways R 1 and R 2 respectively

Raceway 1	Raceway 2
➢ Gravid broods (males) stocking (number) – 100 ➢ Gravid broods (males) stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 2-3% of their body weight 4-5 times weekly ➢ Water discharge maintained – 0.063 L sec^{-1}	➢ Gravid (females) stocked stocing (number) – 100 ➢ Gravid broods (females) stocing (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 2-3% of their body weight 4-5 times weekly ➢ Water discharge maintained – 0.063 L sec^{-1}

Figure 16: Two gravid brood raceways. Current brood raceways R 1 and R 2 that became gravid brood raceways R 1 and R 2 respectively

Happa 1	Happa 2
➢ Gravid broods (males) – 6 ➢ Water discharge maintained – 0.063 L sec^{-1}	➢ Gravid broods (females) – 12 ➢ Water discharge maintained – 0.063 L sec^{-1}

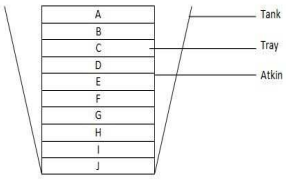
Figure 17: Gravid broods – males in Happa 1 and females in Happa 2

Stripping cum **fertilization** cum **mixing tray**

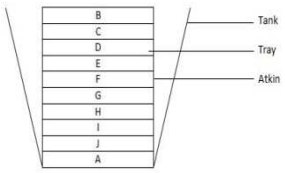
- Eggs – Weight (g) recorded
- Milt – Volume (ml) recorded
- Fertilization – eggs : milt :: numerical : numerical

Figure 18: Stripping and fertilization was done at 4:00 p.m. creating darkness in the hatchery and recording air temperature. Stripping was done only once

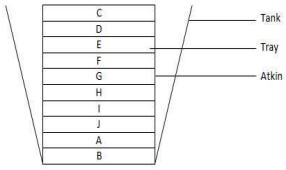
Incubation, cleaning and hatching of fertilized eggs into sac fries



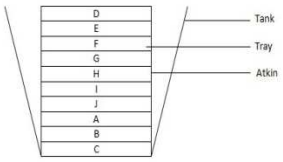
Incubation of fertilized eggs



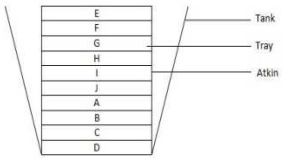
First cleaning and readjustment



Second cleaning and readjustment



Third cleaning and readjustment



Hatching of incubated eggs into sac fries

Figure 19: Incubation of fertilized eggs in ten locally-made incubation cum hatching trays (A, B, C, D, E, F, G, H, I and J) stacked in an atkins put inside incubation cum hatching raceway cum tank maintaining water discharge of 0.033 L sec^{-1} and creating darkness in the hatchery; weekly cleaning of spoilt eggs, recording of their mortality to calculate survivability and readjustment of the trays thrice to maintain their equilibrium as shown in the figure; ultimate hatching of the fertilized eggs into sac fries; and measurement of the length, weight and weight of the yolk-sac of the sac fries

Experimental design of second phase (contd.)

Hatching cage 1

- Sac fry stocked – 1000 per hatching cage
- Water discharge maintained – 0.033 L sec^{-1}

Hatching cage 2

*Sac fry stocked – 1000 per hatching cage

*Water discharge maintained – 0.033 L sec^{-1}

Figure 20: Setting of sac fries for incubation, passing through endogenous feeding in hatching cages up to the hatching of free swimming fries. Two hatching cages – Hatching cage 1 and Hatching cage 2 which could be adjusted in one hatching raceway cum tank

Exogenous feeding experiment of artificial feed

1 T_1	**3** T_3	**5** T_4	**7** T_1	**9** T_3	**11** T_3	**13** T_4	**15** T_2
2 T_2	**4** T_4	**6** T_3	**8** T_4	**10** T_1	**12** T_2	**14** T_2	**16** T_1

Figure 21: Exogenous feeding of three formulated and one control diets of free swimming fries, fries, and fingerlings in sixteen nursing cum feeding cum rearing cages (1 to 16) kept in nursing cum feeding cum rearing raceway cum tank for 150 days. The silkworm pupae diet was Treatment 1 referred to as T_1, silkworm moths diet Treatment 2 referred to as T_2, synthetic amino acids diet Treatment 3 referred to as T_3 and shrimp meals diet Treatment 4 referred to as T_4 in random setting of the cages as shown in the figure

3.7.3 Third phase of experimental work

This phase was deployed for one year starting from June 2011 and completing in May 2012. Every month measurement of water quality parameters of the raceway pond water and the vicinity was performed for 12 months. Broods were fed as per protocol given in the fourth paragraph of the sub-chapter 3.7.2.1 **Management of the rainbow trout brood for captive propagation.** 1.0^+ spent up broods (first spawners) of the second phase was stocked in Raceway 1 (R 1) and Raceway 2 (R 2) for seven months and were known as experimental brood and the raceway called experimental brood raceway (Figure 22). The maturity of the gonads, mortality/survival, growth, health and behaviour of the broods were studied. Experimental broods of the first phase were treated as **future broods** – half number of which were left in the Raceway 1 (**R 1)** and rest half were stocked in Raceway 2 (**R 2)** (Figure 23) for two months (June and July). Future broods became **broods** when males developed rough upper surface on pectoral fins and females swollen belly and future brood raceways became **brood raceways** after two months. Broods were kept in the **R 1** and **R 2** (Figure 24) for one month (August). Broods became **segregated broods** when males were bright and brilliant in

colour with compressed abdomen and elongated lower jaw, sometimes curved upwards like a hook and some appearing darker in colour, almost black and females comparatively light-coloured than males but with swollen and enlarged abdomen containing slightly reddish vent and brood raceways became **segregated brood raceways** after one month. Broods which were segregated sex-wise were called segregated broods and were put into two separate segregated brood raceways: male in **R 1** and female **in R 2** (Figure 25) for two months (September and October). Segregated broods became **current broods** when segregated males oozed milt on pressing their abdomen and females eggs and segregated brood raceways became **current brood raceways** after two months. Current broods were left in two current brood raceways **R 1** and **R 2** (Figure 26) during November. Current broods became **gravid broods** (2.0^{+} broods or second spawners) when males and females developed complete sign of readiness for breeding and current brood raceways became **gravid brood raceways** before breeding and were left in gravid brood raceways **R 1** and **R 2** (Figure-27) during 1^{st} and 2^{nd} week of November 2010 and 2011 respectively. Fifty gravid broods were collected out of which six gravid males and twelve gravid females were selected for the artificial propagation and put into happas, **Happa 1** and **Happa 2** (Figure 28).

Experimental design of third phase

Raceway 1

- Spent up broods stocking (number) – 100
- Spent up broods stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2})
- Fed 45% CP containing artificial pellet feed @ 2-3% of their body weight twice daily
- Water discharge – 0.042 L sec^{-1}

Raceway 2

- Spent up broods stocking (number) – 100
- Spent up broods stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2})
- Fed 45% CP containing artificial pellet feed @ 2-3% of their body weight twice daily
- Water discharge – 0.042 L sec^{-1}

Figure 22: Two spent up cum experimental brood raceways – Raceway 1 (R 1) on the left side and Raceway 2 (R 2) on the right side

Experimental design of third phase (contd.)

Raceway 1	Raceway 2
➢ Future broods stocking (number) – 75 ➢ Future broods stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 35% CP containing artificial pellet feed @ 2-3% of their body weight twice daily ➢ Water discharge – 0.42 L sec^{-1}	➢ Future broods stocking (number) – 75 ➢ Future broods stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 35% CP containing artificial pellet feed @ 2-3% of their body weight twice daily ➢ Water discharge – 0.42 L sec^{-1}

Figure 23: Two future brood raceways – Raceway-1 (R-1) on the left side and Raceway-2 (R-2) on the right side. R 1 was the one in which spent up broods were stocked in the second phase of experimental work and the R 2 was newly arranged as per requirement

Raceway 1	Raceway 2
➢ Broods stocking (number) – 75 ➢ Broods stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 2-3% of their body weight twice daily ➢ Water discharge – 0.52 L sec^{-1}	➢ Broods stocking (number) – 75 ➢ Broods stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 2-3% of their body weight twice daily ➢ Water discharge – 0.52 L sec^{-1}

Figure 24: Two brood raceways. Future brood raceways R 1 and R 2 that became brood raceways R 1 and R 2 respectively

Raceway 1	Raceway 2
➢ Segregated males stocking (number) – 75 ➢ Segregated males stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 1-2% of their body weight twice daily ➢ Water discharge – 0.63 L sec^{-1}	➢ Segregated females stocking (number) – 75 ➢ Segregated females stocking (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 1-2% of their body weight twice daily ➢ Water discharge – 0.63 L sec^{-1}

Figure 25: Two segregated brood raceways – R 1 for males and R 2 for females

Raceway 1	Raceway 2
➢ Current broods stocking (males) (number) – 75 ➢ Current broods stocking (males) (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 1% of their body weight 4-5 times weekly ➢ Water discharge – 0.63 L sec^{-1}	➢ Current broods stocking (females) (number) – 75 ➢ Current broods stocking (females) (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 1% of their body weight 4-5 times weekly ➢ Water discharge – 0.63 L sec^{-1}

Figure 26: Two current brood raceways. Segregated brood raceways R 1 and R 2 that became current brood raceways R 1 and R 2 respectively

Raceway 1	Raceway 2
➢ Gravid broods stocking (males) (number) – 75 ➢ Gravid broods stocking (males) (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 45% CP containing artificial pellet feed @ 1% of their body weight 4-5 times weekly ➢ Water discharge – 0.63 L sec^{-1}	➢ Gravid broods stocking (females) (number) – 75 ➢ Gravid broods stocking (females) (rate) – 5 to 10 kg m^{-2} (15 to 20 trout m^{-2}) ➢ Fed 40% CP containing artificial pellet feed @ 1% of their body weight 4-5 times weekly ➢ Water discharge – 0.63 L sec^{-1}

Figure 27: Two gravid brood raceways. Current brood raceways R 1 and R 2 that became gravid brood raceways R 1 and R 2 respectively

Experimental design of third phase (contd.)

Happa 1	Happa 2
➢ Gravid broods (males) – 6 ➢ Water discharge – 0.063 L sec^{-1}	➢ Gravid broods (females) – 12 ➢ Water discharge – 0.063 L sec^{-1}

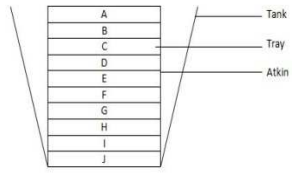
Figure 28: Gravid broods – males in Happa 1 and females in Happa 2

Stripping cum fertilization cum mixing tray

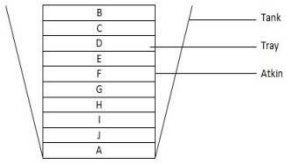
➢ Eggs – Weight (g) recorded
➢ Milt – Volume (ml) recorded
➢ Fertilization – eggs : milt :: numerical : numerical

Figure 29: Stripping and fertilization was done at 4:00 p.m. creating darkness in the hatchery and recording air temperature. Stripping was done only once

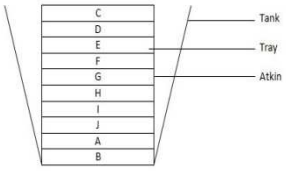
Incubation, cleaning and hatching of fertilized eggs into sac fries



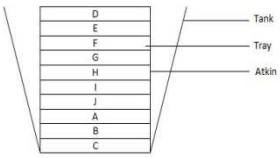
Incubation of fertilized eggs



First cleaning and readjustment



Second cleaning and readjustment



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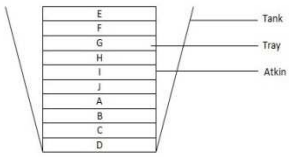
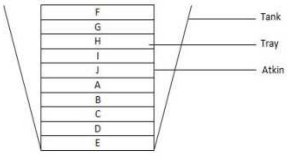


Fourth cleaning and readjustment



Hatching of incubated eggs into sac fries

Figure 30: Incubation of fertilized eggs in ten locally-made incubation cum hatching trays (A, B, C, D, E, F, G, H, I and J) stacked in an atkins put inside incubation cum hatching raceway cum tank maintaining water discharge of 0.033 L sec^{-1} and creating darkness in the hatchery; weekly cleaning of spoilt eggs, recording of their mortality to calculate survivability and readjustment of the trays thrice to maintain their equilibrium as shown in the figure; ultimate hatching of the fertilized eggs into sac fries; and measurement of the length, weight and weight of the yolk-sac of the sac fries

Hatching cage 1	Hatching cage 2
➢ Sac fry stocked – 1000 per hatching cage ➢ Water discharge maintained – 0.033 L sec^{-1}	➢ Sac fry stocked – 1000 per hatching cage ➢ Water discharge maintained – 0.033 L sec^{-1}

Figure 31: Setting of sac-fries for incubation, passing through endogenous feeding in hatching cages up to the hatching of free swimming fries. Two hatching cages – Hatching cage 1 and Hatching cage 2 that could be adjusted in one hatching raceway cum tank

Experimental design of third phase (contd.)

Exogenous feeding experiment of artificial feed

1 T_1	3 T_3	5 T_4	7 T_1	9 T_3	11 T_3	13 T_4	15 T_2
2 T_2	4 T_4	6 T_3	8 T_4	10 T_1	12 T_2	14 T_2	16 T_1

Figure 32: Exogenous feeding of three formulated and one control diets of free swimming fries, fries, and fingerlings in sixteen nursing cum feeding cum rearing cages (1 to 16) kept in nursing cum feeding cum rearing raceway cum tank for 150 days. The silkworm pupae diet was Treatment 1 referred to as T_1, silkworm moths diet Treatment 2 referred to as T_2, synthetic amino acids diet Treatment 3 referred to as T_3 and shrimp meals diet Treatment 4 referred to as T_4 in random setting of the cages as shown in the figure

3.8 Methods for examination of the present work

The methods for spatio-temporal dynamics of the raceway water and its surroundings, captive propagation of the rainbow trout and feeding of the artificial feed have been given below:

3.8.1 Methodology for physico-chemical parameters

Two parameters such as altitude and water resource were measured and observed respectively at the beginning of the study. Altitude which was measured with the help of altimeter and expressed in metre above sea level (masl) was the average of five locations in and around raceways' premises. Water resource supplying the raceways was confirmed through observation. The data of relative humidity and rainfall which

were measured with the help of hygrometer and rain gauze respectively were taken from the Department of Meteorology, Government of Nepal.

3.8.1.1 Water sample collection for the analyses of water quality variables

Water sample collections were made at monthly intervals for two years from June 2010 to May 2012 each year representing four seasons – monsoon (June to August), post-monsoon (September to November), winter (December to February) and pre-monsoon (March to May). Collections were made at five different locations in raceways – at the entry point, at the outlet, and at three locations in between the two, to get an average. The samples were collected in between 8:00 to 9:00 a.m.

During water sampling, emphasis was also given on noting down the colour (whether colourless, silty, or hazy) and transparency (whether clean or less clean or dirty) of the raceway water respectively through observation and Secchi disc. Further, the odour (whether odourless, less pungent or pungent) and taste or flavor (whether tasteless, less tasty or tasty) of the raceway water was determined by Flavour Rating Assessment (FRA) as mentioned by APHA (2005).

3.8.1.2 Water analyses procedure

Water quality variables were measured and the method applied for the determination of the water quality parameters in the present work has been summarized.

3.8.1.2.1 Air and water temperature

Air temperature and water temperature were measured on the spot using a calibrated Germany made standard mercury-in-glass thermometer, graduated (0 to 50 $^{\circ}$C) with an accuracy of 0.1 $^{\circ}$C, avoiding direct sunlight and expressed in degree Celsius ($^{\circ}$C). For air temperature, the thermometer was held upright in the air with the help of fingers and with the lower part exposed to the air for about five minutes (Adoni *et al*. 1985). For water temperature, the thermometer was immersed in water 6 cm below

the water surface and left to stabilize for about five minutes (Adoni *et al*. 1985). The average values of air temperature and water temperature were recorded.

3.8.1.2.2 Water velocity

To measure water velocity on the spot, a distance of 10 m at the sampling site in the feeding channel of raceways was taken. A float (an orange-coloured cork) was released at the initial position and the time taken to travel the distance was measured with the help of stopwatch (Adoni *et al*., 1985). The velocity was calculated and expressed in metre per second (m sec^{-1}).

3.8.1.2.3 Water discharge

To measure water discharge (bucket method) on the spot, a plastic tank of 100 L in the sampling site was taken and kept below the feeding channel of the raceways to fill it up with flowing water and at the same time, the time taken to fill the water was measured with the help of stopwatch (Klunne, 2009). The water discharge (volume ÷ time) was calculated and expressed in litre per second (L sec^{-1}).

3.8.1.2.4 Turbidity

The turbidity was determined by sample collection according to Svobodova *et al*. (1993) and analyzing the sample in the laboratory using a Hach-made turbidometer (model 2001A). The turbidity was expressed in Nephelo-turbidity unit (NTU).

3.8.1.2.5 pH and electrical conductivity

pH and electrical conductivity were measured on the spot by Hanna-make battery operated pocket pH and conductivity meter (211–Microprocessor) and expressed in numerical (1-14) and micro-Siemens per centimetre (µS cm^{-1}) respectively.

3.8.1.2.6 Dissolved oxygen, free carbon dioxide, total alkalinity and total hardness

The chemical parameters like dissolved oxygen (Standard Winkler's method), free carbon dioxide (Nassler's method), total alkalinity (Titration method using

phenolphthalein and methyl orange indicators) and total hardness (EDTA Titration method) were determined by sample collection according to Svobodova *et al*. (1993) and analyzing the sample in the laboratory through titration methods following Standard Methods (APHA, 2005) and expressed in milligram per litre (mg L^{-1}).

3.8.1.2.7 Nitrate, ammonium and phosphate

The nutrients like nitrate, ammonium and phosphate were determined by sample collection according to Svobodova *et al*. (1993) and analyzing the sample in the laboratory by the aid of spectrophotometer (Syntronics-model 106). The concentration of nitrate (Brucine sulphate method) was determined as described by Trivedy and Goel (1984) and expressed in milligram per litre (mg L^{-1}). Again, by the help of spectrophotometer (Syntronics-model 106), the concentration of ammonium (Phenol-hypochloride or Phenate method) and phosphate (Stannous chloride method) were determined following APHA (2005) and expressed in milligram per litre (mg L^{-1}).

3.8.2 Breeding of rainbow trout to get free swimming fries

Free swimming fries, for the present investigation, were obtained by the captive breeding of rainbow trout.

3.8.2.1 Management of rainbow trout brood for captive propagation

The data collection of the maturity of gonads of the broods (based on the study of their pectoral fins, belly and exuding of milts and eggs) to know their breeding condition; mortality to record their survivability; growth (difference in length and weight) to manage crude protein percent of the diet (based on the stage of brood), rate of feeding (based on body weight), feeding frequency (based on the condition of brood) and water discharge of the raceway (based on the stage of brood); disease surveillance (Figure 33 and Figure 34), in terms of parasites and pathogens, to keep

the broods healthy and any change in behavioural activity like activeness, competition, cannibalism and so on (Figure 35) were done as per the present protocol.

Fingerlings were stocked for one year in a farmer's raceway to make them experimental broods (Figure 36) for the present work. They were fed 45% crude protein containing diet at the rate of 8 to 10% of their body weight 8 to 10 times day^{-1} for three months in water discharge of 0.083 L sec^{-1} m^{-2} (0.42 L sec^{-1} in the raceway), 45% crude protein containing diet at the rate of 5 to 8% of their body weight 6 to 8 times day^{-1} for another three months in water discharge of 0.1 L sec^{-1} m^{-2} (0.5 L sec^{-1} in the raceway), 40% crude protein containing diet at the rate of 3 to 5% of their body weight 4 to 6 times day^{-1} for the next three months in water discharge of 0.2 L sec^{-1} m^{-2} (1 L sec^{-1} in the raceway) and 35% crude protein containing diet at the rate of 2-3% of their body weight 3 to 4 times day^{-1} for the last three months in water discharge 0.3 L sec^{-1} m^{-2} (1.5 L sec^{-1} in the raceway). The first spawners or 1.0^{+} spent up broods were taken as experimental broods and selected as future broods of the next year.

Figure 33: Collection of rainbow trout broods for disease surveillance to check for parasites and pathogens

Figure 34: Carrying rainbow trout broods to the laboratory for disease surveillance to check for parasites and pathogens

Figure 35: Actively swimming fingerlings for the feeding of artificial feed

Figure 36: Fingerlings stocked to use them as experimental broods

Future broods (Figure 37), broods (Figure 38), segregated broods (Figure 39), current broods (Figure 40), and gravid broods (Figure 41 and Figure 42), for the research experiment, were maintained based on spawning time (November to February in Nepal). Broods were selected based on selection criteria through observation of the external appearance of the whole body; confirmed on the basis of confirmation criteria through observation of the external appearance of the pectoral fins, abdomen and vent; stocked (weight m^{-2} and number m^{-2}) as per mentioned in experimental design; kept in water discharge as given in experimental design; measured total growth (total length and total weight) and actual growth (difference in length and difference in weight) and fed crude protein containing artificial pellet feed based on the stages of the broods as mentioned below:

Figure 37: Future rainbow trout brood

Figure 38: Rainbow trout brood

Future broods (Figure 37) were fed 35% crude protein containing artificial feed at the rate of 2 to 3% of their live body weight 2 times day^{-1}, broods (Figure 38) 40% crude protein containing artificial feed at the rate of 2 to 3% of their body weight 2 times day^{-1}, segregated broods (Figure 39) 40% crude protein containing artificial feed at the rate of 1 to 2% of their body weight 2 times day^{-1}, current broods (Figure 40) 45% crude protein containing artificial feed at the rate of 1% of their live body weight 4 to 5 times $week^{-1}$ and gravid broods (Figure 41 and Figure 42) 45% crude protein containing artificial feed at the rate of 1% of their live body weight 4 to 5 times $week^{-1}$. Gravid males (Figure 41) and females (Figure 42) called 1.0^{+} broods or first spawners and 2.0^{+} broods or second spawners, after the complete ripening of their gonads (when with a gentle pressure on vent a female and male brood started oozing ova and milt respectively) became ready for breeding in captivity.

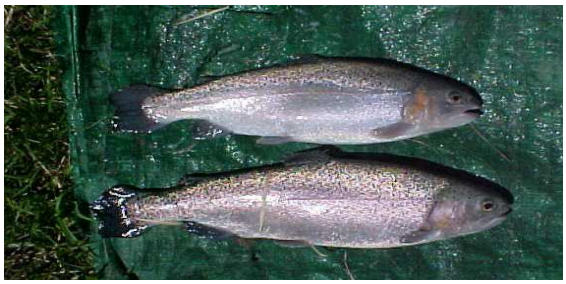


Figure 39: Segregated rainbow trout brood (upper male and lower female)

Figure 40: Current rainbow trout brood

Figure 41: Gravid rainbow trout brood (male)

Figure 42: Gravid rainbow trout brood (female)

3.8.2.2 Procedure of rainbow trout breeding

3.8.2.2.1 Stripping of egg and milt from gravid rainbow trout brood

Striping, also known as dry stripping method, was done to collect eggs (ova) from females (Figure 44) and milts (sperms) from male broods (Figure 43). Dark condition was created during stripping to assure more viability of eggs and milts. The quality and quantity of eggs and milts were assessed. Eggs of two females were poured in fertilization cum mixing tray and then milt of one male was poured over it. The mixing of eggs with that of milts was done for fertilization during evening creating dark condition in the hatchery. The dark condition was created during fertilization to ensure more viability of the eggs and milts and more rate of fertilization. After fertilization, fertilized eggs were segregated from the non-fertilized ones based on the colour. First spawners or 1.0^{+} spent up broods were procured with disinfectants and stocked for another year breeding to come however, second spawners or 2.0^{+} spent up broods were stocked in table fish raceway pond.

Figure 43: Stripping of eggs from female rainbow trout brood on a sieve to drip any water coming along with eggs

Figure 44: Stripping of milt from one male rainbow trout brood on the eggs of two female rainbow trout brood and then fertilization

3.8.2.2.2 Incubation and hatching of fertilized egg into sac fry

One thousand fertilized eggs were kept in incubation cum hatching trays (Figure 45 and Figure 46) of the size of 33 cm × 35 cm × 2.5 cm having screened bottom of the mesh size of 3 mm diameter. Ten such trays (numbering A to J) were stacked in each atkin (Figure 47). Five such atkins were put inside an incubation raceway cum tank

(Figure 48) in the hatchery. During incubation, water discharge of 0.017 L sec^{-1} (for 1st week), 0.033 L sec^{-1} (for 2nd week), and 0.05 L sec^{-1} (for 3rd, 4th and rest weeks) per 10,000 fertilized eggs was maintained in the incubation raceways. During whole incubation period, all the trays were cleaned and readjusted weekly-wise (3 to 4 times during incubation) as per requirement (Figure-49). Dead spoilt eggs were removed and by readjusting them perfect equilibrium was maintained in all the trays for hatchability, survival and growth of fertilized eggs into eyed-eggs, and then into sac fries. Observing eyed-eggs would confirm the tentative date of hatching of sac fries. Mortality of the fertilized eggs kept for incubation was counted at every cleaning and readjustment and expressed in number and percentage. All the procedure was done in the evening on the same day of fertilization creating dark condition in the hatchery. Dark condition was created as development and hatching of sac fries would be better.

Figure 45: Putting fertilized eggs on incubation cum hatching tray

Figure 46: Spreading fertilized eggs on incubation cum hatching tray with the help of a feather

Figure 47: Stacking ten incubation trays at one place to adjust in an atkin

Figure 48: Putting atkins in an incubation raceway cum tank

Now, hatching referred to the emergence of sac-fries (Figure 50). The days and cumulative water temperature (sum of water temperature of all the days) of hatching of sac fries were recorded and expressed in number of days and cumulative figures in $^{\circ}$C respectively. Sac fries were counted, their size (length, weight and weight of the yolk-sacs) measured, percentage of hatchability calculated, conversion and change calculated, and yolk (energy) utilized during the developmental process of conversion of fertilized eggs into sac fries and somatic growth of the sac fries was also calculated. The sac fries, thus, released were further procured into hatching cages for the release of free swimming fries.

Figure 49: Last cleaning and readjustment of incubation tray with eyed eggs

Figure 50: Hatching of sac-fries in hatching trays

3.8.2.2.3 Hatching of sac fry into free swimming fry

One thousand sac fries were put inside one hatching cage (Figure 51) of the size of 100 cm × 50 cm × 45 cm having polythene screened side and bottom of the mesh size of 0.3 cm freely hanging from a light wooden frame of 100 cm × 50 cm. Sac fries were transferred into hatching cages with the help of forceps. Two such hatching cages were kept inside one hatching raceway cum tank of the hatchery. During this setting, water discharge of 0.067 L sec^{-1} per 10,000 sac-fries was maintained in the hatching raceway. It was done in the morning at 8:00 to 9:00 a.m. creating dark condition in the hatchery. The dark condition was created as the development would

be better. The days of hatching or endogenous feeding period of the sac fries was noted down. Mortality of the sac-fries was counted and expressed in number and percentage. Free swimming fries were hatched (Figure 52) from the sac fries. Free swimming fries were counted, their size (length and weight) measured, percentage of hatchability calculated, conversion and change calculated, and yolk (energy) utilized during the developmental process of conversion of sac-fries into free swimming fries and somatic growth also calculated.

Figure 51: Setting of sac-fries in hatching cages for incubation passing through endogenous feeding

Figure 52: Hatching of free swimming fries in hatching cages

3.8.3 Artificial feed for rainbow trout brood and free swimming fry, fry and fingerling for the research work

No diet was formulated for broods as their artificial feed was based on the animal protein of shrimp meals only. However, three diets, first with silkworm pupae, second with silkworm moths and the third with synthetic protein of synthetic amino acids, as per aims and objectives based on the present research protocol, were formulated for the utility assessment and compared with that of the fourth diet with highly costly animal protein of shrimp meals acting as control.

3.8.3.1 Utility assessment and comparison of formulated and control artificial feed of rainbow trout for the research work

The artificial feed formulation is not an easy task. So, while formulating the diet of any fish including rainbow trout, the knowledge of its nutrient requirement is necessary. The nutrient requirement can be fulfilled through nutrient supplements. So, the information of nutrient supplements of rainbow trout becomes a must. Again, the nutrient supplement can be compensated through different feed ingredients. Therefore, the knowledge of feed ingredients is quite obvious. The feed formulations of free swimming fries, fries and fingerlings, according to the present protocol, were based on the utilization of different protein supplements especially non-conventional animal proteins and unconventional synthetic proteins but including conventional animal protein of shrimp meals for the experimental comparison with the previous ones however, plant protein supplements, energy supplements and mineral and vitamin supplements were common to the four diets prepared (three formulated and one control diets).

3.8.3.2 Feed ingredient of the feed formulations of rainbow trout

The feed ingredients of broods and free swimming fries, fries and fingerlings of rainbow trout as protein supplements, in the present work, were animal proteins of silkworm pupae (natural, non-conventional, cheaper ingredient, easily available, and substitute animal proteins), silkworm moths (natural, non-conventional, cheapest ingredient, easily available, and substitute animal proteins) and shrimp meals (natural, conventional, highly costly, available, and traditional animal proteins); synthetic proteins of synthetic amino acids (synthetic, unconventional, less costly, less available, alternative to animal proteins) having lysine and methionine; and plant proteins of soybean (costly ingredient) and mustard oilcake (less costly ingredient); as energy supplements comprised wheat and rice bran (less costly ingredient); as mineral

supplements consisted of mineral pre-mixes (costly ingredient); and as vitamin supplements contained vitamin pre-mixes (costly ingredient). Both minerals and vitamins are also called additives.

After choosing the feed ingredients (silkworm pupae, silkworm moths, lysine, methionine, shrimp meals, roasted soybean, mustard oilcake, wheat flour, and rice bran), their proximate analyses with reference to crude protein, crude fibre, crude lipid, ash and moisture were done following Kjeldahl Protein Analysis Method, Organic Residue Left Method, Soxhlet Extraction Method, Moful Furnace Method, and Loss in Weight Method (AOAC, 1995).

After proximate analyses, the diets were composed based on Pearson's square method. The diet for the experimental brood, future brood, brood, segregated brood, current brood, gravid brood, and spent up brood, which contained crude protein percent as mentioned in the second and fourth paragraphs of the sub-chapter **3.8.2.1 Management of the rainbow trout brood for captive propagation,** were composed by utilizing shrimp meals, soya bean, mustard oilcake, wheat, rice bran, mineral pre-mixes and vitamin pre-mixes. The three formulated and one control diets of free swimming fries, fries and fingerlings of rainbow trout, which comprised 45% crude protein percent, were composed by taking silkworm pupae as the first formulated diet, silkworm moths as the second formulated diet, lysine and methionine (3 : 1) as the third formulated diet and shrimp meals as the fourth but control diet with soybean, wheat, mineral and vitamin pre-mixes common to all the four diets.

After the composition of diets, their nutrition percentage was calculated to see whether nutrient requirements of the diets composed were fulfilled or not. Ultimately after the calculation of nutrition percentage, the artificial diets were processed.

3.8.3.3 Feed processing of the feed ingredient of formulated and control diet of rainbow trout

Feed processing of the ingredients of the artificial feed of rainbow trout was carried out. The feed processing comprised steps in the following orders – drying (Sun- or air-), heating, grinding, sieving, and screening. The feed ingredients like shrimp meals, mustard oilcake, wheat, and rice bran were sundried (Sun-drying) to decrease their moisture content. The ingredients like soybean were first sundried and then roasted (heating). The ingredients like silkworm pupae and silkworm moths, after they were brought from the sericulture farm, were first of all, air dried (air-drying) and then heated. The heating was done in an oven in the temperature of 300 to 350 $^{\circ}$C until moisture came from the ingredients. The dried ingredients were carried for grinding or milling thus converting shrimp meals into shrimp meals powder, mustard oilcake into mustard oilcake powder, wheat into wheat flour, rice bran into rice bran powder, roasted soybean into soybean powder, silkworm pupae into silkworm pupae powder, and silkworm moths into silkworm moths powder. Because lysine, methionine, mineral and vitamin pre-mixes were already in their dry and powdery form, they need no processing. However, mineral and vitamin pre-mixes were fortified in double quantity to compensate the loss during feed processing, preparation and storage. Sieving for the removal of chitin from silkworm pupae, silkworm moths, and shrimp meals and other unwanted materials, if any, from the ingredients was done. The ingredients were, then, passed (screening) through the steel sieve of 180 μ. The screening (Table 1) of the ingredients was done to make their particles uniformity in size. After processing of the ingredients, the diets were prepared.

Table 1: Screening of the artificial feed, that is, crumble feed (CF) for the free swimming fry and fry and pellet feed (PF) for the fingerling after final dough making and before feed preparation along with the feeding rate, feeding frequency and stocking density during exogenous feeding period.

Size of the steel sieve / Artificial feed	Diameter of the wire of the steel sieve (μ)	Size of the aperture of the steel sieve (μ)	Feeding rate (%) at the rate of body weight day^{-1}	Feeding frequency (times day^{-1})	Stocking density (number m^{-2})	Remarks
CF^{1}	126	180	15	12	250	FSFs
CF^{2}	171	240	15	12	-	FSFs
CF^{3}	290	425	12	10	-	FSFs
CF^{4}	340	500	12	10	-	FSFs
CF^{5}	390	600	12	10	-	Fries
CF^{6}	450	710	12	10	-	Fries
CF^{7}	523	800	10	8	-	Fries
CF^{8}	588	1000	10	8	-	Fries
PF^{1}	717	1400	10	8	-	Fingerlings
PF^{2}	840	1700	10	8	-	Fingerlings

CF = crumble feed, PF = pellet feed, CF^{1} = crumble feed one, CF^{2} = crumble feed two, CF^{3} = crumble feed three, CF^{4} = crumble feed four, CF^{5} = crumble feed five, CF^{6} = crumble feed six, CF^{7} = crumble feed seven, CF^{8} = crumble feed eight, PF^{1} = pellet feed one, PF^{2} = pellet feed two and FSFs = free swimming fries.

3.8.3.4 Feed preparation for brood and free swimming fry, fry and fingerling of rainbow trout

After feed processing, the feed ingredients were taken by weight for the preparation of artificial feed. The feed preparation involves steps in the following orders like mixing, agglomerating, drying, heating, screening, crumbling or pelleting. The ingredients were taken in a container and mixed manually. The manually mixed ingredients were put inside a mixer for thorough mixing and proper agglomerating. The properly agglomerated feed ingredients were taken out from the mixer and added with some

quantity of water to make dough. Attention and care were taken not to add more quantity of water at a time making loose dough of no use but little water slowly but continuously along with mixing until hard dough was made. The dough was, initially, procured manually and then inside the mixer. The dough was, finally, put under screening, that is, passing the dough through steel sieve of required size (Table 1), followed by crumbling (for crumble feed) or pelleting (for pellet feed) in the crumble and pellet feed preparing machine. The artificial feed was then put into drying and ultimately into heating. It was dried in 50 $^{\circ}$C in a constant temperature oven and then bagged, tagged and stored in deep freezer until used. The diet, thus prepared, for the broods was called diet of broods (Figure 53) and for the free swimming fries, fries and fingerlings was known as formulated and control diets (Figure 54).

Figure 53: Upper row: artificial pellet feed for fingerlings to gravid broods. Lower row: crumble feed for free swimming fries to fingerlings.

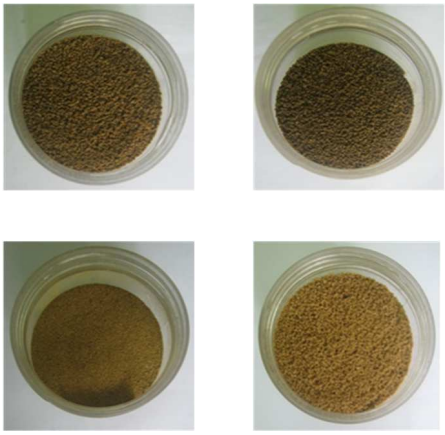


Figure 54: Formulated diets of silkworm pupae, silkworm moths and synthetic amino acids and control diet of shrimp meals respectively after processing and preparation

The artificial feed was prepared in two forms – crumble feed (CF) and pellet feed (PF). The CF was prepared for free swimming fries and fries whereas the PF for fingerlings and broods. The CF prepared for the free swimming fries were of four sizes from CF^1 to CF^4 based on the size of the gape of the mouth. The crumble feed

from CF^1 to CF^4 are also called starter feed. The CF prepared for the fries were also of four sizes from CF^5 to CF^8 again based on the size of the gape of the mouth. The PF prepared for the fingerlings were again of two sizes from PF^1 to PF^2 further based on the size of the gape of the mouth (Figure-54). The PF prepared for the broods were further of two sizes from PF^3 to PF^4 again based on the size of the gape of the mouth. The PF are also known as main feed.

After preparation of the artificial feed, the proximate analyses and feed cost (FC) were done and calculated respectively. The proximate analyses of the diets with reference to crude protein, crude fibre, crude lipid, ash and moisture were done following Kjeldahl Protein Analysis Method, Organic Residue Left Method, Soxhlet Extraction Method, Moful Furnace Method, and Loss in Weight Method (AOAC, 1995) to know whether crude protein percent of the diets prepared coincide with the required crude protein percent or not. In this way, besides preparation of the diet of the broods, four types of artificial feed including three formulated and one control diets were prepared for free swimming fries, fries and fingerlings of rainbow trout.

The price kg^{-1} of the feed ingredients was noted down from the nearby retail market and also verified from the concerned wholesale market. The feed cost (FC) was calculated from kg^{-1} price of the feed ingredients preparing 1 kg of the artificial feed. The production cost (PC) was calculated from the feed cost (FC) and feed conversion ratio (FCR). Further, the cost analyses (CA) was done by the comparison of production cost (PC) with that of feed cost (FC).

3.8.3.5 Formulated and control diets prepared for free swimming fry, fry and fingerling of rainbow trout for exogenous feeding

The three formulated diets were silkworm pupae diet (Treatment 1 abbreviated as T_1), silkworm moths diet (Treatment 2 abbreviated as T_2) and synthetic amino acids diet

(Treatment 3 abbreviated as T_3) and the control diet was shrimp meals diet (Treatment 4 abbreviated as T_4). Each treatment had quadruplicates, i.e., four replicas. The T_1, T_2, T_3 and T_4 (Figure 53) were fed to the free swimming fries, fries and fingerlings during exogenous feeding period in locally-made nursing cum feeding cum rearing cages kept inside nursing cum feeding cum rearing raceway cum tank respectively during nursing, feeding and rearing for a period of 150 days in two consecutive years, the first year (from December 2010 to May 2011) and the second year (from December 2011 to May 2012) as per the protocol mentioned in the experimental design. Two hundred and fifty (250) free swimming fries m^{-2} were stocked inside one nursing cage of the size of 100 cm × 100 cm × 100 cm having polythene screened sides and bottom of the mesh size of 3 mm diameter freely hanging from a light wooden frame of 100 cm × 100 cm. Free swimming fries were transferred into nursing cages with the help of small but handy scoop net. Sixteen such nursing cages (numbering 1 to 16) were kept inside one nursing raceway cum tank. All the treatments with their quadruplicates were set in the sixteen nursing cages by random sampling method and continued upto the last feeding experiment.

3.8.3.5.1 Methodology for the nursing of free swimming fry in nursing cage

Nursing was the procurement of free swimming fries (Figure 55) in locally-made nursing cages put into nursing raceway cum tank for the starting of exogenous feeding of the artificial feed. Nursing was done up to 2 months. Free swimming fries were fed 45% crude protein containing formulated diets of silkworm pupae (Treatment 1), silkworm moths (Treatment 2), and synthetic amino acids (Treatment 3) and the control diet of shrimp meals (Treatment 4) in the form of crumble feed (CF^1 and CF^2) at the rate of 15% of their body weight 12 times daily for 1 month (CF^1 for 15 days and CF^2 for 15 days) and crumble feed (CF^3 and CF^4) at the rate of 12% of their body

weight 10 times daily for the another one month (CF^3 for 15 days and CF^4 for 15 days) (Table 1). During nursing, water discharge was maintained 0.083 L sec^{-1} m^{-2}. The monthly-wise water quality parameters like water temperature, pH, dissolved oxygen, and free carbon dioxide were noted down. Mortality, survivability, total feed intake (TFI) and growth of the free swimming fries was recorded. The mortality of the free swimming fries was counted and expressed in number. The percentage of survivability was calculated by deducting mortality. The total feed intake (TFI) of free swimming fries was calculated and expressed. The growth through length and weight of free swimming fries were calculated by taking a sample of 10 from each nursing cage (Ricker, 1975). The free swimming fries growing to the size of 2.5 cm were called fries.

Figure 55: Nursing of free swimming fries in nursing cages

Figure 56: Release of fries in nursing cages, now called feeding cages

3.8.3.5.2 Procedure for the feeding of fry in feeding cage

Feeding was the procurement of fries in locally-made feeding cages (Figure 56) kept into feeding raceway cum tank for feeding of the artificial feed during exogenous feeding period. The nursing cages then became feeding cages. Feeding (Figure 57) was done up to 2 months. Fries were also fed 45% crude protein containing formulated diets of silkworm pupae (T_1), silkworm moths (T_2) and synthetic amino acids (T_3), and control diets of shrimp meals (T_4) in the form of crumble feed (CF^5

and CF^6) at the rate of 12% of their body weight 10 times daily for 1 month (CF^5 for 15 days and CF^6 for 15 days) and crumble feed (CF^7 and CF^8) at the rate of 10% of their body weight 8 times daily for another the 1 month (CF^7 for 15 days and CF^8 for 15 days) (Table 1). During feeding, water discharge was maintained 0.083 L sec^{-1} m^{-2}. The monthly-wise physico-chemical parameters like water temperature, pH, dissolved oxygen, and free swimming fries were noted down. Mortality, survivability, total feed intake (TFI) and growth of the fries was recorded. The mortality of the fries was counted and expressed in number. The percentage of survivability was calculated by deducting mortality. The total feed intake (TFI) of fries was calculated and expressed. The growth through length and weight of fries (Figure 57 and Figure 58) were calculated by taking a sample of 10 from each feeding cage (Ricker, 1975). The fries growing to the size of 7.5 cm are known as fingerlings.

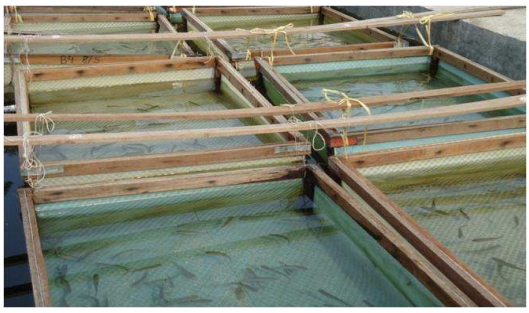


Figure 57: Feeding of fries in feeding cages

Figure 58: Collection of fries from feeding cages for length and weight measurement

3.8.3.5.3 Methods for the rearing of fingerling in rearing cage

Rearing (Figure 60) was the procurement of fingerlings in locally-made rearing cages (Figure 61) put into rearing raceway cum tank for the consumption of the artificial feed during exogenous feeding period. The feeding cages then became rearing cages. Rearing was done up to 1 month. Fingerlings were fed 45% crude protein containing formulated diets of T_1, T_2 and T_3 and the control diet of T_4 in the form of crumble feed

(PF^1 and PF^2) at the rate of 10% of their body weight 8 times daily for 1 month (PF^1 for 15 days and PF^2 for 15 days) (Table 1). During feeding, water discharge was maintained 0.083 L sec^{-1} m^{-2}. The monthly-wise water quality variables like water temperature, pH, dissolved oxygen, and free carbon dioxide were noted down. Mortality, survivability, total feed intake (TFI) and growth of fingerlings were recorded. The mortality of the fingerlings was counted and expressed in number. The percentage of survivability was calculated by deducting mortality. The TFI of fingerlings was calculated and expressed. The growth through length (Figure 62) and weight (Figure-63) of fingerlings (Figure-59) were calculated by taking a sample of

10 from each feeding cage (Ricker, 1975). Advanced fingerlings were released (Figure-64) in stocking raceway pond.

Figure 59: Length and weight measurement of fries

Figure 60: Release of fingerlings in feeding cages, now called rearing cages

Figure 61: Rearing of fingerlings in rearing cages

Figure 62: Length measurement of fingerlings

Figure 63: Weight measurement of fingerlings

Figure 64: Release of advanced fingerlings in rearing cages to be transferred in the stocking raceways

3.8.3.6 Survival and growth of free swimming fry, fry and fingerling of rainbow trout during exogenous feeding period

The utility of the three formulated diets with cheapest animal protein source of silkworm pupae (Treatment 1), cheapest animal protein source of silkworm moths (Treatment 2), and less costly synthetic proteins of synthetic amino acids (Treatment 3) were assessed; the three formulated diets, mentioned above, were compared to the control diet with highly costly animal protein source of shrimp meals (Treatment 4); the diets of animal proteins containing silkworm pupae, silkworm moths and shrimp meals were evaluated against the diet containing synthetic proteins of synthetic amino acids; the impact of the age of the broods, and size of the broods, eggs, sac-fries, and free swimming fries were compared to the physico-chemical parameters and the artificial feed; and the diet formulated with cheap animal protein source of silkworm pupae proved low cost or not compared to rest of the diets with silkworm moths, synthetic amino acids and shrimp meals through **survival** (SR) (Figure 65 and Figure 66) and **growth** (GR) (Figure 67 and Figure 68) of the free swimming fries, fries, and fingerlings of rainbow trout during exogenous feeding period.

Figure 65: Recording of the mortality and mortality percentage

Figure 66: Calculation of survivability and survival percentage

Figure 67: Measurement of length for the measurement of growth

Figure 68: Measurement of weight for the measurement of growth

3.8.3.6.1 Survival of free swimming fry, fry and fingerling due to three formulated and one control diets

The data of mortality (MR) were recorded at every 15 days (one fortnight) intervals up to 5 months in two consecutive years (first year from December 2010 to May 2011 and the second year from December 2011 to May 2012) and expressed through calculation. The survivability (SR) was calculated from mortality (MR) through the formulae (Ricker, 1975) given below:

1. MR (%) = {number of dead fish ÷ number of stocked fish} × 100
2. SR (%) = {number of survived fish ÷ number of stocked fish} × 100

3.8.3.6.2 Growth of free swimming fry, fry and fingerling due to three formulated and one control diets

The data of growth (GR) and total feed intake (TFI) were recorded at every 15 days (one fortnight) intervals up to 5 months in two consecutive years (first year from December 2010 to May 2011 and the second year from December 2011 to May 2012) and expressed through calculation. The growth is measured in two ways – total growth (total length and total weight) and actual growth (difference in length and difference in weight). The actual growth, also called growth (GR), which is the difference between final and initial length and between final and initial weight, was measured by taking 10 samples for each (Ricker, 1975) and calculated by the formulae given below:

1. GR (cm) = final length (L_2) – initial length (L_1)
2. GR (g) = final weight (W_2) – initial weight (W_1)
3. TFI (g $fish^{-1}$) = [{Weight (g) of fish × Percentage (%) of diet supplied} ÷ 100] × number of days

The growth (GR) was further assessed through total protein intake (TPI) along with growth parameters also called feed efficiency indicators of 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) including the feed cost (FC), production cost (PC) and highest growth period (HGP). The cost analysis (CA) was done by the comparison of PC with that of FC. The HGP was recorded by the comparison of growth (GR) of different months. The survival (MR and SR) and growth (TFI, TPI, FE, PER, AGR, SGR, RGR, *K*, FCR and PPV) were recorded, measured and calculated following Ricker (1975), Dogan and Bircan (2015) and Wang *et al*. (2015), the FC, PC and CA were studied following Ijaiya and Eko (2009) and the HGP by the

comparison of growth (length and weight) of different months. All the growth parameters and other indicators were calculated according to the formulae given below:

1. TPI (g $fish^{-1}$) = {total feed intake (g) × crude proteins (g) in the diet } ÷ 100
2. FE (%) = [{final weight W_2 (g) ─ initial weight W_1 (g)} ÷ mass (dry) of total feed intake (g)] × 100
3. PER (numerical) = {final weight (W_2) – initial weight (W_1) in g} ÷ total protein intake (g)
4. AGR (g day^{-1} and g $month^{-1}$) = {final weight W_2 (g) ─ initial weight W_1 (g)} ÷ time t between weightings
5. SGR (% day^{-1} and % $month^{-1}$) = [{final weight W_2 (g) ─ initial weight W_1 (g)} ÷ time t between weightings] × 100
6. RGR (%) = [{final weight W_2 (g) ─ initial weight W_1 (g)} ÷ final weight W_2 (g)] × 100
7. *K* (%) = {final weight W_2 (g) ÷ total length L^3 (cm)} × 100
8. FCR (numerical) = mass (dry) of total feed intake (g) ÷ increase in mass (wet) of animal product (g)
9. PPV = total protein intake (g) ÷ total feed intake (g) × 100
10. FC (kg^{-1} feed) (NRs) = cost of all ingredients preparing 1kg of feed
11. PC (kg^{-1} trout production) (NRs) = FCR × FC (kg^{-1} feed) (in NRs)
12. CA = comparison of PC (kg^{-1} rainbow trout) with that of FC (kg^{-1} feed)
13. HGP (months) = comparison of GR (in length and weight) of different months

3.8.4 Statistical analysis

The data of physico-chemical parameters, artificial breeding and artificial feed along with survival and growth (whether due to the impact of artificial feed or spatio-temporal dynamics or age of the broods, and size of the broods, eggs, sac fries and free swimming fries) were subjected to statistical analyses. Range (minimum – maximum), mean and standard error were calculated using MS-Excel statistical function of computer software (Windows 7). For the examination of individual

relationship between water quality variables, Pearson's correlation matrix was calculated using statistical software SPSS version 20. A one way analysis of variance (ANOVA) and two way analyses of variance (ANOVA without replication) and (ANOVA with replication) in MS-Excel were done to know significant differences in water quality variables, artificial breeding and artificial feed including survival and growth due to artificial feed, physico-chemical properties and age of broods, size of broods, eggs, sac fries and free swimming fries. The significant differences to compare and rank the means were done following t-test and to compare variances following F-test in MS-Excel. A level of significance $P < 0.05$ was used. The degree of linear relationship between water quality parameters, brood and breeding of rainbow trout and survival and growth were done using simple regression analyses of ANOVA in MS-Excel.