Comparative Growth performance of Genetically improved, Chinese, and Local Strains of Rainbow trout (Oncorhynchus mykiss) in Mid-Hill Nepal

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ABSTRACT

Aims: This study aimed to evaluate the growth performance of rainbow trout fry (*Oncorhynchus mykiss*) derived from the best performing families in a previous experiment.
Study design: Experimental
Place and Duration of Study: Fisheries Research Station (FRS), Trishuli Nepal, between April 2021 and June 2021.
Methodology:A total of 2700 fry with similar initial weights were randomly assigned to three groups based on their origin: Farmers Trout (T1:1.39±0.16g), Chinese Trout (T2: 1.40±0.06g), and Genetically Improved Trout (T3: 1.19±0.1g), each group replicated thrice with 300 fish per tank. The parameters assessed were weight gain, feed conversion ratio, and survival rate. All fish were fed farm-made feed for 90 days with fortnightly sampling. **Results:**At the experiment's conclusion, the Genetically Improved trout group exhibited the highest weight gain (14.22±0.51g), followed by the Chinese trout (13.08±0.24g), and Farmers trout (10.77±0.82g) groups (P=.01). The specific growth rate followed a similar trend, with the Genetic group showing the highest value of 4.28±0.08% and the Farmers group showing the lowest value of 3.60±0.07% (P=.01). However, there was no significant difference in the feed conversion ratio among the groups, which ranged from 0.77 to 1.07. Conversely, the Chinese group's fry demonstrated a significantly higher survival rate (77.94±3.63%) compared to the Genetic (72.33±1.09%) and Farmers (59.28±4.6%) groups (P=.02).
Conclusion: Therefore, it is concluded that the fries after selective breeding are genetically improved and, therefore exhibited superior growth traits. Hence genetically improved rainbow trout is recommended for distribution to local farmers and breeders to boost their productivity.

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Keywords: Chinese trout, Genetic Improvement, Selective breeding, Suiki-1, Hybrid

1. INTRODUCTION

Rainbow trout (Oncorhynchus mykiss) belongs to the Salmonidae family and is a valuable cold-water species that originates from the North Pacific Ocean and its tributaries in western North America and Eastern Asia [1]. It is reported that rainbow trout was first introduced in Nepal in 1988 from Japan, but the attempt failed due to the lack of technical expertise on its culture. In 2000, two strains of rainbow trout, namely Oncorhynchus mykiss Mera and Oncorh mykissDonalson, were introduced from Japan again along with their culture technology. These strains are now coll referred to as Japanese Trout in Nepal [2]. The objective of this introduction was to breed and commercialize rainbow trout in Nepal [2]. Likewise, two new strains of rainbow trout have been recently introduced from China, wh

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Oncorhynchus mykissSuiki-1, introduced in 2019, and Oncorhynchus mykiss Danasen introduced in 2021. These strains imported from China are known as Chinese trout in Nepal [2].

Following the introduction of Rainbow trout in Nepal and its various strains over time, Fishery Research Station (FRS) Trishuli (Nuwakot), Rainbow Trout Fishery Research Station (RTFRS) Dhunche (Rasuwa), and National Fishery Research Station (Godawari) have been conducting research on rainbow trout breeding, seed production technology, nutrition, feed, and health management aspects. Consequently, raceway, breeding, feeding, disease management and water supply technologies for this fish were developed and demonstrated to be suitable for Nepalese agro-ecological conditions. So far, out of 56 potential districts identified for rainbow trout farming, this technology has reached 38 districts, and this trend is growing every year in Nepal [3]. Since its introduction, rainbow trout has been popular among consumers and farmers, increasing its demand and has been adopted by more than 120 farmers of this region [2]. However, the dissemination of trout culture package is limited to a few districts and the majority of farmers remain small-scale operators. Consequently, the production of rainbow trout has not yet reached its full potential, resulting in a relatively low yield of approximately 400-420 metric tons every year [2-3]. This indicates that the sector has considerable room for growth and development.

In Nepal, rainbow trout production is entirely reliant on the seed supply from only ten to twenty trout hatcheries, both public and private, dispersed throughout the hilly region [2]. The lack of sufficient hatcheries and the need for validation of the technological package for trout farming in several potential districts have consistently hindered the expansion of this industry in Nepal. Additionally, farmers have recently reported issues with the slow growth rate of trout, particularly during the warmer summer months when temperatures surpass 20°C [2]. Apart from the increase in temperature, the main reason behind the poor performance of rainbow trout observed recently among farmers could be due to loss of genetic vigor because of inbreeding. It is because most of the hatcheries have their own small number of brood fish which they do not exchange between farms consequently resulting in isolated and genetically closed breeding system [4]. In a selectively bred, closed population, the probability of interbreeding increases due to the limited number of progenitors [5]. As generations progress, the diminished effective population size results in inbreeding depression, characterized by decreased growth rates, fertility reduction, and sub-optimal survival [6]. Consequently, there is an immediate need to increase the population size by crossing the broods of different location and to pinpoint the strains that exhibit superior growth and can withstand temperatures beyond their optimal range [2]. Owing to the fact that species in aquaculture are characterized by their rapid reproduction and significant phenotypic diversity [7], techniques such as mass selection and hybridization are considered efficient approaches for the genetic enhancement of fish [8]. A study in Chinook salmon (Oncorhynchus tshawytscha) has demonstrated that traits such as growth, feed intake, feed conversion ratio, and condition factor have low to moderate heritability and can be improved through selection [9]. Similarly, a study conducted in tilapias (Oreochromis sp.) showed that growth rate, survival, and FCR of hybrids of O. aureus and red tilapia (Oreochromis sp) brood fish females were enhanced when they were crossed with male of O. niloticus. In the same experiment, the author showed that the cold tolerance trait of O. aureus was inherited into a cold sensitive population of red tilapia after back crossing [10]. Such research has indicated that the hybrids resulting from crossbreeding possess significant commercial potential in the realm of fish farming in terms of growth and tolerance to adverse environmental conditions while minimizing inbreeding losses.

The primary objective of this research was to conduct a thorough assessment of the progeny derived from the crossbreeding of Japanese rainbow trout (Oncorhynchus mykiss.) collected from various regions in the mid-hills of Nepal. This assessment aimed to juxtapose their characteristics with those of the rainbow trout introduced from China (Chinese trout) and those cultivated locally (farmer's trout), with a particular emphasis on growth performance, feed conversion ratio, and survival rate. Consequently, the overarching aim was to identify, select, and advocate for the most efficient rainbow trout available in Nepal, thereby benefiting local farmers and breeders. This initiative is anticipated to significantly enhance trout farming practices in the region.

2. MATERIAL AND METHODS

2.1 Fish used and experimental design

The study was conducted at the Fishery Research Station (FRS), Trishuli Nepal. Every year, under the genetic improvement project, FRS Trishuli compares the performance of the fry obtained from selectively bred brood fish obtained from various locations of Mid-hill Nepal. To achieve this, FRS Trishuli have produced various

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Comment [MOU8]: Give a mapofexperimental location in country map trout families by switching the male and female brood (for crossing) sourced from Mardi (Kaski), Dhunche (Rasuwa), Danam (Makwanpur), Sankhani (Dhading) and Amare and Kimtang area of Nuwakot district in Nepal between 2017 and 2021. During the first year, FRS created 3 families (Family 1-3) by collecting brood fish from Mardi, Amare and Rasuwa. Later, an additional 4 families (Family 4-9) were created with the broods obtained from Daman, Sankhani, and Kimtang. During the first year the fry obtained from first three families of average weight 3.6g were cultured for 3 months while in the second phase fingerlings of 26.4g were cultured for one production cycle. At the end of the culture period, the preliminary analysis of the data showed that Family 3 (Mardi Female and Rasuwa Male) from first phase, and Family 8 (Daman Female and Sunkhani Male) had the highest specific growth rate [2]. For the current study, the fries of these two best performing families were referred to genetically improved and mixed together to form a treatment group. Similarly, the fry of Chinese trout (Danasen strain), and fries of local strain of Japanese trout grown by farmers (referred to Farmer's trout hereafter) were another two group for comparison. As such, current study was designed as a supplementary to the continuous genetic improvement project at FRS to compare the growth performance of Farmer's trout (T1) and Chinese trout (T2) against Genetically Improved trout (T3). For this, 2700 fry of average weight 1.32g in all three treatments were randomly distributed into 9 raceway tanks (2.0m × 0.55m × 0.25m) and fed with same farm-made diet (45% crude protein) by thoroughly grounding and mixing the ingredients given in Table 1 at the rate of 5% body weight twice a day for 90 days. Proximate analysis of the diet was done according to Official Method of Analysis 11 at the National Animal Nutrition Research Centre, Khumaltar Lalitpur.

Basic water quality parameters such as water temperature, dissolved oxygen, and pH were measured every week to ensure the optimum water quality available for the rainbow trout fry. Water temperature throughout the culture period was measured 16.45±0.35 ℃, dissolved oxygen was 7.05±0.49 mg/L and pH was measured to be 7.7±0.14.

Table 1: Feed composition and results of proximate analysis of experimental diets

Feed ingredients	g/kg	Proximate analysis	%
Shrimp meal	500.00	Moisture (%)	10.3
Soybean full fat	180.00	Ash (%)	10.5
Wheat flour	200.00	Crude protein (%)	45
Mustard oil cake	80.00	Lipid (%)	7.7
Rice bran	50.00	Crude fiber (%)	3.3
Vitamins mix[a]	10.00		
Mineral mix[b]	10.00		

[a] Vitamin mixture/kg premix containing the following: 33000IU vitamin A, 3300IU, vitamin D3, 410IU vitamin E, 2660mg Vitamin BI, 133mg vitamin B2, 580mg vitamin B6, 41mg vitamin B12, 50mg biotin, 9330mg choline chloride, 4000mg vitamin C, 2660mg Inositol, 330mg para-amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid. [b] Mineral mixture/kg premix containing the following: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5mg Cobalt.

2.2Sampling of fish for growth parameters

Fish were sampled bi-weekly for their length and weight, and the feeding ration was adjusted accordingly. At the end of experiment, growth performance was assessed in terms of weight gain, specific growth rate, feed conversion ratio, and survival rate according to Aqmasjed et al. [12].

WG (g) = Final weight (g) - Initial weight (g)
SGR (%) = 100 × [In (final weight) – In (initial weight)]/days
K = 100 × [final weight/ (final length)3]
FCR = Dry feed intake (g)/ weight gain (g)
SR (%) = 100 × (final number of fish/initial number of fish)

2.3Statistical analysis

The data collected during the sampling were recorded, calculated for the desired parameters, and tabulated using the MS-Excel in windows computer. All the results obtained were presented as Mean ± SE mean, and were analyzed using SPSS software (Version 25, IMB, Armonk, NY, USA) for the difference among the mean using one-way ANOVA. Before that, data were checked for normality and homogeneity of variance with the Shapiro-Wilk and Levene tests, respectively. When significant difference was detected, Tukey's multiple comparison test was conducted to compare the means among treatments. Means were regarded as significantly different when $P < .05$.

3. RESULTS AND DISCUSSION

The growth performance of the rainbow trout fry in all groups was assessed based on their final body weight, average body weight gain, specific growth rate (SGR), and survival rate (%). The weight of the fish in all the treatment groups exhibited a rise over a 90-day culture period, starting from their statistically similar original weight of 1.19-1.40g. The growth parameters, including final weight, weight gain, and specific growth rate (SGR), were significantly different across the treatment groups ($P=.05$). The corresponding data are provided in Table 2. The average weight gain of fry in the Genetically Improved group (T3) was 14.22±0.51g, while the Chinese group (T2) had a weight gain of 13.08±0.24g. Both numbers are significantly higher than the weight gain by Farmer's trout group (T1), which only reached 10.77±0.82g ($P<0.01$). An analogous trend was noted in the final body weight of juvenile fish in this investigation. Similarly, the specific growth rate (SGR) observed in the Genetically Improved group (4.28±0.08) was the greatest, but it was statistically similar to the Chinese group (3.92±0.16). However, the specific growth rate (SGR) observed in the Farmer's trout was significantly lower (3.60±0.07) compared to the Genetic groups ($P<0.01$), but there was no significant difference observed when compared to the Chinese trout. In addition, the feed conversion ratio (FCR) was highest in the Genetically Improved group (1.07±0.18), followed by the Chinese group (1.01±0.1). The lowest observed value (0.77 ±0.06) in the Farmer's trout group did not reveal any significant difference when compared to the other groups ($P>.05$).

The survival rate in this experiment varied from 59.28% to 72.33%, and there was a significant difference across the groups ($P=.05$). The survival ability of Farmer's trout was significantly lower compared to Chinese trout, with a rate of 77.94±3.63%. Nevertheless, its capacity to survive was similar to that of the fry of Genetically Improved group (72.33±1.09%). While the Genetic group had superior growth performance, it was unable to surpass the survival capabilities of the Farmers trout.

Table 2. Growth performance of progeny of genetically improved, Chinese, and Farmers trout

Parameters	Treatment groups			
	Farmers (T1)	**Chinese (T2)**	**Genetic (T3)**	**p-value**
Initial weight (g)	1.40±0.06 [a]	1.39±0.16 [a]	1.19±0.11[a]	0.38
Final weight (g)	12.17±0.86[b]	14.47±0.32 [a]	15.4±0.61[a]	0.03
Weight gain (g)	10.77±0.82[b]	13.08±0.24 [a]	14.22±0.51[a]	0.01
SGR (%)	3.60±0.07[b]	3.92±0.16 [ab]	4.28±0.08 [a]	0.01
FCR	0.77±0.06 [a]	1.01±0.1 [a]	1.07±0.18 [a]	0.28
Survival rate (%)	59.28±4.6[b]	77.94±3.63 [a]	72.33±1.09[b]	0.02

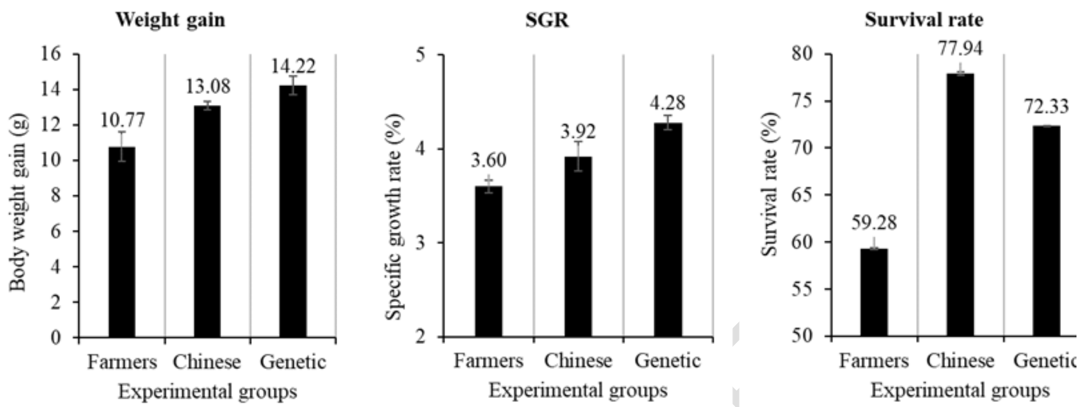

Fig. 1. Weight gain (g), specific growth rate (%), and survival rate (%) of progeny of genetically improved, Chinese, and Farmers trout reared for 90 days at FRS, Trishuli.
*Test groups: significant from normal control, * P < 0.05; ** P < 0.001*
Mean ± S.E.M = Mean values ± Standard error of means of six experiments

In aquaculture, several approaches are available for the genetic improvement of the cultured species. Some of these approaches include crossbreeding and hybridization, sex-control, chromosome manipulation, transgenesis, and selective breeding [13]. However, their relative practical applicability has not been described well in the publications on the field of aquaculture except for selective breeding approach. It is believed that the first experiment of selective breeding of fish was done on goldfish in China and later in Japan following which the goldfish have been changed in a spectacular manner in its important aspects such as color, scale, and fin. It is said that selective breeding is the only approach that allowed continued genetic gain and can be made permanent. It is because the gain achieved in this way is transmitted from parents to their offspring and from there to thousands or millions of descendants [14]. Among the selective breeding approaches, one may find the individual or mass selection be a simplest and cost-effective approach because it can provide us with rapid improvement in the traits if the heritability of that particular trait is very high [13]. However, in such conditions, high risk of inbreeding and genetic drift can be expected in offsprings in next few generations if fewer number of parents were used for breeding program. For instance, there was no improvement in the growth rate of Nile Tilapia (Oreochromis niloticus) when mass selection was carried out for two generations by Hulata et al. [15]. Similarly, response to mass selection declined sharply after fifth generation in Silver barb (Barbonymusgonionotus), and Common carp (Cyprinus carpio) as reported by Lind et al. [13]. These results suggest that for the mass selection to be successful, there should be some sort of controlling structure that ensures the parental contribution to the scions. One of the control measures for unstructured mass selection could be the controlled pair matting method of Basten and Olesen which showed that keeping the minimum of 50 pairs for matting, can lower the inbreeding rates to 1 percent given that the standardized number of progenies for test is maintained to 30-50 [16]. However, keeping such a large number of pairs for matting and sample the standard number of progenies can pose significant hurdles, especially when the farmers or breeders have limited resources. Therefore, as a remedy to poor brood stock management and genetic deterioration due to inbreeding in the hatcheries, mating of individual broods from the different groups or location can be performed [13]. In other words, the selection within cohorts and exchange of breeders can be a solution. This method was used by McPhee et al. [17] for selection based on weight of redclaw crayfish (Cheraxquadricarinatus) and also a method adopted in the current study for weight base selection of rainbow trout.

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Mating was carried out in current study using individuals gathered from several places (rotational matting), including Mardi, Amare, Kimgtang, Sunkhani, Daman, and hatcheries in Rasuwa when this project began few years ago. The fish thus obtained from various locations was termed as family and maintained in separate tanks without tagging. A selection line was then created by matting the best performing (harvest weight) individual from each family with best performing individual from another family and the progeny were grown in separate raceway tanks at FRS, Trishuli. When selecting parents for the following generation, the heaviest-weighing group growth in the same environment with other cohorts was chosen at harvest. In agreement with the findings of McPhee et al. [17], the final weight and growth rate of rainbow trout was observed highest in the selection line after consecutive generations of selection breeding in the current study.

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In addition, the observed higher growth in the selection breeding group compared to control in the current study could be attributed to the exchange of breeders between the locations, i.e. using a male brood of one location to fertilize the female eggs at another location and vise-versa. Similar conditions were also described by Nomura and Yonezawa [18]. Therefore, it is indicated that with in family-selection combined with the rotational matting resulted in improved growth of the rainbow trout. In fact, family-wise selection method was recommended for Asian countries by Uraiwan and Doyle [19]. Later, the same approach was adopted to improve the stain of Tilapia in Philippines at Freshwater Aquaculture Centre (FAC) and had achieved 12.4% genetic gain in harvest weight per generation after twelve generations [19]. Moreover, Camacho et al. also concluded that the within-family selection coupled with the rotational matting is easy to manage [20] and eliminates the requirement of tagging large numbers of individuals while avoiding the inbreeding in the meantime [13].

4. CONCLUSION

Ultimately, genetically improved strains are crucial for the advancement of aquaculture to cope with the effects of climate change and the rising demand for animal protein. And the current research demonstrated that the rainbow trout fry resulting from the selective breeding of brood sourced from various locations exhibited superior growth performance in terms of weight gain and specific growth rate compared to the Farmer's trout and the strain imported from China. This result suggests that selective breeding and rotational matting amalgamates the significant characteristics from the parents, thereby enhancing the growth traits of the rainbow trout. This amalgamation did not significantly enhance the feed conversion ratio in the current study; however, it did improve the survival rate in the offsprings. Because selective breeding improved growth and survival capability of the fry, it is anticipated that these fries are genetically improved and ready to boost the overall productivity of local farmers and breeders. Thus, we recommend these genetically improved batches of fry to farmers and breeders to expand their production capabilities.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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