ISSN: 2347-5129
IJFAS 2014; 1(3): 184-188

www.fisheriesjournal.com
Received: 30-10-2013
Accepted: 05-11-2013

Shyam Narayan Labh
Research Management Cell, Amrit Campus, Tribhuvan University, GPO Box: 102, Kathmandu, Nepal.
Email: snlabh@gmail.com
Tel: +977-1-4411637

Archana Prasad
Lecturer, Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal.

Rahul Ranjan
Chief Scientific Officer
Corona of Agriculture.
Email: mail4rahulrnjn@gmail.com

Correspondence:
Shyam Narayan Labh
Research Management Cell, Amrit Campus, Tribhuvan University, GPO Box: 102, Kathmandu, Nepal.
Email: snlabh@gmail.com
Tel: +977-1-4411637

Effects of dietary protein concentrations on growth and RNA: DNA ratio of rainbow trout (*Oncorhynchus mykiss*) cultured in Nuwakot district of Nepal.

Shyam Narayan Labh, Archana Prasad, Rahul Ranjan

ABSTRACT
The aim of this study was to determine the effects of dietary protein on growth performance of rainbow trout (*Oncorhynchus mykiss*) in relation to RNA: DNA ratio. Fifty days old rainbow trout larvae (weighing 0.1846±0.0055 g), were cultured in "*Trout Village Fish Farm*" in Doman of Nuwakot district, Nepal, during February-March, 2011. Culture of trout was done in small netted cages (50 cm X 50 cm X 50 cm) which were maintained in the raceways of fish farm and the stocking density was 320 fish/m3 i.e. 40 fish in each cage. Altogether, three raceways were used for the experimental purpose each with three cages set as three replicates for each feeding regime. Fish in three raceways were fed with three different types of feed viz.: diet containing 40% protein (D_1), 50% protein (D_2) and regular commercial diet considered as control (C) respectively. Size of pellet for all type of feed was 60 microns for the whole study period. Temperature, pH and dissolved oxygen were recorded at regular intervals and trout were fed twice (at 9:00 am and 4:00 pm) a day at the ratio of 3% of their body weight and for 6 weeks. Sampling was taken at weekly interval and final sampling was completed on 5th sampling of feeding trial. The effects were assessed by comparing the treated groups of rout to that of control group. The protein supplementation exerted significant (P<0.05) effects on weight gain and the specific growth rate peaked in the group of D_2 diet fed trout. Significant changes in the RNA: DNA ratio was observed in D_2 diet fed trout as compared to controlled diet fed trout. Trout larvae fed with 50% protein diet showed better growth performance as compared to fish larvae fed with 40% protein diet and control.

Keywords: Rainbow trout fry, RNA: DNA ratio, protein-effects of feeding

1. Introduction
Many studies have demonstrated that the RNA/DNA ratio is one of the techniques to indicate physiological condition of aquatic organisms, particularly for fish larvae and fry [1, 2, 3, 4, 5, 6, 7, 8]. Poor nutritional condition contributes to low protein synthesis and slow growth, resulting in a low RNA/DNA ratio [9, 10, 11]. Analytical techniques for determining RNA/DNA concentrations have been well established for small quantities of white muscle and pooled larva [12, 13, 14 ,2] and more recently sensitive assays have been developed for single fish larvae [16, 6, 8]. We must continue development of new methods for assessing larval nutritional status and growth in order to answer fundamental questions of fish condition [16]. Measurement of the RNA/DNA ratio of the cells of individual tissues may provide a more accurate index of physiological condition than whole fish homogenate, since the growth rate of different organs may vary, and therefore, interpretation of the RNA/DNA ratio in whole fish homogenate as an index of physiological condition may be difficult [17]. We have chosen, however, whole fish homogenate to analyse nucleic acid concentration to compare with other physiological parameters, such as weight gain and specific growth rate (SGR) increment. This study determined nutritional status of the rainbow trout fry fed continuously to compare physiological condition.

2. Materials and Methods
Fifty days old rainbow trout larvae, *Oncorbynchus mykiss*, were cultured in "*Trout Village Fish Farm*" in Doman of Nuwakot district, Nepal during February-March, 2011. Fish were cultured in outdoor natural conditions under three feeding regimes in order to find out the effects of different dietary proportion of protein content in food on RNA: DNA ratio and growth

performance of the fish. Culture was done in small netted cages (50 cm X 50 cm X 50 cm) which were previously used for hatchery purpose by the farmer. The cages were maintained in the raceways of fish farm and the stocking density was 320 fish/m3 i.e. 40 fish in each cage. Water from a natural source was passed through these raceways. Three raceways were used for the experimental purpose each with three cages set as three replicates for each feeding regime. Fish in three raceways were fed with three different types of feed viz.: diet containing 40% protein (D_1), 50% protein (D_2) and regular commercial diet considered as control (C) respectively. Size of pellet for all type of feed was 60 microns for the whole study period. Temperature, pH and dissolved oxygen were recorded at regular intervals and trout were fed twice (at 9:00 am and 4:00 pm) a day at the ratio of 3% of their body weight and for 6 weeks. Samplings were taken @ weekly interval and final sampling was completed on 6 weeks of feeding trial. The effects were assessed by comparing the treated groups of rout to that of control group. Total wet weight, W, in mg and standard length, L, in mm were recorded (Bu- sacker *et al.,* 1990). Specific growth rate was obtained by: (InW_2 - InW_1) x100/ (t_2-t_1), where W_1 and t_1 were total wet weight and time at the beginning of experiment and W_2 and t_2 final wet weight and time [19]. Nucleic acid contents of spleen was assayed [20, 21] and followed by RNA and DNA were determined by using diphenylamine and orcinol, respectively [22].

3. Results and Discussion

There was no significant (P>0.05) difference in the average wet weight of the rainbow trout fed varied proportion of protein diets (C, D_1, and D_2) at the beginning of the study but after six weeks of feeding trials, the final average weight was significantly (P<0.05) higher in the trout fed with D_2 diet followed by D_1 and C diets fed fish (Fig. 1). Consequently, specific growth rate (SGR) increased by 33.5% in the D_2 diet fed trout as compared to control diet fed larvae (Fig. 2).

These results were in agreement with the number of researchers [23] who have investigated the biochemical parameters especially RNA/DNA ratio to evaluate the growth of fish. Significant (P<0.05) difference found in the RNA concentration (µg/mg) of trout larvae among the trout larvae fed with different diets. RNA content was 4.40±0.02 µg/mg in the beginning of the experiment which increased upto 6.027±0.050 in control, 6.053±0.069 in D_1 and 6.743±0.039 in D_2 diet fed larvae respectively (Fig. 3). In case of DNA, there was no significant (P>0.05) difference in average DNA content among trout larvae fed with different diets from 1st week to 6th week. Average DNA content of trout larvae was 2.919±0.011 µg/mg in the beginning of experiment while the average DNA content of trout larvae increased 3.043±0.003, 3.038±0.003 and 3.042±0.008 for larvae fed with diets C, D_1 and D_2 respectively (Fig. 4).Similarly, in the present study the data indicated that trout larvae fed with high dietary protein (D_2) showed higher growth rate and higher RNA: DNA ratio than trout larvae fed with a low protein diet (D_1) and control diet (C) fed larvae (Fig. 5). The increase in RNA concentration appears to be the result of a more efficient utilization of dietary protein intake leading subsequently to an increased protein synthesis and thus growth.

Correlations between RNA concentrations, RNA: DNA ratio and growth rate have been observed for a wide variety of organisms [2, 24]. The direct positive relationship between the RNA: DNA ratio and growth rate has also been observed for adult golden shiners [2] in small and mouth bass and carp [25] and in the muscle of catfish [26]. The changes in RNA concentration and protein concentration maintained same trend which can be elaborated with the help of earlier findings [27], emphasizing the role of RNA as the organizer of protein synthesis [22]. Trout larvae fed with 50% protein in feed grow better than other groups [27], though they have concluded that a low protein containing feed can be used during the unavailability of high protein feed. Many other authors [28] have also found that increase in dietary protein level have often been associated with higher growth rates in many species [29].

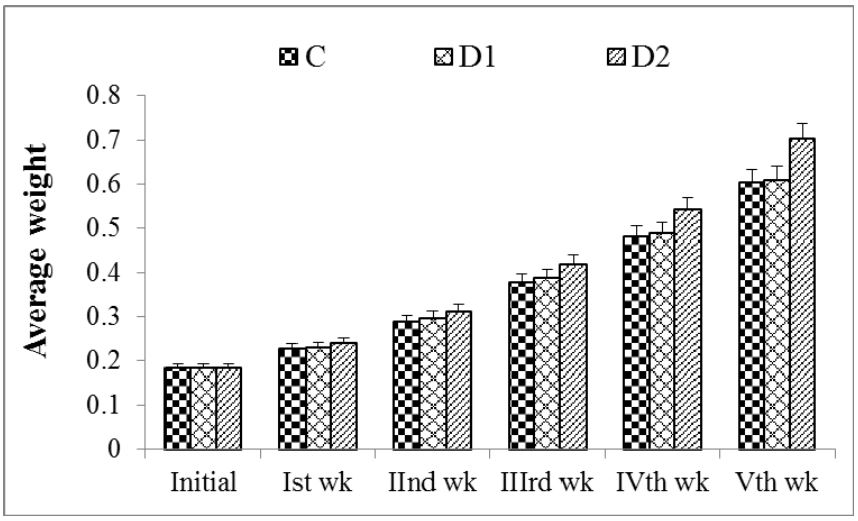

Fig 1: Average weight of rainbow trout (*Oncorhynchus mykiss*) larvae fed varied proportion of supplemented protein diet.

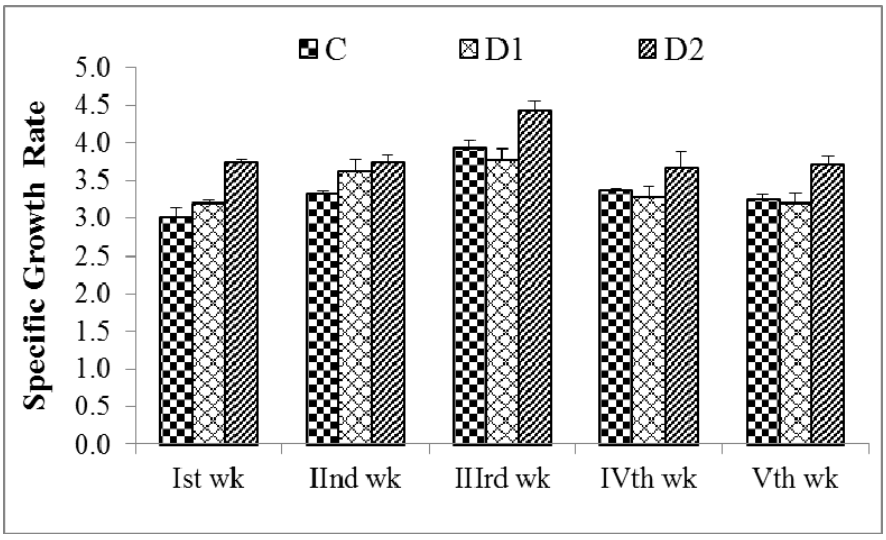

Fig 2: Specific growth rate of rainbow trout (*Oncorhynchus mykiss*) larvae fed varied proportion of supplemented protein diet.

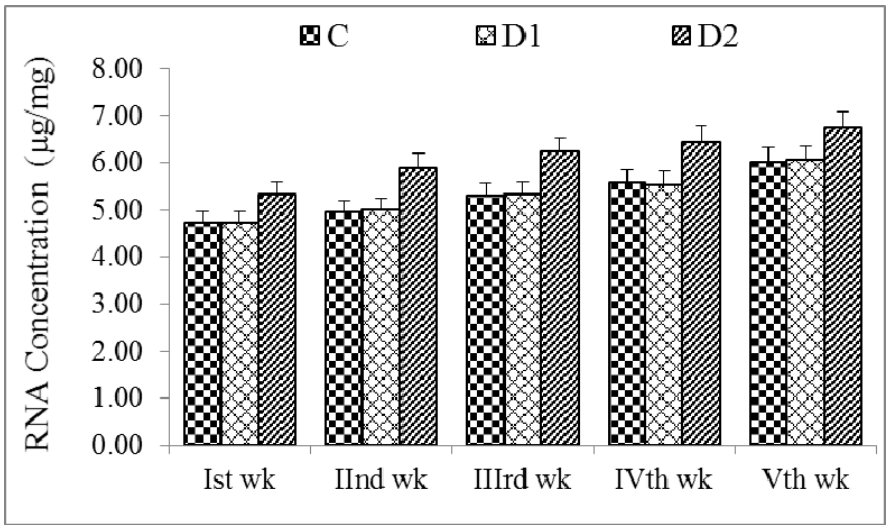

Fig 3: RNA concentration (µg/mg) of rainbow trout (*Oncorhynchus mykiss*) larvae fed varied proportion of supplemented protein diet

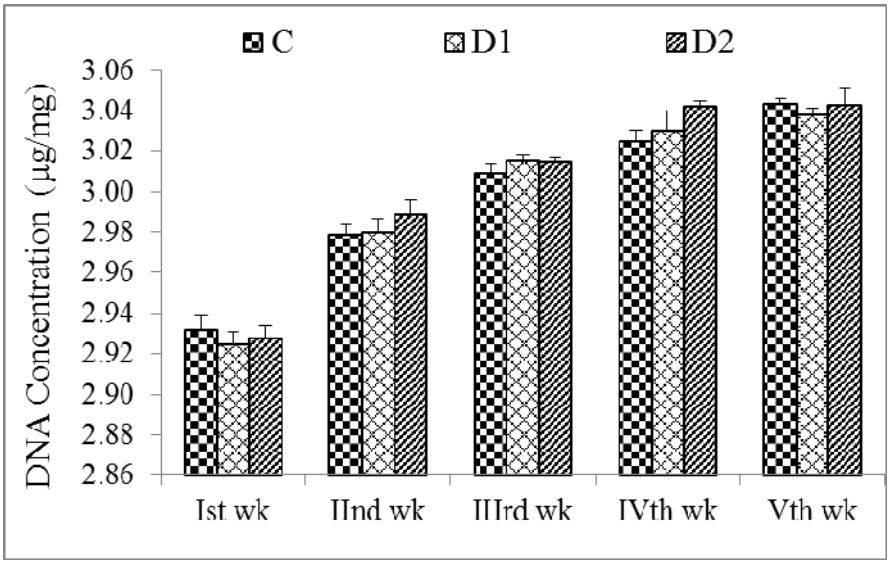

Fig 4: DNA concentration (µg/mg) of rainbow trout (*Oncorhynchus mykiss*) larvae fed varied proportion of supplemented protein diet

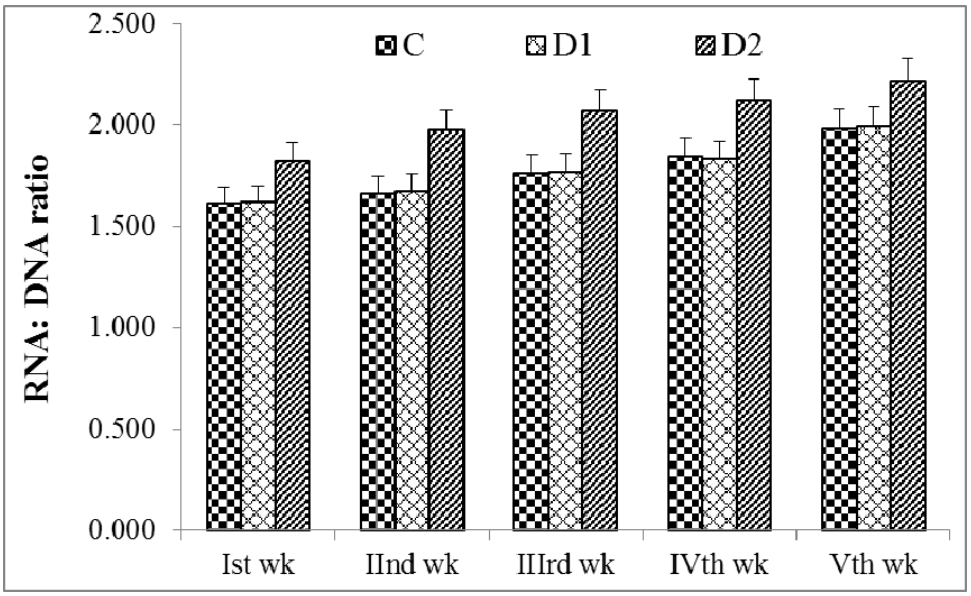

Fig 5: RNA: DNA ratio of rainbow trout (*Oncorhynchus mykiss*) larvae fed varied proportion of supplemented protein diet

4. Conclusion

Trout larvae fed with 50% protein diet showed better growth performance as compared to fish larvae fed with 40% protein diet and control. Similarly, the biochemical indices i.e. RNA: DNA ratio of fish fed with 50% protein diet were found to be better as compared to fish fed with other two diets. Finally, total body weight changes and specific growth rate (SGR) indicated that nutritional status is an important factor determining RNA-DNA ratio as an index of physiological condition of rainbow trout fry.

5. Acknowledgements

The authors are pleased to acknowledge Prof. Dr. Ranjana Gupta, Head of Central Department of Zoology, Tribhuvan University, Nepal for the use of her Department's facilities and Mr. Birendra Chaudhary of MV Polyclinic and Diagnostic Centre for providing facility during biochemical analysis

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