International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

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IJBSM November 2022, 13(11):1302-1311 Print ISSN 0976-3988 Online ISSN 0976-4038 **Article** AR3113a

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2022.3113a

Assessment of the Growth Performance by RNA:DNA Ratio in *Cyprinus carpio* var. *communis* (Scale carp) Using Different Dietary Protein Levels

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ABSTRACT

The study was conducted at wet laboratory of Faculty of Fisheries, Rangil, Ganderbal, SKUAST-Kashmir, India from the time period of March to June, 2019 on scale carp fingerlings (*Cyprinus carpio* var. *communis*) to determine the growth performance by measuring RNA:DNA ratio in scale carp after fed with dietary protein level. The fingerlings were fed with three different levels of silkworm pupae supplement [17.51 g 100 g^{-1} (T_1), 14.42 g 100 g^{-1} (T_2), 11.33 g 100 g^{-1} (T_3)] and a control in which no silkworm pupae was included. RNA:DNA ratio were highest in T_1 (1.609), followed by T_2 (1.526) and T_3 (1.239) while the control showed the lowest ratio (0.929). Similarly, fingerlings fed with Silkworm pupae supplemented diets (T_1, T_2 and T_3) exhibited relatively better growth in terms of body weight gain, % weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) as compared to the control. The highest weight gain of 2.4 g was recorded in T_1 followed by T_2 (2.09 g) and T_3 (1.52 g) while the control achieved the lowest weight gain of 0.72 g. Overall, the results revealed that T_1 performed better as compared to the rest treatments and control. In general, the RNA:DNA ratio and growth results correlate with each other in different treatments and control. So, RNA:DNA ratio seems to be as an effective and rapid indicator of growth performance.

KEYWORDS: RNA:DNA ratio, silkworm pupae, growth, protein

***Citation* (VANCOUVER):** Bhat et al., Assessment of the Growth Performance by RNA:DNA Ratio in *Cyprinus carpio* var. *communis* (Scale carp) Using Different Dietary Protein Levels. *International Journal of Bio-resource and Stress Management,* 2022; 13(11), 1302-1311. HTTPS://DOI.ORG/10.23910/1.2022.3113a.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 31st May 2022 RECEIVED in revised form on 28th October 2022 ACCEPTED in final form on 13th November 2022 PUBLISHED on 27th November 2022

1. INTRODUCTION

Aquaculture production has the potential to break the unavailability of the food line and can provide a nutritious diet to the growing population (Troell et al., 2014). The growth of aquaculture demands more use of feed with high protein content for the proper growth and functioning of fish. At the same time, the high cost of the feed due to expensive protein sources limits the start of new fish farms which necessitates the search for a cheap protein source (Luthada-Raswiswi et al., 2021). The Silkworm *Bombyx mori* L. is a nocturnal moth and pupae containing 48.7% protein (Rao, 1999). Carp fishes like all other animals must consume protein to maintain a continuous supply of amino acids. In the present study, the effect of silkworm pupae on the growth of common carp, (*Cyprinus carpio* Linnaeus, 1758) was evaluated.

Nucleic acids (i.e., RNA and DNA) play a major role in the growth and development of organisms (Clemmesen, 1994). The RNA: DNA ratio or simply the quantification of a nucleic acid ratio is a simple technique available that provides a short-term measure of the condition of fish (Bulow, 1970, Gwak and Tanaka, 2001). The technique is based on the simple notion that within individual cells, DNA concentrations remain fairly constant and it is RNA that varies which in turn increases the protein synthesis (Buckley, 1980, Ferron and Leggett, 1994, Suthers et al., 1996). Thus RNA:DNA ratio is an indicator of the protein-synthesizing potential of a cell (Sivaraman et al., 2011). In general, RNA:DNA is relatively high in a well-fed, metabolically active, growing fish compared to a starving, sluggish and metabolically inactive individual (Richard et al., 1991). The measurement of RNA:DNA ratios have several advantages over other methods to quantify the condition of the fish and more importantly it exactly provides insights into the recent health status of a fish (Jena et al., 2011,). This prevents the cumbersome traditional measures of growth and condition in which the history of feeding is integrated with the energetic utilization over the whole lifetime of an organism (Buckley et al., 1999).

There are multiple sources of variations that reflect the RNA: DNA ratio and the primary sources include methodological, ontogenetic, and temperature effects (Foley et al., 2016). Temperature influences physiological processes and affects RNA/DNA ratios and the somatic growth rate in a variety of larval fish species (Buckley, 1984, Buckley et al., 1984, Buckley et al., 1990, Clemmesen, 1996, Folkvord et al., 1996, Garcia et al., 1998, Kono et al., 2003). The fish may show cyclical differences in the ratio throughout the day due to different feeding regimes and endocrine activity rather than changes in temperature (Rooker and Holt, 1996, Ching et al., 2012).

RNA: DNA ratio has been used in many studies to study the growth rate of fish like, mrigal, *C. mrigala* (Zehra and Khan, 2017a), Nile tilapia, *O. niloticus* (Zehra and Khan, 2017b), rohu, *L. rohita* (Abidi and Khan, 2009, Siddiqua and Khan, 2022), and catla, *C. catla* (Zehra and Khan, 2013).

Keeping in view the advantages of the RNA:DNA as an indicator of growth in animals and its scarce use in the fishes of Jammu and Kashmir, the present work was carried out in *Cyprinus carpio* var. *communis*. The fish was fed with different graded levels of proteins from silk-worm pupae and the changes in RNA:DNA ratio was measured to indicate changes in the growth.

2. MATERIALS AND METHODS

2.1. Experiment

The study was carried out at wet laboratory of the Faculty of Fisheries, Rangil, Ganderbal, SKUAST-Kashmir, India from the time period of March to June, 2019. The study was conducted in different phases to achieve the desired objectives.

2.2. Fish procurement and transportation

The scale carp fingerlings of uniform size (4±1 g) were procured from the Faculty of Fisheries, Shuhama fish farm, SKUAST-K.

2.3. Fish rearing and experimental design

The experimental design consisted of 3 treatments and a control. All the treatments and control were supported by 4 replicates (R1, R2, R3 and R4) with 10 fish in each replicate following a CRD (Completely Randomized Design). The aeration was maintained in all the experimental tubs for 24 h throughout the experiment. The experimental trials were conducted for 60 days. Feed was given @ 5%, twice daily at 8:00 and 18:00 h. Faecal matter and uneaten feed were siphoned out on alternative days with about 30% water exchange. The diet regime was changed after every 15 days corresponding to the body weight of the fish.The initial weight of fishes was taken on day one of the start of the feeding trial, subsequently; the weight was monitored after every 15 days over a period of 60 days.

Important water quality parameters like Water temperature, dissolved oxygen, pH and total alkalinity were recorded fortnightly.

2.4. Feed formulation

4 experimental diets with 35% crude protein were formulated (Table 1). Four different diets with different pupae concentrations were prepared. The diet control (C) had no pupae in it, similarly three treatment diets designated as T_1, T_2 and T_3 were formulated containing silkworm pupae

Table 1: Ingredient composition of experimental diets

Ingredient	Inclusion rate (%)			
	Control (C)	T_1	T_2	T_3
Fish meal	20.6	3.09	6.18	9.27
Silkworm pupae	0	17.51	14.42	11.33
Mustard oil cake	32.88	32.88	32.88	32.88
Rice bran	20.26	20.26	20.26	20.26
Wheat flour	20.26	20.26	20.26	20.26
Vegetable oil	5	5	5	5
Vitamin and mineral mixture	1	1	1	1

at the rate of 15%, 30% and 45% respectively in addition to the basal feed ingredients.

2.5. Sample collection

The ethical guidelines for handling the fish were strictly followed. Initially one fish from each replicate were anaesthetized by using clove oil (50 µl) (Misra et al., 2006) and sacrificed for muscle tissue collection. Similarly, the muscle tissue from all the treatments was collected after 15, 30, 45 and 60 days for the extraction of DNA and RNA respectively. After each sampling, the tissues were immediately processed for nucleic acid extraction.

2.6. Extraction of DNA and RNA

DNA extraction was carried out as per Ke et al. (2008). Fresh Fish muscle was excised and processed for DNA extraction. 0.3 g of tissue was homogenized in 430 ul of extraction buffer (1 mM EDTA, 50 mM Tris, 0.05% Tween 20). 250 ul of denaturing solution (4 M Guanidium thiocyanate, 25 mM Sodium citrate pH 7.0, 0.5% Sodium dodecyl sulfate and 0.1 Beta-mercaptoethanol) and 20 ul of 20 mg ml^{-1} Proteinase K was added. Followed by incubation at 56°C overnight and 100 ul of 2M Sodium acetate was added and mixed. Vortexed again with an equal volume of a Phenol: Chloroform: Isoamyl alcohol (25:24:1) mixture for 10 s and kept on ice for 15 m and centrifuged at 10,000 g for 10 m at 4°C.. The next day, DNA was pelleted by centrifuging at 10,000 g for 15 m at 4°C. The pellet was washed with 1 ml of pre-chilled 70% ethanol dried at room temperature and then dissolved in 50 ul of distilled water.

RNA Extraction was carried out as per Chomczynski and Sacchi (2006). Fresh Fish muscle was excised and processed for RNA extraction. 0.3 g of tissue was taken and homogenised by motor and pestle and the tissue suspension was transferred in 1.5 ml Eppendorf tube. An equal volume of solution containing, sodium citrate, guanidium thiocyanate and sodium dodecyl sulphate supplemented with 3.6 ul of 2-mercaptoethanol was added. Vortexed for a few seconds and 100 ul of 2 M sodium acetate was added and mixed. The mixture was vortexed again and an equal volume of a Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added and kept on ice for 15 m and centrifuged at 10,000 g for 10 m at 4°C. The next day, RNA was pelleted by centrifuging at 10,000 g for 15 m at 4°C. The pellet was washed with 1 ml of pre-chilled 70% ethanol dried at room temperature and then dissolved in 30 ul of distilled water.

2.7. Quality check and quantification of nucleic acids

2.7.1. Quality of DNA & RNA

Quality of nucleic acids was analysed on Agarose gel electrophoresis as per Lee et al. (2012). Agarose gel electrophoresis of DNA samples was carried out using 1–2% agarose in 1X Tris Borate-EDTA buffer (TBE, 10.8 g l^{-1} tris base, 5.5 g l^{-1} boric acid, 5 ml (0.5 M) EDTA pH 8.0, containing 5 µg ml^{-1} of ethidium bromide in both gel and running buffer. Electrophoresis was carried out at 60 V for 2 h. DNA was visualized by illumination with UV light (310 nm) and images were recorded as TIF files using a digital 33-gel documentation system. *Quantification of DNA and RNA*

Quantity of isolated DNA and RNA was determined by UV spectrophotometer (Human corporation/systronicsas per Barbasas et al.(2007).

2.8. Growth parameters

The recorded data on weight were used for the calculation of Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR). On each sampling day, the SGR or % body weight increase day^{-1} and FCR for all the experimental groups was calculated according to Ricker (1979) as follows,

SGR=(Ln of Final weight-Ln of Initial weight)/time interval in days×100……..(1)

FCR=(Feed given (Dry weight))/Weight gain(wet weight).....(2)

PER=(wet body weight gain (g)/(protein intake (g)) …..(3)

2.9. Statistical analysis of the experimental data

The data were statistically analysed by SPSS version 20 (USA). Experimental data were subjecte the statistical analysis following the (Completely Randomized Design) CRD. Data were subjectea d to one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test. $p<0.05$ was considered as statistically significant. The results were expressed as the mean±standard deviation.

3. RESULTS AND DISCUSSION

3.1. Water quality parameters

The water quality conditions remained congenial throughout the study period (Table 2). The present results of water quality parameters fall in the optimum range required for the proper growth of fish (DeLong et al., 2009).

Table 2: Water quality parameters recorded during the experimental period (March–April, 2019)

Parameters	March	April
Water Temperature	12–18°C	16–20°C
pH	7.0–7.5	7.0–7.7
Dissolved oxygen	8.0–9.1 mg l^{-1}	8.5–9.3 mg l^{-1}
Total alkalinity	250–272 mg l^{-1}	255–278 mg l^{-1}

3.2. Gel Electrophoresis and quantification of RNA and DNA

The bands of RNA were clear and crisp indicating good integrity of extracted RNA (Figure 1). Similarly, DNA showed clear bands which indicated its integrity was maintained during the extraction process (Figure 2). Agarose gel electrophoresis has proven to be an effective and rapid way to separate nucleic acids like DNA and RNA to check the quality of extracted nucleic acid (Lee et al., 2012). After the analysis in gel, the nucleic acids were quantified by using a spectrophotometer (Table 3 and 4).

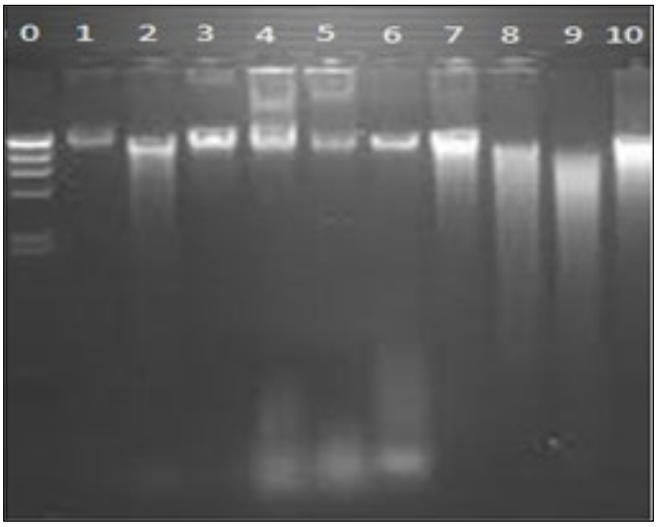

Figure 1: Gel Electrophoresis of RNA. 0 indicate the ladder (1 kb) and numbers indicate the different samples

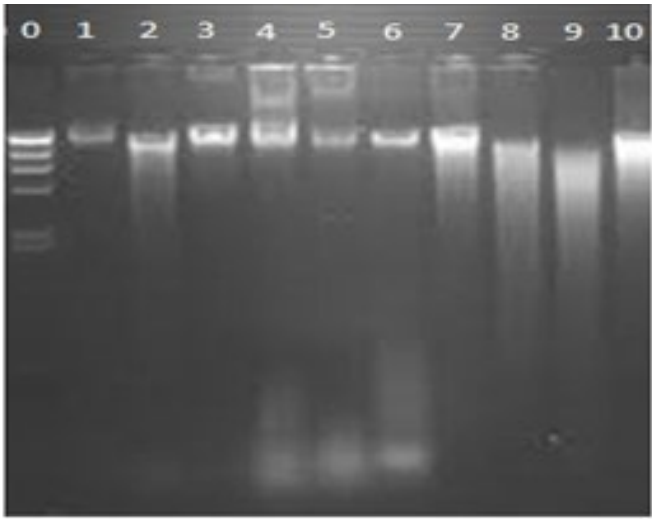

Figure 2: Gel Electrophoresis of DNA. 0 indicate the ladder (1 kb) and numbers indicate the different samples

3.3. RNA:DNA ratio

In control, a non-significant increase in the ratio was

Table 3: RNA concentration (g l^{-1}) during experimental study

0 Days	15 Days	30 Days	45 Days	60 Days
6.2	6.3	6.36	6.44	6.48
6.36	6.3	6.4	6.48	6.52
6.36	6.3	6.44	6.52	6.56
7	6.9	7.04	6.64	6.8
7.32	7.2	7.28	7.4	7.4
7.32	7.24	7.28	7.44	7.44
7.44	7.4	7.44	7.48	7.52
7.48	7.44	7.32	7.4	7.44
6.92	7.32	7.4	7.52	7.8
7.08	7.24	7.44	7.62	7.64
6.92	7.32	7.44	7.6	7.84
7.28	7.48	7.56	7.6	7.96
6.88	7.28	7.28	7.28	7.32
7.2	7.2	7.2	7.24	7.32
7.2	7.24	7.2	7.24	7.28
7.2	7.2	7.2	7.26	7.28

Table 4: DNA concentration (g l^{-1}) during experimental study

0 Days	15 Days	30 Days	45 Days	60 Days
7.8	7.8	7.9	8	8.1
7.5	7.5	7.9	7.9	8
7.6	7.6	8	8	7.9
8.15	8.1	8.2	8.1	8.1
8.1	8.6	8.7	8.7	8.8
8.15	8.6	8.6	8.7	8.85
8.1	8.6	8.7	8.9	8.9
8.3	8.8	8.8	8.9	8.9
8.3	8.8	8.8	8.9	9
8.4	8.9	8.9	9	9
8.4	8.8	8.9	8.9	9
8.3	8.5	8.85	8.9	8.95
8.0	8.5	8.65	8.7	8.8
8.7	8.8	8.85	8.9	8.9
8.4	8.5	8.65	8.7	8.8
8.2	8.4	8.5	8.6	8.7

noticed, whereas in T_1 after 30 days a significant difference was detected which continued up to 60 days. Similarly, T_2 group showed an increase in the ratio but was lower than T_1 after 45 and 60 days. T_3 also exhibited a similar trend but the ratio was low compared to T_2. In general, T_1 showed better results compared to rest treatments and control. The RNA/DNA ratio results are presented in (Figure 3). In the present study, the RNA:DNA ratio showed an increasing trend in all the treatments including control with time. Initially, the ratio was similar in all the treatment groups

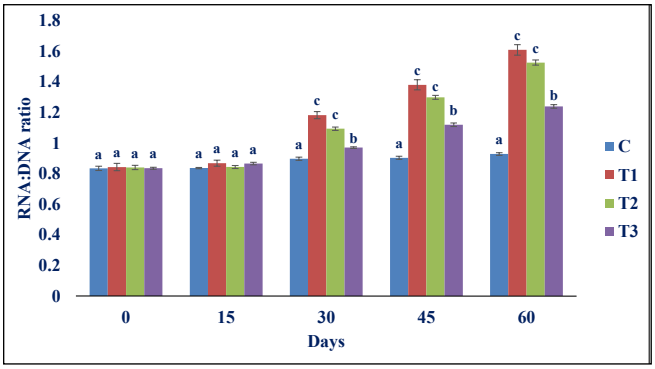

Figure 3: RNA:DNA ratio in different treatments and control at different time points. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

and with changes in dietary protein levels and time of the experiment significant changes were noticed. The ratio was more in T_1 compared to the rest treatments and control. It proportionately increased with the increase in body weight of fish, highest in T_1 followed by T_2. The results varied with the % of silkworm pupae used in the feed which was highest in T_1. The present value of ratio ranged from 0.834±0.08 to 1.609±0.017 which falls in the expected range i.e., the lowest is for the control and highest for the higher % of silkworm pupae fed diet. The ratio was found to be high in the treatment having 50% protein compared to the treatments having a protein level below this and followed the increasing trend as the protein level was increased (Labh, 2015). Comparing these results to our findings a similar trend was followed with the highest in the treatment in which more concentration of silkworm pupae was fed followed by a decreasing trend in the rest of the treatments. In the present study, the fingerlings of Common carp were used so the effect on RNA:DNA ratio with varied diets could be felt. The DNA and RNA were isolated by simple procedures and the ratio was considered as a simple tool to evaluate the growth status of fish.

3.4. Comparison of RNA: DNA ratio and growth

The growth rate was also recorded for a period of 60 days at an interval of 15 days (Figure 4) to correlate the results of

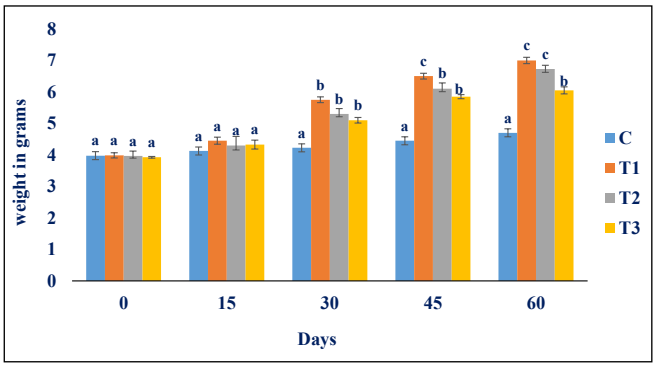

Figure 4: Growth rate in different treatments and control at different time points. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

RNA: DNA ratio with that of weight. The results revealed that the growth pattern and RNA:DNA ratio results coincide with the highest in T_1 compared to rest treatments and control. In the present study, the weight of fish showed an increasing trend in all the treatments including control with time. Initially, the weight of fish was similar in all the treatment groups, and with changes in dietary protein levels and time of the experiment, significant changes were noticed. The weight of fish was more in T_1 compared to the rest treatments and control. The results varied with the % of silkworm pupae used in the feed which was highest in T_1. The present value of body weight ranged from 3.97±0.08 to 7.0±0.11gm which falls in the expected range i.e., the lowest is for the control and the highest for the highest % of silkworm pupae fed as was found in RNA:DNA ratios indicating that the weight of fishes and RNA:DNA ratios complement each other. Like this study, Malloy and Targett (1994) and Rooker et al. (1997) obtained higher correlations between growth in weight and RNA: DNA ratio (r_2=0.66) in the white muscle tissue wild-caught juvenile summer flounder *Paralichthys dentatus*. In another study, a strong correlation between RNA/DNA ratio and growth was observed in a variety of species such as, *Clupea harengus*, *Ammodyles spp*, *Theragrachalco gramma*, *Paralichthys dentatus*, *Pseudopleuronectes americanus*, *Gadus morhua*, *Scombers combrus* and *Morone saxatilis* (Buckley, 1984).

3.5. Growth parameters

After acclimatization, the fishes were weighed to obtain their initial weight and subsequent weighing was carried out every two weeks. The scale carp fingerlings fed with silkworm pupae supplemented diet showed an appreciable increase in growth, measured in terms of weight gain, % weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio than that of the control feed diet. From the present study, it was observed that silkworm pupae are an efficient growth promoter for fish. In a previous

study, the highest growth rate was observed in *Clarias gariepinus* fingerlings when fed with a diet containing a high level of silkworm pupae (Olaniyi and Babasanmi, 2013). Rahmasari et al. (2014) found that the diet containing silkworm pupae had better growth performance. Tomotake et al. (2010) reported that the silkworm pupae are good sources of high-quality protein in fishes. In another study, when Mahseer fingerlings (*Tor khudree*) were fed with silkworm pupae rich diet, better growth and survival rates were observed (Sunder et al., 1993).

The three treatments viz., T_1, T_2 and T_3 which contain 17.51, 14.42 and 11.33 g of silkworm pupae extract showed a significant increase in weight gain (T_1=2.4 g, T_2=2.09 g and T_3=1.52 g) than control (0.72 g) without silkworm pupae supplement. The highest weight gain was found in T_1 treatment as shown in Figure 5. The results indicate that the higher protein promotes more growth. The statistical analysis of change in weight in control and different treatments is presented in table 5.

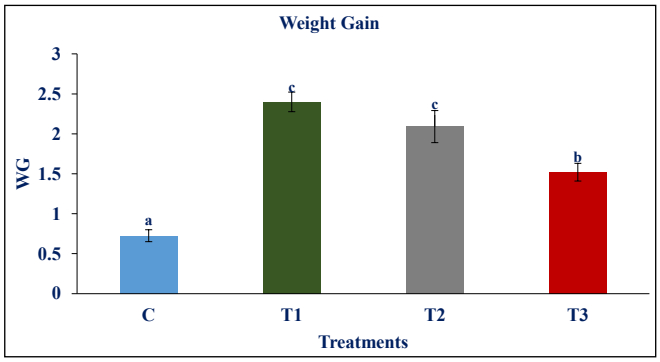

Figure 5: Weight gain of the fish in different treatments and control. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

The highest weight gain in % body weight (52.37) was recorded in T_1 group while the lowest (18.37) was recorded in the control. T_2 and T_3 groups showed a weight gain in % body weight of 44.78 and 23.00, respectively (Figure 6). The high specific growth rate was recorded in T_1 (0.70) and it varied significantly with treatments T_2 (0.61) and T_3 (0.44). The Control group exhibited low SGR (0.28) compared to the rest treatments (Figure 7).

Lowest FCR (2.52) was found in T_1 and it differs from all other treatments. Contrary to the control treatment, all other treatments viz., T_1, T_2 and T_3 showed better results with the highest in T_1 (Figure 8). The highest protein efficiency ratio (1.14) was found in T_1 treatment while the lowest (0.41) was recorded in control. T_2 and T_3 groups showed a protein efficiency ratio of 1.01 and 0.71, respectively (Figure 9). In present study, the silkworm

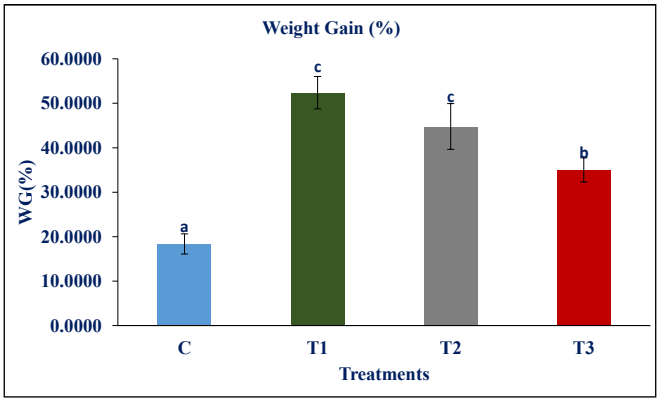

Figure 6: Weight gain (%) of the fish in different treatments and control. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

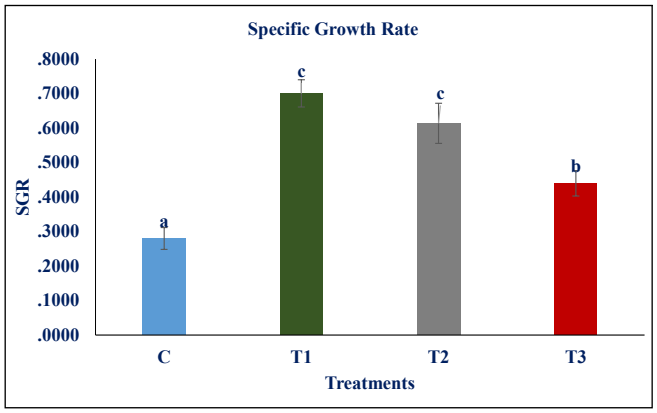

Figure 7: Specific growth rate of the fish in different treatments and control. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

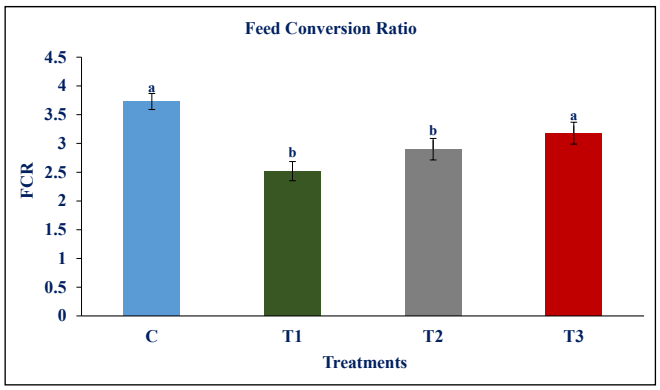

Figure 8: Feed conversion ratio of the fish in different treatments and control. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

pupae rich diet improved the SGR, FCR and PER results, and the same is reported in the previous reports (Nazerath Nisha et al., 2014).

Table 5: Statistical analysis of growth rate in terms of weight in control and different treatments

			Number of Fish (n)	Average weight	Std. Deviation	
0 h	Control		4	3,975	0,170783	0,085391
	T_1		4	3,986	0,221736	0,110868
	T_2		4	3,97	0,182574	0,091287
	T_3		4	3,925	0,182574	0,091287
	Total	16	3,96	0,401663	0,100416	
	Model	Fixed Effects		0,190394	0,047599	
		Random Effects			0,203357	
		Number of fish (n)	Average weight			
15 days	Control		4	4,125	0,15	0,075
	T_1		4	4,45	0,287228	0,143614
	T_2		4	4,3	0,173205	0,086603
	T_3		4	4,325	0,182574	0,091287
	Total		16	4,3	0,121633	0,104083
	Model	Fixed Effects		0,205142	0,051286	
		Random Effects			0,208916	
		Number of fish (n)	Average weight			
30 days	Control		4	4,225	0,05	0,025
	T_1		4	5,75	0,282843	0,141421
	T_2		4	5,3	0,173205	0,086603
	T_3		4	5,1	0,129099	0,06455
	Total		16	5,09	0,443048	0,110762
	Model	Fixed Effects		0,179699	0,044925	
		Random Effects			0,230799	
		Number of fish (n)	Average weight			
45 days	Control		4	4,45	0,057735	0,028868
	T_1		4	6,5	0,191485	0,095743
	T_2		4	6,1	0,170783	0,085391
	T_3		4	5,85	0,08165	0,040825
	Total		16	5,72	0,467217	0,116804
	Model	Fixed Effects		0,137689	0,034422	
		Random Effects			0,251946	
		Number of fish (n)	Average weight			
60 days	Control		4	4,7	0,08165	0,040825
	T_1		4	7	0,191485	0,095743
	T_2		4	6,725	0,208167	0,104083
	T_3		4	6,05	0,05	0,025
	Total		16	6,1	0,524047	0,131012
	Model	Fixed Effects		0,149304	0,037326	
		Random Effects			0,28328	

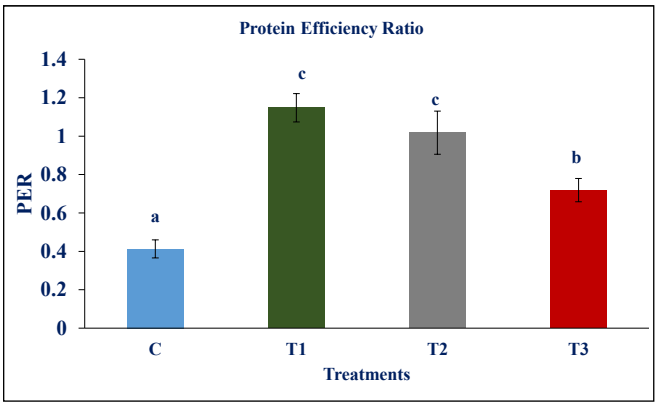

Figure 9: Protein efficiency ratio of the fish in different treatments and control. Data were expressed as mean±S.D (n=4). The same letter used between the groups indicates no significant difference (p<0.05)

4. CONCLUSION

Silkworm pupae-supplemented diets significantly improved the growth performance of experimental fish *Cyprinus carpio* var. *communis* as revealed by the higher values of RNA:DNA ratios. The weight gain was correlated with the RNA:DNA ratios which exhibited a positive relationship between the two. From the results, it was concluded that supplementation of Silkworm pupae powder in the feed @ 17.51 g kg^{-1} resulted in better growth performance of the fish.

5. REFERENCES

Abidi, S.F., Khan, M.A., 2009. Dietary arginine requirement of fingerling Indian major carp, *Labeo rohita* (Hamilton) based on growth, nutrient retention efficiencies, RNA/DNA ratio and body composition. Journal of Applied Ichthyology 25(6), 707–714.

Barbas III, C.F., Burton, D.R., Scott, J.K., Silverman, G.J., 2007. Quantification of DNA and RNA. Cold Spring Harbon Protocols. Available at https://www.researchgate.net/publication/50226084_Quantitation_of_DNA_and_RNA.

Buckley, L.J., 1980. Changes in ribonucleic acid, deoxyribonucleic acids and protein content during ontogenesis in Winter Flounder, *Pseudopleuronectes americanus*, and the effect of starvation. Fish Bulletin 77, 703–708.

Buckley, L.J., 1984. RNA-DNA ratio: An index of larval fish growth in the sea. Marine Biology 80, 291–298.

Buckley, L.J., Caldarone, E., Ong, T.L., 1999. RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Hydrobiologia 401, 265–277.

Buckley, L.J., Smigielski, A.S., Halavik, T.A., Laurence, G.C., 1990. Effects of water temperature on size and biochemical-composition of Winter Flounder *Pseudopleuro nectes americanus* at hatching and feeding initiation. Fishery Bulletin NOAA 88, 419–428.

Buckley, L.J., Turner, S.I., Halavik, T.A., Smigielski, A.S., Drew, S.M., Laurence, G.C., 1984. Effects of temperature and food availability on growth, survival and RNA-DNA ratio of larval sand lance (*Ammodytes americanus*). Marine Ecology Progress Series 15, 91–97.

Bulow, F.J., 1970. RNA–DNA ratios as indicators of recent growth rates of a fish. Journal of the Fisheries Board of Canada 27(12), 2343–2349.

Ching, F.F., Nakagawa, Y., Kato, K., Murata, O., Miyashita, S., 2012. Effects of delayed first feeding on the survival and growth of Tiger Grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775), larvae. Aquaculture Research 43(2), 303–310.

Chomczynski, P., Sacchi, N., 2006. The single-step method of RNA isolation by acid Guanidium Thiocyanate-Phenol-Chloroform extraction. Nature Protocols 1(2), 581–585.

Clemmesen, C., 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured Herring larvae laboratory calibration. Marine Biology 118, 377–382.

Clemmesen, C., 1996. Importance and limits of RNA/DNA ratios as a measure of nutritional condition in fish larvae. In: Watanabe, Y., Yamashita, Y.A., Oozeki, Y. (Eds), Survival strategies in early life stages of marine resources (1st Edn.). CRC Press, 67–82.

DeLong, D.P., Losordo, T.M., Rakocy, J.E., 2009. Tank culture of tilapia. SRAC Publication No. 282. Available at https://wkrec.ca.uky.edu/files/tilapiatankculture.pdf.

Ferron, A., Leggett, W.C., 1994. An appraisal of condition measures for marine fish larvae. Advances in Marine Biology 30, 217–303.

Foley, C.J., Bradley, D.L., Hook, T.O., 2016. A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae. Ecological Indicators 60, 346–357.

Folkvord, A., Ystanes, L., Johannessen, A., Moksness, E., 1996. RNA/DNA ratios and growth of herring (*Clupea harengus*) larvae reared in mesocosms. Marine Biology 126, 591–602.

Garcia, A., Cortes, D., Ramirez, T., 1998. Daily larval growth and RNA and DNA content of the NW Mediterranean Anchovy *Engraulis Encrasicolus* and their relations to the environment. Marine Progress Series 166, 237–245.

Gwak, W.S., Tanaka, M., 2001. Developmental change

in RNA: DNA ratios of fed and starved laboratory-reared Japanese flounder larvae and juveniles, and its application to assessment of nutritional condition for wild fish. Journal of Fish Biology 59(4), 902–915.

Jena, A.K., Biswas, P., Arambam, K., Parhi, J., Pandey, P.K., 2019. Effects of Dietary Protein Levels on Haemato-biochemical Response and DNA: RNA ratio of pabda, *Ompok bimaculatus* (Bloch, 1794). Fishery Technology 56(3). Available at https://epubs.icar.org.in/index.php/FT/article/view/92239.

Ke, X.L., Wang, J.G., Go, Z.N., Zhang, J.Y., Li, M., Gong, X.L., 2008. Study on total DNA Extraction methods of Saprolegniaceae. Acta Hydrobiologica Sinica 32, 67–72.

Kono, N., Tsukamoto, Y., Zenitani., H., 2003. RNA/DNA ratio for diagnosis of the nutritional condition of Japanese anchovy *Engraulis japonicus* larvae during the first-feeding stage. Fisheries Science 69(6), 1096–1102.

Labh, S.N., 2015. RNA: DNA ratio and growth performance of rohu *Labeo rohita* (Hamilton) fed varied proportion of protein diet during intensive aquaculture. International Journal of Life Sciences 9(6), 113–122.

Lee, P.Y., Hsu, C.Y., Kim, Y.H., 2012. Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visual Experiments 20(62), 3923.

Luthada-Raswiswi, R., Mukaratirwa, S., O'Brien, G., 2021. Animal protein sources as a substitute for fishmeal in aquaculture diets: A systematic review and meta-analysis. Applied Sciences 11(9), 3854.

Malloy, K.D., Targett, T.E., 1994. The use of RNA: DNA ratios to predict growth limitations of juvenile summer flounder (*Paralichthys dentatus*) from Delaware and North Carolina. Marine Biology 118, 367–375.

Misra, S., Sahu, N.P., Pal, A.K., Xavier, B., Kumar, S., Mukherjee, S.C., 2006. Pre- and post-challenge immuno-haematological changes in *Labeo rohita* juveniles fed gelatinised Ornongelatinised carbohydrate with N-3 PUFA. Fish Shellfish Immunology 21(4), 346–356.

Nazerath Nisha, S.B., Geetha, Anbujothi, B., 2014. Growth performance and haematological parameter of the ornamental fish, *Maylandia estherae*, fed varying inclusion of Silkworm pupae meal Advances in Biological Research 8(6), 268–273.

Olaniyi, C.O., Babasanmi, G.O., 2013. Performance characteristics of African cat fish (*Clarias gariepinus*) fed varying inclusion levels of Silk worm Pupae (*Anaphein fracta*). Bangladesh Journal of Animal Science 42(1), 76–80.

Rahmasari, R., Sumiati, S., Astuti, D.A., 2014. The effect of Silkworm pupae (*Bombyx mori*) meal to substitute fish meal on production and physical quality of quail eggs (*Cortunix cortunix japonica*). Journal of the Indonesian Tropical Animal Agriculture 39(3), 180–187.

Rao, P.U., 1994. Chemical composition and nutritional evaluation of spent silkworm pupae. Journal of Agricultural and Food Chemistry 42(10), 2201–2203.

Richard, P., Bergeron, J.P., Boulhic, M., Galois, R., Person-Le Ruyet, J., 1991. Effect of starvation on RNA, DNA and Protein content of laboratory-reared larvae and juveniles of *Solea solea*. Marine Ecology Progress Series 72, 69–77.

Ricker, W.E., 1979. Growth rates and models. In: Hoar, W.S., Randall, P.J., Brett, J.R. (Eds.), Fish physiology. Academic Press, New York, 677–743.

Rooker, J.R., Holt, G.J., Holt, S.A., 1997. Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. Marine Biology 127, 387–394.

Siddiqua, K.S., Khan, M.A., 2022. Effects of dietary lipid levels on growth, feed utilization, RNA/DNA ratio, digestive tract enzyme activity, non-specific immune response and optimum inclusion in feeds for fingerlings of rohu, *Labeo rohita* (Hamilton). Aquaculture 554, 738114.

Sivaraman, G.K., Barat, A., Ali, S., Mahanta, P.C., 2011. Prediction of fish growth rate and food availability in the Himalayan Waterbodies by estimation of RNA/DNA ratios. The IUP Journal of Genetics & Evolution 4(3), 15–19.

Sunder, S., Mohan, M., Raina, H.S., Singh, R., Haldar, R.S., 1993. Culture of golden mahseer, *Tor putitora* (Ham.) in Kumaon Himalaya. 1. Mass scale production of stocking material. In: Proceedings of Third Indian Fisheries Forum. Mangalore, 11–14 October.

Suthers, I.M., Cleary, J.J., Battaglene, S.C., Evans, R., 1996. Relative RNA content as a measure of condition in larval and juvenile fish. Marine Freshwater Research 47(2), 301–307.

Tomotake, H., Katagiri, M., Yamato, M., 2010. Silkworm pupae (*Bombyx mori*) are new sources of high quality protein and lipid. Journal of Nutrition Science and Vitaminology 56(6), 446–448.

Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., Arrow, K.J., Barrett, S., Crépin, A.S., Ehrlich, P.R., Gren, Å., 2014. Does aquaculture add resilience to the global food system? Proceedings of the National Academy of Sciences 111(37), 13257–13263.

Zehra, S., Khan, M.A., 2013. Dietary lysine requirement of fingerling *Catla catla* (Hamilton) based on growth, protein deposition, lysine retention efficiency, RNA/

DNA ratio and carcass composition. Fish Physiology and Biochemistry 39(3), 503–512.

Zehra, S., Khan, M.A., 2017a. Dietary thiamin and pyridoxine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). Aquaculture Research 48(9), 4945–4957.

Zehra, S., Khan, M.A., 2017b. Dietary leucine requirement for growth and maintenance, and its utilization efficiency for fingerling of Nile tilapia, *Oreochromis niloticus* (Linnaeus). Borneo Journal of Marine Science and Aquaculture 1, 16–24.