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Evaluation of DNA/RNA Ratio of Soybean Keal Replacement Diet with Cottonseed Meal in Thai - Chitralada Strain of *Oreochromis Niloticus* (L)

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***Abstract*: An experiment was conducted at Fisheries College and Research Institute, Thoothukudi, Tamil Nadu in 2017-18. Eight (average 300 l) tanks with four different diets as soybean meal (SSM) replaced with cottoned meal (CSM) T1 - 25 % CSM, T2 - 30 % CSM, T3 - 35 % CSM and control diet (without CSM) of protein contents were used to conduct the experiment properly to determine the varied proportion of dietary protein on the RNA: DNA ratio of Thai – Chitralada strain of Oreochromis niloticus (L). After 90 days of culture DNA-RNA content increased rapidly with age. The average DNA -RNA content increased highest in 35 % CSM diet fed fish. RNA: DNA ratio was recorded highest in 35 % CSM diet fed fish. RNA: DNA ratio, an indicator of protein synthesis and have been used to accurately estimate the growth rate and feeding condition of fish hence, as the dose of protein increased RNA and DNA contents also increased with age of Thai – Chitralada strain of Oreochromis niloticus (L) cultured during experiment. Thus, it was clear from this study that the incorporation of protein in diet enhances the growth of fish regardless of species weight groups and the doses, as the average weight of fish was significantly lower in control diet fed fish as compared to the treated one.**

***Keywords*: RNA:DNA ratio, Thai, Chitralada strain of Oreochromis niloticus (L)growth, Thai, Chitralada strain of Oreochromis niloticus (L), protein, CSM**

1. Introduction

Aquaculture sector is steadily growing and ensures that all fish produced are consumed by human kind. Consumption of fish provides energy, protein and a range of essential nutrients required for steady growth. Eating fish is part of the cultural traditions of many peoples and fish and fishery products are a major source of food and essential nutrients for some populations. In many cases, there may be no alternative affordable food sources available for many of these essential nutrients (FAO, 2014). The cost of feed is largely influenced by the level and sources of protein which is the most expensive component of a fish diet. It is the major dietary component which influences growth of fish and insufficient as well as excess level of protein in feed is not desirable. Protein is the most important nutrient for fish growth and plays a central role in the structure and functioning of all living organism. According to Shang, (1996) fish is an important component of total human food consumption and a principal source of animal protein for more than half of the world's population.

RNA is directly involved in protein synthesis and therefore increases in RNA content are observed during periods of rapid growth, whereas DNA content is usually stable making the RNA:DNA ratio an indicator of protein synthesis capacity per cell. RNA:DNA ratio is thus a frequently measured indicator of growth rate. RNA:DNA ratios were used successfully to predict growth and nutritional state in a multitude of studies on a variety of organisms such as bacteria, phytoplankton, insects , zooplankton , marine invertebrates, fish , reptiles and humans . Thus, in the present study Thai–Chitralada strain of Oreochromis niloticus (L) fed varied proportion of protein diets and RNA:DNA ratios were measured during experiment period.

2. Materials and methods

A. Formulation of experimental diets

Altogether four experimental diets containing protein percentages of 25% 30% and 35% had been used as T1 - 25 % CSM, T2 - 30 % CSM, T3 - 35 % CSM and control diet respectively. The ingredients were dried well and powdered. The major ingredients used for the preparation of feed were soybean meal and cottonseed meal. These major ingredients were mixed in the feed at four different concentration viz., CSM 25%, 30% & 35%. The control feed was prepared without adding CSM. The ingredients composition of feeds used for different experiments including control feed is presented in Table 1.

All the ingredients and feed additives like (soybean meal and cottonseed meal) except vitamin and mineral mixture were mixed well as per the ratio and made it as a ball and cooked in a pressure cooker for 10-15 minutes. Even distributions of additives were ensured by vigorous kneading. The cooked paste was cooled and vitamin and mineral mixture was added. Then each dough was pelletized separately by using the manual pelletizer. The each pelletized feeds were dried separately at 60°C for twelve hours and stored in different airtight containers for the experiment.

Table 1
Ingredient composition of feed with cottonseed meal

S. No.	Ingredients	Percentage of inclusion		
		25%	30%	35%
1	Soybean meal	35.70	30.70	25.70
2	Cottonseed meal	25.00	30.00	35.00
3	Cassava starch	27.20	27.20	27.20
4	Rice bran oil	4.40	4.40	4.40
5	Fish oil	1.00	1.00	1.00
6	Fish hydrolysate	3.00	3.00	3.00
7	Mono calcium phosphate	1.70	1.70	1.70
8	Vitamin premix	0.50	0.50	0.50
9	Mineral premix	0.50	0.50	0.50
10	Common salt	1.00	1.00	1.00
11	Total	100.00	100.00	100.00

B. Experimental design and feeding trial

Experiment were conducted in order to find out the effects of varied proportion of protein content in food on RNA: DNA ratio of the fish. The experimental fish (Thai-Chitralada tilapia) were procured from Svara Biotechnovations fish farm, Madurai, Tami Nadu. All the fish seeds were properly acclimatized in cement tanks and were nursed for 15 days with commercial diet. All the fishes were graded according to their weight prior to the experiment. An average of 2.650 gram sized 240 numbers of Thai-Chitralada tilapia were selected for the experimental trial.

The growth experiment was carried out in Tilapia hatchery unit at the Department of Fisheries Biotechnology, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu. The experimental set up comprised of three sets of treatments and one sets of control with two replicates, over 90 day's growth trial. Experiment was conducted in rectangular cement tanks (water volume: 1000 l; length: 1.4732 m, height: 0.8636 m, breadth: 0.8636 m) installed with 14 fish hapas of the size 0.30 m3 (length: 0.73 m, height: 0.59 m, breadth: 0.71 m, water volume: 300 l) were used for the experiment. Before starting the experiment, all the cement tanks were cleaned and disinfected. Water was filled in these tanks up to ¾ of its volume. All the tanks were provided with proper aeration facility using air compressor.

The experimental fishes were fed with experimental feed (with cottonseed meal) and control diet was given to the control group of fishes. Every day, the fishes were fed at the rate of 5 % of their body weight. The feeding ration was divided into three equal quantities and given thrice a day viz., morning, afternoon and evening. T1 - 25 % CSM fed with (protein), T2 - 30 % CSM fed with (protein), T3 - 35 % CSM fed with (protein) and control diet fed with (protein). On 45th day and 90th day DNA/ RNA ratio were estimated to determine the growth of Thai-Chitralada tilapia.

C. Examination procedure

During and after the completion of experiment, fishes were collected from each tank. Difference in number of fish between the time at stocking and at harvest has been determined for estimation of fish survival. Biochemical analyses for RNA content and DNA content estimation were done. Isolation of nucleic acids from the fish larvae were done by the method developed by Schneider (1945) and followed by Mustafa (1979). Estimation of RNA and DNA in fish larvae were done by method developed by Buckley and Bulow (1987). Estimation of DNA was done spectrophotometrically by Diphenylamine method. Estimation of RNA was done spectrophotometrically using Orcinol method. At the end statistical analysis of data was done using one way ANOVA followed by Duncan's Multiple Range Test at $P<0.05$ level of significance. The data were processed as per SPSS software to compare the target parameters.

1) Extraction of nucleic acids

In the laboratory, larvae were thawed and measured (to the nearest 0.1 mm) under a dissecting microscope equipped with an ocular micrometer. The procedures outlined by Caldarone and Buckley (1991) [conventional fluorimetric analysis (CFA)] and developed by Clemmesen (1988, 1993) and further modified by Chıcharo (1996) [modified ´ fluorimetric analysis (MFA)] were used to quantify nucleic acids in individual fish larvae. Fish larvae were extracted in 0.15 ml of 1% sarcosine (sodium N-lauroylsarcosine) in Tris–EDTA buffer (Trizma, pH 8.0) to give a final concentration of 0.1%. After centrifugation, aliquots of the supernatant were used for further analyses.

2) CFA

A 0.2-ml aliquot of extracted sample was combined with 0.4 ml of Tris–NaCl 21 (Trizma, pH 7.5) and 0.05 ml of ethidium bromide (EB) (0.1 mg /ml). Another 0.2-ml aliquot of the same extracted sample was combined with 0.35 ml of Tris–NaCl 21 and 0.05 ml of ribonuclease A (Type-II A, 0.12 mg / ml). This mixture was incubated at 378C for 30 min, allowed to reach room temperature for at least 15 min, and stained with 0.05 ml of EB.

3) MFA

For purification of nucleic acids, a third 0.6 ml aliquot of the extracted sample was washed first with 0.6 ml of phenol–chloroform–isoamyl alcohol (49.5:49.5:1, v/v) and then with 0.3 ml of chloroform–isoamyl alcohol (24:1, v/v). After these purification steps, 0.2-ml aliquots of the supernatant were treated as above for CFA.

4) Fluorescence assays

Calculations of nucleic acids concentration were identical for both procedures. Endogenous sample fluorescence (blank) was subtracted from total sample-EB dye fluorescence. The fluorescence due to total RNA, mainly ribosomal, was calculated as the difference between total fluorescence (RNA and DNA) and the fluorescence measured after ribonuclease treatment, which is assumed to be due to DNA. Fluorescence was determined by exciting at 365 nm and reading at 590 nm with a spectrofluorometer (Hitachi Model 650-10). Concentrations were determined by running standard curves of DNA–EB and RNA–EB every day with known concentrations of 21 21 l-DNA (0.25 mg / ml) and 16s–23s RNA (4 mg / ml), in the appropriate range of values. All chemicals used in the procedures described above were analytical grade.

The limit of detection, i.e. the analyte concentration giving a signal equal to the blank signal plus 2 standard deviations of the blank (Miller and Miller, 1988), was 0.16 21 21 mg/ml for DNA and 0.46 mg / ml for RNA. Percent recovery of added l-DNA to eight larvae homogenates (DNA spike) was 95.3% for CFA and 88.8% for MFA, and the recovery of added 16s 1 23s RNA (RNA spike) was 105.6% for CFA and 62.8% for MFA. Total amounts of nucleic acids were corrected based on these values. The coefficient of variation (Zar, 1996) calculated for estimates from eight homogenate samples was: (1) 1.5% for DNA and 3.5% for RNA when using CFA, and (2) 14.8% for DNA and 17.8% for RNA when using MFA.

5) Data analysis

Predicted nucleic acids contents were log-transformed to correct for non-normality. Normality was then evaluated using the Kolmogorov–Smirnov k test (Zar, 1996). Differences between rivers and methods were compared using Student's t-tests (Zar, 1996). The relationships between log RNA, log DNA, and log (RNA/DNA) predicted by the two methods were studied using correlation coefficients (Zar, 1996). Functional relationships between variables were derived using geometric mean regression analysis (Sokal and Rohlf, 1981).

3. Results and discussion

Dietary protein is always considered to be of primary importance in fish feeding, thus sufficient supply of dietary protein is needed for rapid growth of Thai – Chitralada strain of Oreochromis niloticus (L). In the present study, results revealed that the optimum dietary protein level was 35% for Thai – Chitralada strain of Oreochromis niloticus (L). The high protein level significantly enhance the fish growth. Hamza and Kenawy (1997) found out that 40% protein was more potent than other levels for Nile tilapia growth. Al-Hafedh (1999) and Al-Hafedh et al. (1999) found out that the better growth of fish was obtained at high dietary protein levels (40-45%) rather than 25-35%. Feed input is the single largest operational cost in majority of aquaculture practices. De Silva and Gunasekera (1991) conclusively proved the existence of daily variations in dry matter and protein digestibility and opined that feeding fish everyday with the same level of protein is not economical. The DNA/RNA ratio of different experimental fishes including treatments control group is presented in Table 2.

Salim and Sheri (1999) observed significant influence of high protein diets (50%) on growth performance of *Labeo rohita* fingerlings followed by medium protein diets (45%) and low protein diet (40%) respectively. RNA content increased rapidly with age. The average RNA content of fish fed with control diet was increased up to The DNA / RNA ratio of Thai-Chitralada tilapia in different treatments and control on 45th day was (T1 - 25 % CSM) 0.0636±0.00^{a}, (BT2 - 30 % CSM) 0.066±0.01^{a}, (T3 - 35 % CSM) 0.0812±0.00^{b} and Control - 0.057±0.015^{c}. On 90th day this was recorded as (T1 - 25 % SSM) 0.331±0.03^{ab}, (BT2 - 30 % SSM) 0.364±0.00^{bc}, (T3 - 35 % SSM) 0.3997±0.00^{bcd} and Control - 0.321±0.04^{ab}. The higher DNA / RNA ratio of Thai-Chitralada tilapia was obtained in T3 - 0.3997±0.00^{bcd}, followed by T2 - 0.364±0.00^{bc} and T1 - 0.331±0.03^{ab}. The lower DNA / RNA ratio of Thai-Chitralada was obtained in control - 0.321±0.04^{ab}. Statistically, there is a significant difference in RNA concentration among fish fed with different diets. A highest DNA content were increased in (T3 - 35 % SSM) 0.3997±0.00^{bcd} diet fed fish during the study period. Nucleic acids play a major role in growth and development. The RNA concentration is a sensitive parameter to determine the growth rate of an organism because it is the organizer of protein synthesis. DNA concentration represents an index of cell numbers since cellular DNA content is insensitive to changes in environmental condition. The ratio of RNA to DNA is, therefore, a more accurate index of metabolic activity than RNA concentration alone because the number does not affect this ratio or size of the cells in tissue samples . RNA and DNA are compounds found in all living organisms. DNA is the genetic template; its cellular concentrations may be related to cell size and be relatively insensitive to changes in environmental conditions. In contrast, RNA is involved in protein synthesis, which is required for growth. Its cellular concentration is highly dependent on the growth rate, which is determined in part by environmental conditions.

Measurements of RNA/DNA ratios can provide useful information about the nutritional status of animals. There is usually a significant correlation between nutritional status, RNA/DNA ratios and rates of growth . RNA: DNA ratios in Thai – Chitralada strain of Oreochromis niloticus (L) were positively correlated to the trends in growth as has been indicated by several workers . Wilder and Stanley (1983) confirmed the relationship between growth and RNA: DNA ratios in brook trout, *Salvelinus fontinalis* and *Atlantic salmon*, *Salmo salar*. In the present study, the elevated RNA: DNA ratios were associated with higher levels of RNAand lower levels of DNA. Increased RNA: DNAratios noticed in Thai – Chitralada strain of Oreochromis niloticus (L) corresponding to growth increment are indicative of higher protein synthesis

Table 2
DNA/RNA ratio of different experimental fishes

Treatments Parameters		Control	T1 (25 % CSM)	T2 (30 % CSM)	T3 (35 % CSM)
DNA/RNA Ratio	45th day	0.057±0.015^{c}	0.0636±0.00^{a}	0.066±0.01^{a}	0.0812±0.00^{b}
	90th day	0.321±0.04^{ab}	0.331±0.03^{ab}	0.364±0.00^{bc}	0.3997±0.00^{bcd}

which could be attributed to fermented diets containing more total free amino acids. Moreover, quantification of RNA: DNA ratios is a well-established approach that has been used extensively to examine approximate short-term growth of field-collected larval.

Unlike our study, many previous studies evaluated relationships between growth and RNA: DNA ratios after prolonged periods, e.g., 30-56 d. Stierhoff et al. (2009) used RNA:DNA ratios to investigate the growth response of two juvenile estuarine species (weakfish Cynoscion regalis and summer flounder Paralichthys dentatus) to hypoxia in a coastal Delaware bay and found a strong relationship between RNA:DNA ratios and growth after a 7-d period. Stierhoff et al. (2009) also suggested a response of RNA: DNA ratios occurred after only 1 d without food. The strong effect of temperature on young yellow perch RNA:DNA ratios makes it less straight forward to use this measure to index growth of fish that experience variable temperatures. Similar to conclusions of previous studies and Stierhoff et al. 2009 suggest that it is important to consider temperature when interpreting RNA: DNA ratios of field collected fish.

4. Conclusion

RNA: DNA ratio, an indicator of protein synthesis and have been used to accurately estimate the growth rate and feeding condition of fish hence, RNA and DNA contents increased rapidly with age of Tilapia and the average RNA: DNA ratio increased during the experiment. However, in case of DNA and RNA content increasing trend was observed with the increase in dietary protein level. RNA: DNA ratio also followed the same increasing trend as RNA content along with the increase in dietary protein level during the whole experimental period. Thus, the outcomes from the 90 days of feeding trials indicated a varied growth rate under different treatments and control diet fed fish showed significantly ($P<0.05$) higher growth among the treatments and will be suitable for proper fish growth and can be recommendable to the fish farmers in tropical environment. Increase in the RNA: DNA ratio in recovering fishes can be considered as an indicator of protein synthesis and growth. This was also clear from this study that the incorporation of protein in diet enhances the growth of fish regardless of species weight groups and the doses, as the average weight of fish was significantly lower in control diet fed fish as compared to the treated one. Number of larger weight group of fish were more in protein incorporated diet fed fish, compared to the control diet fed fish.

References

[1] FAO, 2014. The State of World Fisheries and Aquaculture 2014. edited by FAO. Rome: FAO.
[2] Shang, Y. C., 1996. The role of Aquaculture in World Fisheries: Proceeding of the World Fisheries Congress, Theme 6 (T. G. Heggberget Ed.). Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, India. 24-42 pp.
[3] Schneider WC. Phosphorous compounds in Animal tissues. I. Extraction and estimation of deoxyribonucleic acid and ribonucleic acid. J BioI Chem 1945; 161:293.
[4] Mustafa S. RNA and synthesis of protein in relation to 'biological condition' of freshwater teleost Channapunctatus. Comp Physiol Ecol 1979; 4:118-120.
[5] Buckley, L. J. and Bulow, F. J., (1987). Techniques for the estimation of RNA, DNA, and protein in fish. In The age and growth of fish. Edited by R.C. Summerfelt and E. Gordon. The Iowa State University Press, IA, 345-354.
[6] Hamza, A.K. and Kenawy, S.M. 1997. Food potency and growth rate of fresh water teleost, Oreochromis niloticus. Bull. Facult. Sci., Zagazig Univer., 19(1): 258-266.
[7] Al-Hafedh, Y.S. 1999. Effects of dietary protein on growth and body composition of Nile tilapia, Oreochromis niloticus L. Aquaculture Research, 30(5): 385-393.
[8] Al-Hafedh, Y.S., Siddiqui, A.Q. and Al-Saiady, Y. 1999. Effects of dietary protein levels on gonad maturation, size and age at first maturity, fecundity and growth of Nile tilapia.Aquaculture International, 7(5): 319-332.
[9] De-Silva, S. S. and R. M. Gunasekera, 1991. A cultivation of the growth of Indian Chinese major carps in relation to the dietary protein content. Aquaculture, 92(2): 237-241.
[10] Salim, M. and A.N. Sheri, 1999. Influence of protein sources, levels of protein and levels of feeding on growth of rohu (Labeo rohita) fingerlings under intensive system. Pakistan J. Sci. Res., 51: 85–8
[11] Stierhoff, K. L., T. E. Targett, and J. H. Power. 2009. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of smallscale temporal dynamics using RNA: DNA. Canadian Journal of Fisheries and Aquatic Sciences 66:1033–1047.
[12] Caldarone, E.M., Buckley, L.J., 1991. Quantitation of DNA and RNA in crude tissues extracts by flow injection analysis. Anal. Biochem. 199, 137–141.
[13] Clemmesen, C., 1988. A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. Meeresforsch. 32, 134–143.
[14] Chícharo, M.A., 1996. Methods of assessing nutritional status in larvae of Sardina pilchardus (Walbaum, 1792) applied to the study of survival conditions in the wild. Unpublished Ph.D. Thesis, University of Algarve, Faro.
[15] Sokal, R.R, Rohlf, F.J., 1981. Biometry. The Principles and Practice of Statistics Biological Research, 2nd Edition, W.H. Freeman, New York.
[16] Zar, J.H., 1996. Biostatistical Analysis, 3rd Edition, Prentice Hall, New Jersey.