Asian Fisheries Science 4(1991): 75-84.
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.1991.4.1.008

Protein and Nucleic Acid Concentrations in the Muscle of the Catfish *Clarias batrachus* at Different Dietary Protein Levels

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Abstract

Variations in muscle protein and nucleic acid in the catfish *Clarias batrachus* fed iso-caloric diets with varying crude protein levels have been reported. A positive clear relationship between the amount of dietary protein level fed and the muscle protein content was evident up to 35% dietary protein incorporation. Above this level of protein in the diet, muscle protein decreased. Similar changes were observed in RNA concentration and the RNA : DNA ratio. The concentration of DNA, on the other hand, declined progressively up to 35% dietary crude protein level. The view that RNA : DNA ratio can be used as a sensitive tool in monitoring the growth and/or protein deposition in fish has been further strengthened.

Introduction

Protein, the most important nutrient for growth, constitutes the bulk of the diet and is usually the most expensive component in artificial fish feeds. The ultimate aim of artificial feeding in fish farming is to achieve maximum protein deposition with minimum input on feeds and with minimum cost. The quality and quantity of dietary protein strongly influence growth rate in fishes (Mertz 1972; Love 1980; Wilson 1985; Wilson and Halver 1986) and thus protein deposition. In fish, as in other animals, the body proteins are in a continual state of turnover, being broken down and resynthesized in varying degrees. Synthesis is under genetic control, requiring amino acids, particularly essential ones, as raw materials.

It has also been established that protein synthesis is accompanied by an increase in RNA and decrease in DNA concentrations. Bulow (1987) has exhaustively reviewed the relationship between nucleic acid and growth rate of fish. The amount of DNA in each cell nucleus is fixed (Love 1980) and, therefore, it is considered as an index of cell numbers contributing to unit weight of tissue while the concentration of RNA in a cell is related to metabolic functions of a tissue (Leslie 1955; Bulow 1970). The RNA:DNA ratio, therefore, indicates the activity of protein synthesis and could be a more sensitive tool for investigating the effectiveness of different diets in promoting growth compared to conventional growth-assessing parameters. In recent years, considerable interest has been shown in the study of RNA : DNA ratios in fish as a tool for the measurement of growth and/or protein synthesis (Bulow et al. 1981; Fauconneau 1985; Lied and Rosenlund 1984). In the present study, protein and nucleic acid concentrations were measured in the muscle of the catfish *Clarias batrachus* Linnaeus fed variable dietary protein levels to investigate the use of RNA : DNA ratio as a growth-assessing parameter.

C. batrachus is an important species cultured in India singly or as a component of polyculture system with other fish species. It accepts artificial feeds readily. A production level of 7,000 $kg \cdot ha^{-1}$ in 5 months has been reported for the monoculture of this species.

Methods and Materials

Estimation of Muscle Protein and Nucleic Acids

Fish fed different protein level diets were decapitated and replicate samples of white skeletal muscle removed from the epaxial portion in the region of the trunk below the place of origin of dorsal fin, approximately 12 hours after the last feeding. The samples were immediately transferred to a freezer (-20°C) and stored for 1-2 hours.

A weighed quantity of muscle sample was homogenized in distilled water and treated with cold trichloroacetic acid (10%) to precipitate the proteins. The homogenate was centrifuged at 4,000 rpm for about 15 min and the supernatant discarded. The process was repeated for complete removal of acid soluble compounds. The residue was treated with 95% ethanol twice or three times and with

solvent ether several times. After discarding the solvent, the residual mass was dried in an electric hot-air oven to remove solvent traces. The dried fat-free sample in powdered form was used for estimating the protein and nucleic acid concentrations.

Protein was estimated by the method of Lowry et al. (1951). The calibration curve used, relating the optical density to mg of protein, was prepared with highly processed bovine serum albumin as the standard.

RNA was extracted and estimated using the method of Schneider (1957). A calibration curve, relating the optical density to μg of RNA, was prepared using purified yeast RNA as the standard. DNA was extracted by the method of Webb and Levy (1955) and estimated using the technique of Ashwell (1957). The calibration curve used, relating the optical density to μg of DNA, was made using highly polymerized calf thymus DNA as the standard.

Spectrophotometric measurements for the above were carried out on Milton Roy Microprocessor controlled Splitbeam Spectronic 1001 Spectrophotometer. The concentrations of protein, RNA and DNA have been calculated on dry weight basis as the mean of 5-6 determinations.

Composition and Preparation of Experimental Diets

Six different purified test diets within a crude protein range 20-45% were formulated using casein and gelatin as protein sources (Table 1). A preliminary study conducted at this laboratory reflected poor utilization of crystalline amino acids in *C. batrachus*. Therefore, diets were not supplemented with crystalline amino acids. The amino acid profile of the diet was that of the casein-gelatin pattern. Diets were made iso-caloric by adjusting the dextrin and α-cellulose content. Protein-to-energy ratio in the experimental diets ranged from 50.0 to 112.5 $mg \cdot kcal^{-1}$. Gross energy in the test diets was calculated after estimating the energy contents of dietary ingredients in a Gallenkamp ballistic (adiabatic) bomb calorimeter. The vitamin and mineral premixes used and the preparation of diets were according to Halver (1976). Diets in the form of soft cakes were stored in sealed plastic bags at -20°C until used.

Table 1. Composition of isocaloric test diets for *Clarias batrachus*.

Ingredients ($g \cdot 100\ g^{-1}$ diet)	Dietary protein level					
	20%	25%	30%	35%	40%	45%
Gelatin/Casein[1]	23.66	29.58	35.49	41.41	47.32	53.24
Dextrin (white)	54.80	46.42	38.21	30.00	21.68	13.26
Cod liver oil	2.00	2.00	2.00	2.00	2.00	2.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00
Mineral mix[2]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[3]	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl cellulose	5.00	5.00	5.00	5.00	5.00	5.00
α-Cellulose	2.54	5.00	7.30	9.59	12.00	14.50
Water	100.00	100.00	100.00	100.00	100.00	100.00
Protein:energy ratio ($mg \cdot kcal^{-1}$)	50.00	62.50	75.00	87.50	100.00	112.50
% Calories as protein	31.85	39.81	47.78	55.74	63.71	71.61
Gross energy ($kcal \cdot 100\ g^{-1}$)	400.00	400.00	400.00	400.00	400.00	400.00

[1]The ratios and quantities adjusted to crude protein content of the product.
[2,3]Halver (1976).

Feeding Trial

Young fish of 0^+ year-class (average total length 13.0 ± 0.6 cm; average weight 15.14 ± 2.68 g) were taken from a previously acclimated fish stock maintained on a casein-gelatin synthetic diet in a wet laboratory. Feeding trials which lasted for six weeks were conducted in polyvinyl 55-l circular troughs fitted with a continuous water flow-through system. The rate of exchange of water was maintained at 1-1.5 $l \cdot min^{-1}$. Eight fish per trough were stocked in replicate groups. Fish were fed the diet at the rate of 4% of the body weight (dry weight to wet-weight basis) at 1700 hours, except on days of weekly measurements. A natural light : dark cycle was maintained. The ration level and schedule were fixed after carefully watching the dietary intake and feeding behavior of the fish.

The average water temperature and dissolved oxygen over the experimental period were 27 ± 1°C and 6.4 ± 2.0 ppm, respectively.

Results and Discussion

Effects on protein and nucleic acid concentrations in white muscle of *C. batrachus* of iso-caloric diets with different protein levels are depicted in Figs. 1 and 3. A positive clear relationship between

the amount of dietary protein level fed and the muscle protein content was evident up to 35% dietary protein incorporation. The muscle protein content increased from 63.66 to 70.45 mg/100 mg tissue.

The concentrations of RNA (Fig. 1), which increased linearly from 631.74 to 1538.87 ug·100 mg^{-1} tissue, followed the same pattern of variation. The increase in RNA concentration appears to be the result of more efficient utilization of dietary protein up to 35% crude protein intake, leading subsequently to increased protein synthesis. A fall in muscle protein and RNA concentration beyond 35% dietary protein intake strengthens the already established fact that there are limits to the amount of protein that a fish can convert to its body material (Love 1980). Increase in the level of RNA is necessary for active protein synthesis, mainly responsible for fish growth (Bulow 1970).

The present findings were in agreement with the work of Mustafa and Jafri (1977) on growth and feeding in relation to protein and RNA content in *Channa punctatus,* emphasizing the role of RNA as chief organizer of protein biosynthesis. The published information points to a close relationship between the protein intake and RNA levels of tissue (Brachet 1955; Leslie 1955; Bulow 1970, 1971;

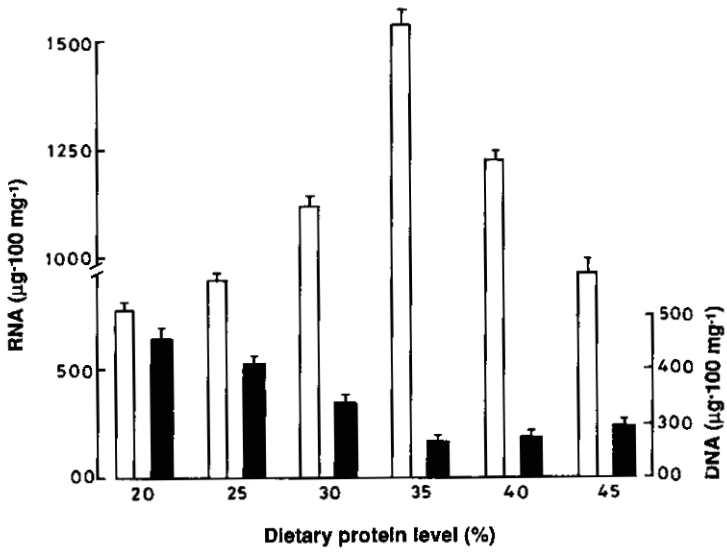

Fig. 1. Concentration of RNA (□) and DNA (■) in the muscle of *C. batrachus* at different dietary protein levels. Vertical lines indicate standard error of mean of five observations.

Mustafa 1977; Buckley 1979, 1980; Mustafa and Mittal 1982). The observed decrease in RNA concentration and the amount of muscle protein in *C. batrachus* beyond 35% dietary protein level may be attributed to the lack of sufficient non-protein energy sources in the diet. The extent of growth increase, measured in terms of specific growth rate of the fish, declined beyond the 35% dietary protein level (Fig. 2). The extra energy expenditure towards deamination and excretion of excessive amounts of amino acids could be another important factor to suppress the muscle protein gain. Any factor that prevents or slows growth is known to be reflected by reduced RNA concentration (Buckley 1979, 1982, 1984; Martin et al. 1985).

DNA concentration in white muscle was found to decline from 454.32 $\mu g \cdot 100\ mg^{-1}$ in fish fed 20% protein diet to 267.31 $\mu g \cdot 100\ mg^{-1}$ in those fed 35% crude protein diet (Fig. 1). The quantity of DNA, however, increased on further increment of dietary protein intake. Since DNA carries the genetic material in each cell and is

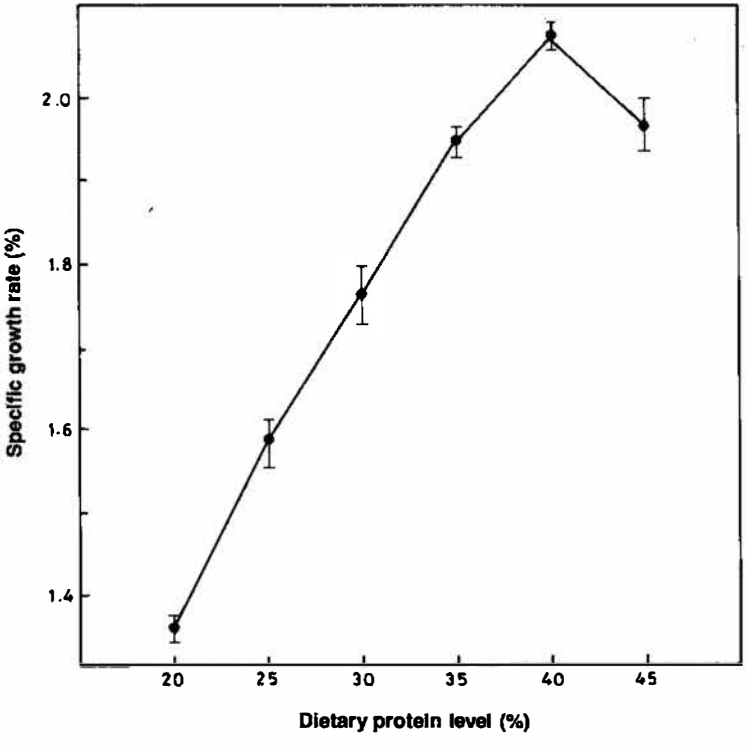

Fig. 2. Specific growth rate (%) of *C. batrachus* at different dietary protein levels. Vertical lines indicate standard error of mean of three observations.

present in the nucleus in fixed quantities (Love 1980), it is considered an index of cell numbers contributing to unit weight of tissue. In fish losing weight, the size of cells decreases and thus number of cells contributing to unit weight of tissue increases, enhancing the number of nuclei and contributing to increased DNA content. In a weight-gaining fish, on the other hand, the DNA content becomes diluted with larger volume of cells per unit weight.

In catfish, *Heteropneustes fossilis,* 7-21 cm long, growth was reported to proceed by increase in size rather than number of the white muscle fibers (Mustafa 1978). Lied and Rosenlund (1984), who observed slightly higher average values of DNA in muscle of Atlantic cod, *Gadus morhua,* fed lower levels of protein, ascribed the change to difference in cell diameter due to reduced synthesis of myofibrillar protein.

These inferences can further be strengthened from the fact that in slow-growing fish like the small shark, *Etmopterus spinax,* bluntnose minnow, longnose dace and bully which grow to a small size and do not exceed 50 cm, growth in muscle results mainly from increase in fiber diameter. This is in contrast to fish-like eels, cod and rainbow trout which have faster growth and larger maximum size, where increase both in number and diameter of muscle fibers occur. Even in such fish, the recruitment of new fibers is reported to cease after attaining a particular size, and somatic growth is continued by fiber diameter increase (Weatherley and Gill 1987; Weatherley et al. 1988). In *C. batrachus,* which is a relatively slow-growing fish attaining a maximum size of 40-50 cm, growth is presumably attained mainly by increase in fiber diameter as reflected by decreased muscle DNA concentration in fish fed high protein diets.

The RNA : DNA ratio is considered a sensitive measure of the growth rate of fish (Love 1980). This concept is based on the fact that since the quantity of RNA varies directly with the activity of protein synthesis, it is expected to be more concentrated in tissues undergoing faster growth or protein synthesis, whereas the amount of DNA per cell remains constant within a species. Thus, the ratio of RNA to DNA which is indicative of the amount of RNA per cell is usually a more accurate index of protein synthetic activity than RNA concentration alone, because the ratio is not affected by differences in cell number.

Fig. 3 represents the changes in RNA : DNA ratio and muscle protein content of *C. batrachus* at varying dietary protein levels. The lowest (1.39) RNA : DNA ratio at 20% dietary protein indicates lowest

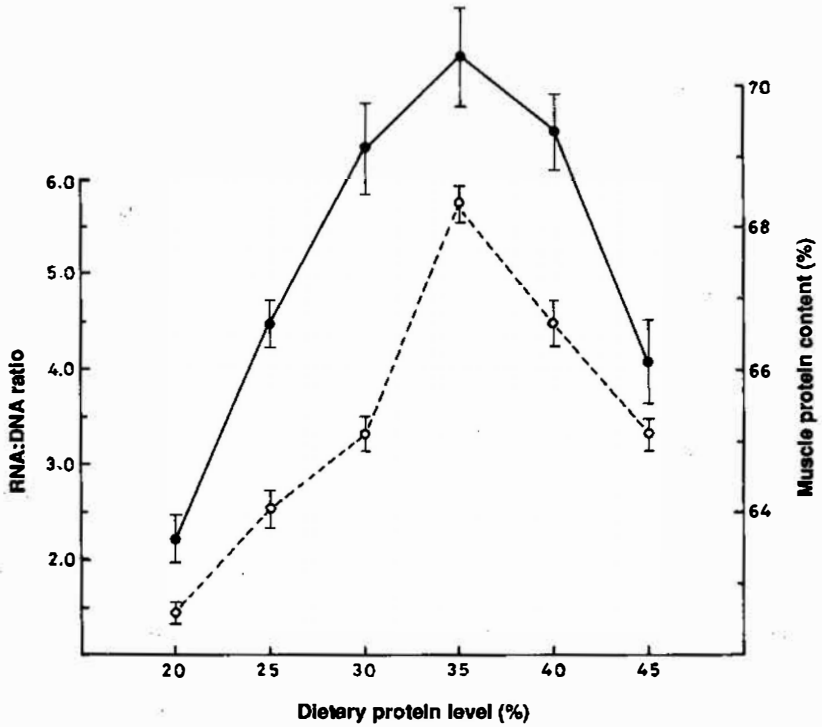

Fig. 3. Relationship between muscle protein content (●—●) and RNA : DNA ratio (o—o) in *C. batrachus* at different dietary protein levels. Vertical lines indicate standard error of mean of three observations.

protein synthesis, as evident from the quantity of tissue protein deposited at this level of dietary protein intake. The data indicate that fish with high (5.75) RNA : DNA ratio at 35% dietary protein level were more actively synthesizing and accumulating protein, resulting in faster growth than fish with low RNA : DNA ratio receiving either low or very high protein diets. This assumption was strongly supported by the maximal value of RNA : protein ratio noted in *C. batrachus* at the above (35%) level of dietary protein. In general, a strong correlation (r = 0.98) was observed between the RNA : DNA ratio and RNA : protein ratio of the fish at varying levels of dietary protein. Houlihan et al. (1989) also observed that the RNA : protein ratio in fish tissues responds directly to increasing levels of protein ration correlating closely with growth and protein synthesis. The RNA : DNA and RNA : protein ratio in fish can thus be used as effective indices for monitoring growth and/or protein deposition.

Acknowledgements

The authors are indebted to the Chairman, Department of Zoology, Aligarh Muslim University, Aligarh, for providing the laboratory facilities; and to the Far Eastern Regional Research Office, United States Department of Agriculture and the Indian Council of Agricultural Research, New Delhi, for making the funds available. The help and advice of Professor John E. Halver, School of Fisheries, University of Washington, Seattle, USA, is gratefully acknowledged. The study forms part of USDA Research Grant No. FG-IN-645.

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Manuscript received 27 September 1989; accepted 25 September 1990.