BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part V.—Influence of Age of Fish on the Distribution of Protein in their Body

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The total nitrogen and non-protein nitrogen (N. P. N.) contents in the meat of young carps like Rohu (*Labeo rohita*), Mrigale (*Cirrhina mrigale*) and Kalbos (*Labeo calbaus*) at two stages of their growth have been determined. The N. P. N. fraction constitutes about 6.3 to 9.43% of the total nitrogen. This decreased whereas the N. P. N. increased with the increase of the body weight. The per cent increase of the N.P.N. was much more than the per cent decrease of the total nitrogen. This caused diminution of the true protein values due to increase of the body weight.

In case of the group of fish ranging from 55.5 to 78.5 g. body weight, the crude protein and true protein levels varied from 93.87 to 96.93% and 86.75 to 90·81% respectively. For higher body weight from 109·5 to 168 g., the crude protein and the true protein contents decreased to the levels of 88.8 to 92.5 % and 81·07 to 84.97% respectively. The significance of these data have been discussed in the light of protein nutrition in young fish and their importance in the technological aspects of the fish processing industries in East Pakistan.

Introduction

In the previous communication by Qudrat-i-Khuda, De and Sharif,[1] it was reported that on spoilage during storage the young fish of large species yielded more tyrosine than the adult fish of small species. In explaining these findings it was suggested that in the younger fish more tissue protein remains in circulation for which there is generation of more tyrosine during autolytic spoilage. In order to verify the validity of this postulate, a series of experiments were carried out to evaluate the difference in the apparent and true protein contents of some large species like carps at the two stages of their growth.

In general practice the protein percentage of any good material is evaluated by multiplication of the total nitrogen with the factor 6.25. But in many food materials, a certain amount of nitrogen remains in non-protein (N.P.N.) stage as ammonia, urea and amide etc. and this is also included in the total nitrogen determined by Kjeldahl's process. Thus the apparent protein calculated on the basis of total nitrogen does not actually represent the true protein. The latter may be determined after deducting the N.P.N. from the total nitrogen. In the present investigation these facts have been taken into consideration and the true protein contents have been calculated accordingly, so as to make a better study of the influence of the size and weight of the fish on the true protein values.

An early report on this line was submitted by Mrs. Hatakoshi[2] who in determining the influence of age on different constituents of fish has noted a decrease of water and protein contents with the progress of age. Phillips[3] in his study of the chemistry of developing brown trout eyed eggs and sac fry has noted continued decrease of the crude protein with the passage of time until the eggs are hatched and fry begins to feed. Sasa[4] has made a survey of the chemical composition of a number of samples of spring herring, and although he has not attempted to correlate the change of body weight with the chemical composition, yet on scrutiny and compilation of his data it appears that immature fish contained more water but less protein, on both wet and dry basis, than the mature ones. But in the absence of any data of N.P.N. the variation of the true protein content could not be judged from these results. In another field on the study of nitrogen metabolism in fish, a group of workers have confined their attention to investigate the distribution of total nitrogen and different non-protein nitrogenous compounds in the meat of various species of fish. Sekin[5] in his experiments on 22 kinds of fish has reported the ammonia N to the extent of 5.3% of the total nitrogen, while Sasa[4] reported a slightly lower value of 4.92% for herring meat. Igarshi[6] on the contrary has obtained a higher value of 9.2% for the same raw meat. Earlier, Clark and Almy[7] have noted a very low content of ammonia nitrogen to the extent of 0.4 to 0.8% of the total nitrogen in various species of fish. In all the above work no attempts had been made to evaluate the true protein contents of the fish by taking into account the ammonia N and other N.P.N. fractions which, however, constitute a fair percentage of the total nitrogen. In view of the above facts it is expected that the present work will throw new light on the storage of true protein in fish meat and its relationship with the N.P.N. fraction due to change in body weight and size.

During the preparation of the present paper, the authors came across a paper by Tanikawa et al.[8] on the chemical study of meat of fish *Theragra chaleogramma* at different stages of their growth. In that paper an experiment has been described in which the adult size fish showed less crude protein and N.P.N. and more true protein than the young ones. In another experiment in the same paper it was shown that the increase of size and body weight (which also relates to the young and adult stage of maturity as before) is followed by increase of crude protein and N.P.N. and decrease of true protein. The above results thus seem to be contradictory. Further, it is not clearly understood how the true protein contents on dry basis from these groups of fish varied by 0.3% when their protein nitrogen content was of the same order of 12.05%. The results of the above work, therefore, do not give a true picture as to the influence of body weight, size and stage of maturity on the levels of crude and true protein and of other constituents in the meat of the fish investigated therein.

Experimental

Three species of fish of carp family as Rohu, Mrigale and Kalbos were selected for the present investigation.

A number of young fish of different ages and body weights were collected from the same batch of the c tch. From each fish sample, the meat underneath the skin of the ventrodorsal region was dissected out and its moisture content, total nitrogen and N.P.N. were determined quickly without allowing any spoilage to occur, so that the N.P.N. fraction might not increase due to formation of ammonia, urea etc. as a result of decomposition. The total nitrogen was determined directly from the meat and the N.P.N. from the filtrate left from the deproteinisation of the meat by sodium tungstate and sulphuric acid. Total nitrogen was determined by Kjeldahl's method and the N.P.N. by the Folin Wu method. The replicate determinations from each sample showed very minor differences and the results in Table 1 represent the average values of these replicate determinations of different samples of each size-group. The apparent protein calculated from the total nitrogen and the true protein from the residual nitrogen fraction obtained after deducting the N.P.N. from the to'al nitrogen are also presented in Table 1. Since the moisture per cent of different sizes varied to a certain degree, all the values form total nitrogen, N.P.N., apparent and true proteins have been expressed on dry basis for comparison of the results in a more precise manner.

Table 2 represents the correlation between the total nitrogen and the non-protein nitrogen and the significance of their distribution with respect to difference in the size and weight of the fish.

Results and Discussion

From the results presented in Table 1 it would appear that moisture, total nitrogen, apparent and true protein values decrease whereas the N.P.N. increases with the increase of the body weight in all the three fishes. On moist basis these differences in the above values due to difference of size and body weight do not appear to be very much significant but appreciable differences are no-

TABLE 2.—THE RELATIONSHIP BETWEEN TOTAL N AND N. P. N. IN THEIR DISTRIBUTION IN FISH DUE TO VARIATION IN BODY WEIGHT AND SIZE.

Name of fish	Size and weight		Total N × N.P.N. (A)	True protein N × N.P.N. (B)	N.P.N. as % of total N (C)	N.P.N. as % of protein N (D)	Decrease of total N as % of original due to increase of body weight (E)	Increase of N.P.N. as % of original due to increase of body weight (F)	Decrease of protein N as % of original due to increase of body weight (G)
	Length in cm.	Weight in g.							
Rohu (*Labeo rohita*)	14·0	55·50	18·42	17·74	7·85	8·51	5·2	14	6·7
	21·5	120·0	20·09	18·17	9·43	10·4			
Mrigale (*Cirrhina mrigale*) ..	16·5	57·60	15·19	14·24	6·31	6·74	4·57	22·8	6·4
	21·5	109·50	17·72	16·35	8·13	8·85			
Kalbos (*Labeo calbasu*)	19·0	78·50	17·15	15·52	7·60	8·23	5·32	11·2	6·8
	24·0	168·0	18·02	16·42	8·93	8·91			

TABLE I.—THE INFLUENCE OF AGE ON THE DISTRIBUTION OF NITROGEN IN YOUNG FISH.

Name of fish local & zoological	Size of fish		Moisture % (A)	Total N % (B)		Total N.P.N. % (C)		Actual protein N(B-C)% (D)		Total crude protein (B × 6.25)% (E))		Decrease of crude protein due to increase of body weight (F)		Total true protein % (D × 6.25) (G)		Decrease of true protein due to increase of body weight (H)		Difference between the crude and true protein (E-G) on dry basis for the same size of fish (I)
	Length in cm.	Weight in g.		On wet basis	On dry basis	On wet basis	On dry basis	On wet basis	On dry basis	On wet basis	On dry basis	Total	% of the original	On wet basis	On dry basis	Total on dry basis	% of the original	
Rohu (*Labeo*	14.0	55.50	80.10	3.06	15.37	0.240	1.206	2.82	14.17	19.12	96.05			17.66	88.56			7.50
rohita)	21.5	120.0	79.64	2.97	14.58	0.280	1.375	2.69	13.22	18.56	91.12	4.93	5.13	16.81	82.62	5.74	6.70	8.50
Mrigale (*Cirr*	16.5	57.50	79.82	3.13	15.51	0.198	0.980	2.93	14.53	19.56	96.93			18.32	90.81			6.12
hina mrigale)	21.5	109.50	79.60	3.02	14.80	0.248	1.204	2.772	15.596	18.97	92.50	4.43	4.57	17.32	84.97	5.84	6.47	7.53
Kalbos (*Labeo*	19.0	78.50	81.7	2.75	15.02	0.209	1.142	2.54	13.88	17.18	93.87			15.87	86.75			7.12
calbasu)	24.0	168.0	81.0	2.70	14.21	0.241	1.270	2.46	12.94	16.87	88.81	5.06	5.39	15.37	81.07	5.68	6.54	7.74

ted when these values are expressed on dry weight basis, and specially when their per cent decrease or increase with respect to the original ones, as presented in columns E, F, and G of Table 2, are critically examined. It is noted from this data that in case of total nitrogen and actual protein N there is diminution by 4.57 to 6.8% from the original value but in case of N.P.N. there is rather a high increase to the extent of 11.2 to 22.8% from the original value due to increase of the body weight and size.

Apparent Protein and Actual Protein

From the survey of the results presented in columns E and G of Table 1 it would further appear that for any particular size of fish the true protein value is less than the crude protein one and the diminution as shown in column 1 of the same table varies from 6.1 to 8.5%. This diminution of true protein values as compared to crude protein ones is mainly due to N.P.N. which constitutes about 6.31 to 9.43% of the total nitrogen and 8.23 to 10.4% of the true protein nitrogen as are shown in column C and D of Table 2. The deduction of this N.P.N. from the total nitrogen causes this fall in the true protein values. When the effect of body weight and size on the apparent protein and true protein levels are considered, it is noted from the data of columns F and H of Table 1 that the total decrease and the decrease as per cent of original value due to increase of body weight and size is higher in case of true protein than in case of apparent protein. This is also, however, due to the influence of the N.P.N. values, the percent increase of which is of much higher order than the percent decrease of the total nitrogen values due to increase of body weight as discussed above. This reciprocal non-linear relationship between the decrease of the total nitrogen and increase of the N.P.N. due to increase of body weight is further shown from the data presented in columns A and B of Table 2. It will be observed from the data that the increase of the body weight is followed by an increase of the multiplication of the values between total N and N.P.N. and protein N and N.P.N. Had the decrease of the total N and the increase of the N.P.N. followed reciprocal linear relationship, the above multiplication of the values would not then have undergone any change due to variation in the body weights. It thus appears from the above discussion that the N.P.N. present in the fish meat of any size and body weight influences the protein level in a converse manner and thus causes considerable decrease in the true protein contents. Moreover, due to increase of body weight, when the crude protein level decreases mainly as a result of decrease of total N, the drop of the true protein level is, on the contrary, effected jointly by the drop of the total nitrogen and by simultaneous increase of the N.P.N. in a greater proportion.

The question arises as to whether the higher concentration of non-protein fraction in the bigger size fish arises as a result of breakdown of the protein already formed in the tissues or by the accumulation of the free amino acids or their deaminated products before they have entered into the synthetic mechanism for tissue protein formation. Further work on this important issue is now being continued. In the meanwhile, it may be summarised from the results so far obtained that from the view point of protein nutrition, young fish of large species is a better source of protein than the adult size ones. But because of high protein content these young fish are more apt to spoilage either by autoproteolysis or by bacterial invasion. Proper care should, therefore, be taken in handling the young fish of large species for preservation and canning operations.

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