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Protein content and amino acid composition of young Atlantic halibut (*Hippoglossus hippoglossus* L.) captured in the autumn in north Norway

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Samples of four tissue-types from immature Atlantic halibut (*Hippoglossus hippoglossus* L.), captured in the autumn, were analysed for total protein content and for free and protein-bound amino acids. The analyses revealed a relatively high total protein content in the muscle tissues, whereas the liver and fin base "notch" had a lower total protein content. The main amino acid residue of the protein was glutamate, with lysine, aspartate, leucine, and arginine also present in considerable amounts. The fraction of free amino acids and ninhydrin-positive substances was dominated by taurine.

The results from the chemical analyses were treated statistically in order to determine possible sexual and/or tissue discrimination. This treatment revealed extensive heterogeneity between tissues, while indications of heterogeneity between sexes were doubtful.

The data presented in this paper suggest a possible test diet for young Atlantic halibut and will serve as a good basis for comparison with captive halibut reared on man-made diets.

Key words: protein, amino acid, halibut.

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Introduction

In recent years a growing interest in introducing the Atlantic halibut (*Hippoglossus hippoglossus*) to aquaculture has emerged. For this reason, several Norwegian research institutions are at present working towards the development of a production line from fertilized eggs to marketable fish. Many important questions relating to halibut aquaculture, however, are as yet unresolved (Haug, 1990). For example, a detailed knowledge of natural halibut biology and chemistry is necessary to evaluate the aquacultural potential of the species, to establish base-line values for relevant parameters (e.g. for comparison between captive and wild specimens), and to formulate a test diet which meets the requirements of young halibut.

In Norwegian coastal waters several halibut nursery areas, inhabited by juveniles aged from 2 to 5 years, are

Table 1. Contents (in per cent of dry weight) of total protein-bound amino acids in various tissues of young Atlantic halibut males and females. Number of fish analysed are given in parentheses.

Tissue type	Males	Females
	Total protein-bound amino acids	
White muscle	88.7 ± 3.5 (10)	90.1 ± 2.5 (8)
Red muscle	77.9 ± 5.3 (10)	81.6 ± 1.1 (8)
Fin base "notch"	58.2 ± 7.0 (10)	61.3 ± 8.5 (7)
Liver	33.8 ± 4.2 (10)	36.4 ± 8.8 (8)
	Total free amino acids	
White muscle	1.5 ± 0.3 (6)	1.4 ± 0.2 (6)
Red muscle	6.0 ± 2.6 (6)	4.3 ± 1.8 (6)
Fin base "notch"	2.0 ± 0.9 (6)	1.6 ± 0.2 (6)
Liver	1.9 ± 0.3 (6)	1.9 ± 0.4 (6)

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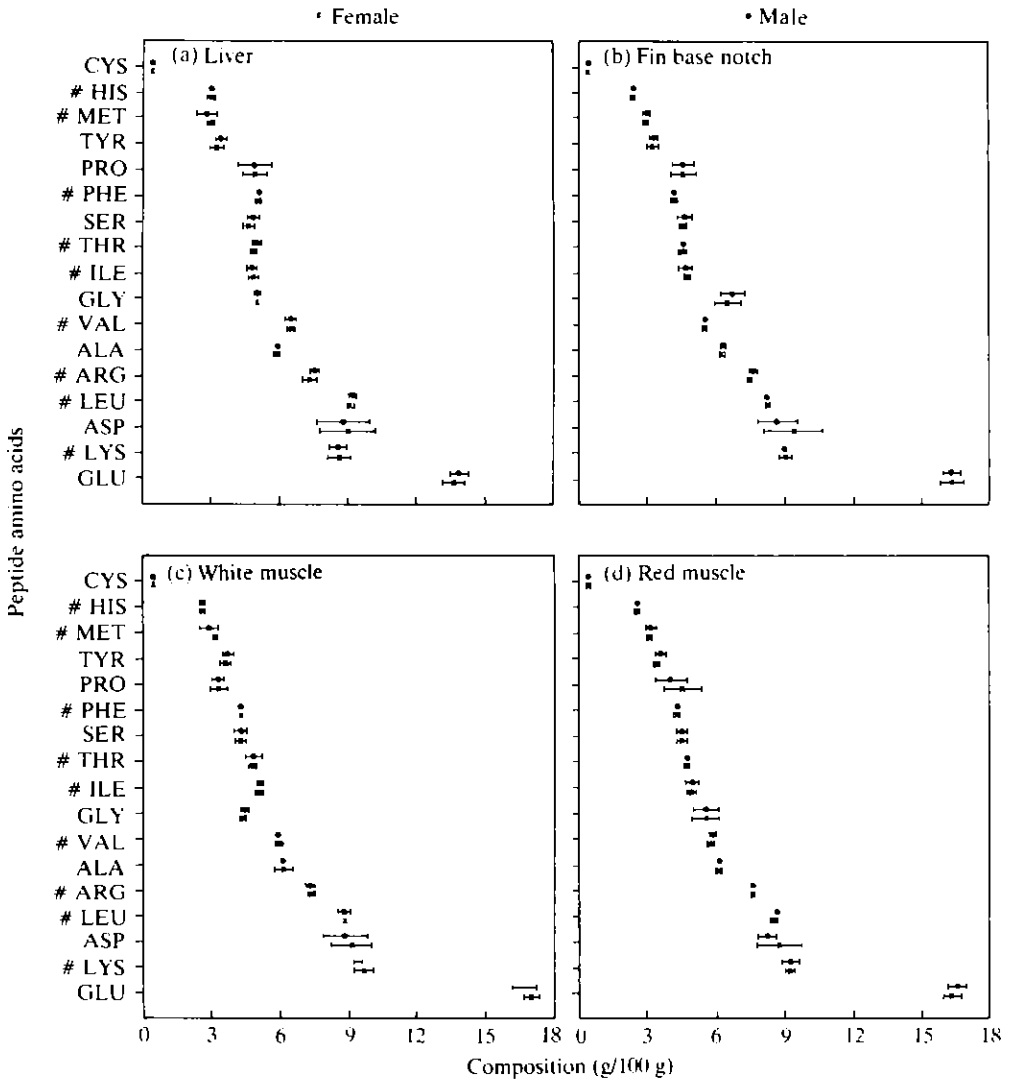

Figure 1. The composition of the protein-bound amino acids (mean ± 95% confidence interval) for males and females in the four tissues analysed. ♯: Amino acids that are considered essential for fish.

Table 2. A/E ratios, (essential amino acid/total essential amino acids) × 1000, for the tissues and sexes.

	White muscle		Red muscle		Fin base "notch"		Liver	
	Male	Female	Male	Female	Male	Female	Male	Female
ARG	142.5	142.4	149.1	150.0	154.8	152.8	142.4	148.7
HIS	51.5	50.9	50.1	50.3	48.8	47.7	56.8	56.1
ILE	100.2	98.3	96.6	97.1	95.2	96.7	90.9	92.6
LEU	172.0	171.1	169.5	168.5	167.0	168.3	174.7	172.3
LYS	184.1	186.6	181.1	182.3	183.0	184.7	167.5	163.1
MET	56.2	60.8	61.7	61.0	60.6	60.4	52.3	55.5
PHE	83.6	83.0	84.8	84.4	84.7	84.8	96.9	96.0
THR	94.1	91.9	92.7	92.4	92.9	92.4	95.0	92.2
VAL	115.9	115.0	114.5	114.0	112.9	112.2	123.6	123.5

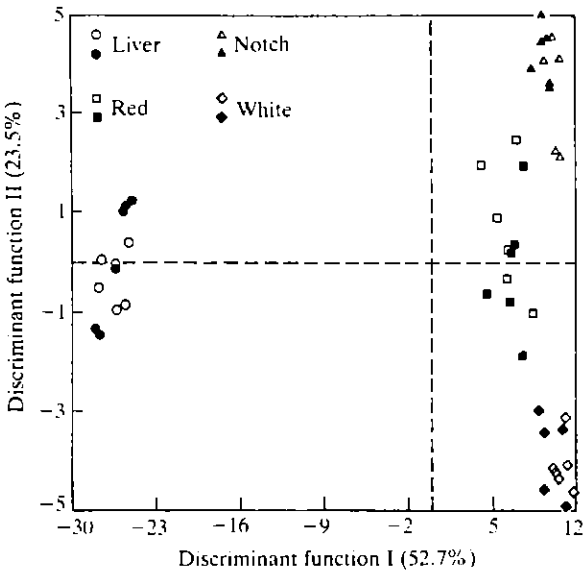

Figure 2. Discriminant score plot of the protein-bound amino acids (discriminant function I and II) for the tissues and sexes analysed. Females: open symbols; males: filled symbols.

known (Godø and Haug, 1988). Sampling of fish from such areas affords an excellent opportunity to study relevant parameters of halibut of the same age as those which probably will be raised in future fish farms. This paper presents results from studies of protein content and amino acid composition of juvenile halibut captured in the autumn, from halibut nursery areas in Finnmark, north Norway, and thus reflect the situation after a period of intensive food intake during the summer (see McIntyre, 1953). Sexual differences in the physiology (in particular in connection with reproduction) and growth energy strategies have been suggested for the halibut (Haug, 1990). Thus, in all our analyses we choose to treat the sexes separately in order to see whether such differences are also manifested in the biochemistry of the species, and to ascertain whether and at what point sexual differences can be detected in the composition of Atlantic halibut. In flatfish, it is generally accepted that both the liver and the carcass itself act as storage sites for energy reserves (Love, 1970). The tissues studied, therefore, included liver as well as red and white muscles. Additionally, the anal fin base "notch" was included as it is known in flatfish to be a site of adipose tissue where energy can be stored in periods of intensive food intake (see Wilson, 1939). The tissues of adult halibut have also been analysed and will be published in a future paper.

Materials and methods

Biological sampling

The material consisted of 18 juvenile Atlantic halibut between 2 and 4 years old (determined by otolith readings, see Devold, 1938) with total lengths ranging from 37 to 55 cm. The specimens were collected during a research cruise, using Danish seines (mesh size 135 mm) conducted in September 1987 in Bølefjord and Galtefjord on the north side of Sørøya (70°45'N, 22°35'E) in north Norway. Fishing depths ranged between 35 and 60 m, with bottom temperatures and salinity at about 7.5°C and 34.1‰ at the time of collection.

Samples of four different tissue types were dissected from 10 males and eight females for total protein analysis, and from six males and six females for amino acid analysis. The tissue samples included red and white myotomal muscles (taken underneath the lateral line of the eye side midway between tail and head), anal fin base "notch" (tissues taken from the base of the unpaired anal fin just behind the gut cavity), and liver. The samples were transferred to 10% trichloroacetic acid (TCA), frozen at −20°C, and stored at this temperature until analysed.

Chemical analyses

The composition of the protein-bound amino acids was determined by hydrolysis of the TCA precipitate with concentrated hydrochloric acid/water (1:1) (1 ml/100 mg protein) for 24 h in sealed tubes overlaid with nitrogen at 110°C, filtered, and adjusted to pH 2.2 and centrifuged for 15 min at 12 000 rpm, in order to remove particulate matter, immediately prior to analysis on a LKB 4151 Alpha Plus amino acid analyser using ninhydrin detection, and equipped with a LKB integrator. A physiological amino acid mixture (Pierce amino acid standards: physiological A/N and B) with D-L norleucine as the internal standard was used both for calibrating the integrator and for identification of the individual amino acids. Tryptophan was not determined as this amino acid is destroyed during acid hydrolysis.

Extracts containing free amino acids were obtained by deproteinization using 10% (w/w) trichloroacetic acid and centrifuged for 30 min at 2250 rpm. The supernatant was collected and the free amino acids were determined as described by Ringø *et al.* (1988), using the above amino acid standards with glutamine and tryptophan added. D-L norleucine was used for calibration and identification.

Standard annotation for the amino acids was used for all tables and figures. Three additional abbreviations were used: AAA (α-aminoadipate), PPS (phosphoserine), and CTT (cystathionine).

Total protein (N × 6.25) of the TCA precipitate was determined by the Kjeldahl method with digestion at 420°C using a Kjeltec 1015 digestor followed by distillation using a Kjeltec 1002 distilling unit.

Statistical treatment

Intersexual comparison of total protein levels was performed using the BMDP programs P1D and P1V (Dixon, 1981) and carried out on a VAX computer.

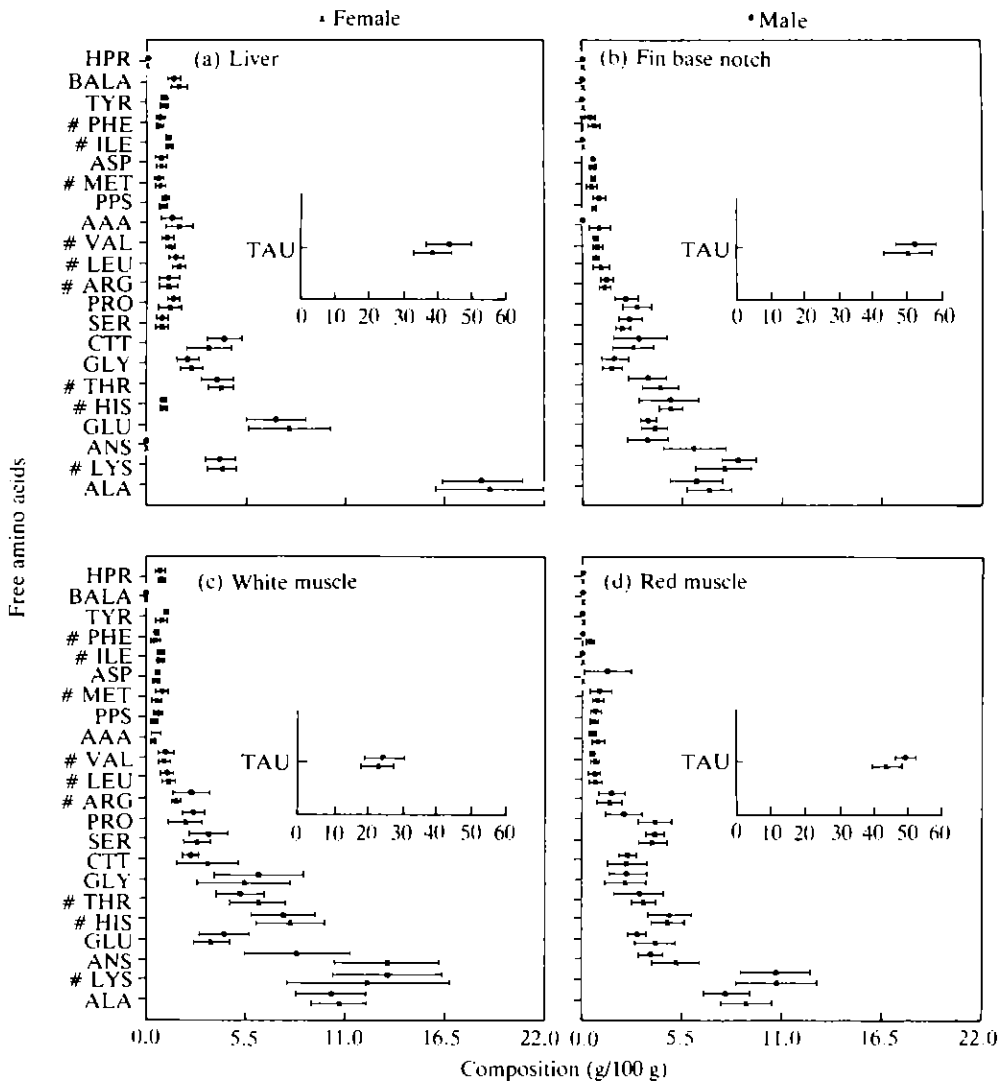

Figure 3. The composition of the free amino acids (mean ± 95% confidence interval) for males and females in the four tissues analysed. Note that the inserted taurine values are given in another scale.

Evaluations of potential differences/similarities in amino acid composition both between the tissues, and between the sexes, were performed using the statistical system SYSTAT (Wilkinson, 1988). The MGLH module (Wilkinson, 1988) was employed to test for differences in amino acid composition (two-way ANOVA) among sexes and tissues (liver, notch, red, and white muscle). Discriminant function analysis (DFA) was used to test whether it was possible to discriminate among eight *a priori* categorized groups (two sex-groups within four tissue-groups = eight groups). Data were treated as normally distributed or transformed to get normality and equal variances. A Box–Cox analysis (Box and Cox, 1964) clearly indicated that a square root transformation was necessary to achieve a standardization of the variance of the free amino acid data set. The Wilk's lambda statistic was used to test for differences between groups.

The success of the discriminant functions can be assessed by constructing a contingency table of "correct" and "incorrect" allocations.

Results

Total protein

None of the tissues showed significant sexual heterogeneity in total protein content (ANOVAs, all $p > 0.05$). As seen from Table I, the total protein level was higher in the white muscle (approximately 90%) than in the other tissue types which contained approximate values of 75%

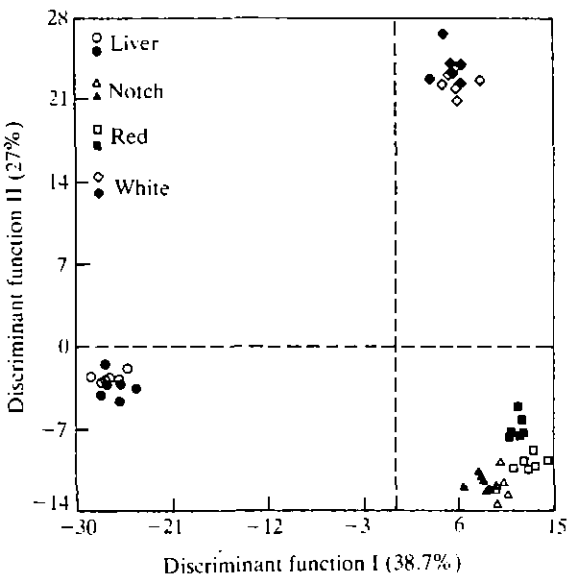

Figure 4. Discriminant score plot of the free amino acids (discriminant function I and II) for the tissues and sexes analysed. Females: open symbols; males: filled symbols.

in red muscle, 60% in fin base notch, and 35% in liver. The large standard deviations suggest considerable individual variation.

Protein-bound amino acids (PAA)

In all tissues examined the amino acid present in greatest amounts in the protein was glutamic acid (13.6–17 g 100 g^{-1} PAA) followed by almost equal amounts (8–9.5 g 100 g^{-1} PAA) of lysine, aspartic acid, leucine, and arginine (Fig. 1).

The ratio of each essential amino acid to the sum of all essential amino acids in a given tissue (Cowey *et al.*, 1970; Ketola, 1982; Wilson, 1989) shows agreement both between the sexes and the tissues, except for the liver which differs slightly (Table 2).

When the amino acids bound in proteins were grouped by two-way ANOVAs with respect to sex and tissue type, the results indicate that, except for tyrosine, there were no significant differences between sexes. There were however, except for methionine, cysteine, and aspartate, highly significant differences ($p<0.01$) in the composition of the amino acids bound in proteins among the four tissues.

In the discriminant analyses all univariate comparisons, except aspartate, cysteine, and methionine, indicate a highly significant group heterogeneity. Good discrimination between tissue-groups is achieved (Wilks' lambda = 0, $F_{119,166}=5.27$, $p<0.001$), where the liver is particularly separate from the other tissues (Fig. 2). Most of the variance is represented by the first two canonical variates (52.7 and 23.5%, respectively). Using the generated classification scores for protein amino acids within the eight groups in question (two sexes × four tissues) revealed a few misclassifications of sex, while no tissue groups were incorrectly classified to other tissues.

Free amino acids (FAA)

The FAA constitute only a small fraction of dry tissue ranging from 1.4% in white to 6.0% of dry tissue in red muscle (Table 1).

The free amino acids (Fig. 3) are dominated by taurine, with a content ranging from 22.42 g 100 g^{-1} FAA in female white muscle to 52.27 g 100 g^{-1} FAA in the male anal fin base. In liver the only amino acid present in appreciable quantity apart from taurine is alanine, while in the other tissues examined considerable amounts of lysine, alanine, and histidine are also found. White muscle contains some anserine which is completely absent in the liver.

When the FAA were grouped by two-way ANOVA with respect to sex and tissues, it appeared that, except for taurine ($p<0.005$) and for anserine and phosphoserine ($p<0.001$), no significant differences were found between sexes. There are, however, except for methionine and tyrosine, highly significant differences in FAA among the four tissues.

In the discriminant analyses, all univariate comparisons except methionine and cystathionine indicate a highly significant group heterogeneity. Good discrimination between tissue groups is achieved (Wilks' lambda = 0, $F_{154,138}=14.0$, $p<0.001$) (Fig. 4). Canonical variate I and II are responsible for, respectively, 38.7% and 27.0% of the variance. Using the generated classification scores for FAA within the eight groups in question (two sexes × four tissues), it appeared that only one fish was misclassified with respect to sex, while no tissue groups were incorrectly misclassified to other tissues.

Discussion

Total protein

The values for protein content of liver and red and white muscle tissue are somewhat higher in this study than those reported for Atlantic halibut by Reay *et al.* (1943) and Brækkan (1959), but are in accordance with the findings of Mannan *et al.* (1961) and Brækkan and Boge (1962). These studies, however, give little information regarding the sex and size of the fish examined, at what time of year they were captured, or whether or not the samples included red muscle or not. Seasonal variations in Atlantic halibut protein content analogous to those described in flounder (Lapin, 1976) and female plaice (Dawson and Grimm, 1980) cannot be excluded. Reay *et al.* (1943) observed that

there appears to be considerable seasonal variation in the fat content of Atlantic halibut, which may imply similar variation in the protein content.

The lower protein content observed in red muscle than in white is consistent with the results of Brækkan (1959) and Mannan *et al.* (1961) and is probably attributable to the different physiological function of these tissues, where the white muscle is designed to give the maximum possible amount of contractile units in a given space (Best and Bone, 1973). The lower protein content observed in the fin base "notch" and liver compared with muscle tissue is not surprising, owing to their function as energy stores (see Love, 1970).

Protein-bound amino acids (PAA)

Some similarity in the protein amino acid composition has been reported for a number of various species of fish, including Atlantic halibut (Brækkan and Boge, 1962; Njaa, 1990). Comparison of the present results with the PAA composition reported for Atlantic halibut in previous studies (Brækkan and Boge, 1962; Rajniakova *et al.*, 1976; Njaa, 1990) reveal only minor differences in the general distribution of amino acids.

Significant differences in PAA composition both between red and white muscle and among other tissues has been demonstrated in a number of other fish species (e.g. Arnesen, 1969; Iwasaki and Harada, 1985; Ostrowski and Divakaran, 1989). Such differences were also revealed in the present study.

The statistics show a significant sexually determined difference in the relative tyrosine content. It is, however, not clear whether this heterogeneity, which in other species has been found to be essential at some levels, has any physiological significance.

Previous nutritional studies have shown that test diets simulating the amino acid profile of whole body tissue of fish would serve as a guideline for those fish whose requirements are not yet known (Ketola, 1982; Wilson and Cowey, 1985; Gatlin, 1987; Wilson and Poe, 1985). However, studies have shown that for some fish 10 specific amino acids are essential (Cowey *et al.*, 1970; Ketola, 1982; Wilson, 1989). It has been suggested that the ratio of each essential amino acid to the total essential amino acids would be a good starting point for a test diet (Cowey and Luquet, 1983; Wilson and Poe, 1985; Wilson, 1989). Assuming that these amino acids are essential for Atlantic halibut, this ratio would serve as a viable test diet formulation for this species as well.

Proline and glycine were found to be more abundant in red muscle than in white, which may indicate more connective tissue in red muscle, and the greater standard deviation in these results indicates variable addition/removal of other substances and thus greater metabolic activity. We therefore suggest that more importance should be attached to this tissue when formulating a diet.

A report from a joint FAO/WHO expert committee suggests that the proportion of total amino acids which must be supplied as essential amino acids for human consumption should be at least 36% of the protein (Anon., 1973). The ratio for Atlantic halibut is approximately 45%, which is slightly higher than that reported by Rajniakova *et al.* (1976) but in agreement with calculations made from the data given by Brækkan and Boge (1962). However, it should be pointed out that the essential amino acids quoted for man (Anon., 1973) are not the same as those given for fish. A comparison with the provisional scoring pattern given by Anon. (1973) shows that all analysed amino acids except cysteine were in excess.

Free amino acids (FAA)

There is good evidence that FAA contribute to the adjustment of intracellular osmotic pressure (see Love, 1980). The concentration of the individual FAA furthermore depends on species and dietary history (Kaushik and Luquet, 1979; Timoshina, 1970), time and site of sampling (Kjosbakken and Larsen, 1981), and age (Siddiqui *et al.*, 1973; Ostrowski and Divakaran, 1989).

The composition of FAA in the present study reveals some deviation from the results (for comparative reasons recalculated) previously reported for Atlantic halibut white muscle by Partmann and Schlaszus (1973). The relative levels of lysine and histidine are respectively nine and 10 times higher in the present study and the relative glycine level is approximately 6% compared with 28% in the Partmann and Schlaszus (1973) study. Less conspicuous deviations were observed in taurine, threonine, proline, and anserine. The material of this previous study, however, was obtained off the coast of west Greenland in May and no information offered as to the age of the fish. These factors may explain the seemingly large discrepancies.

The present study reveals significant differences in the FAA composition between individual types of tissue. This is consistent with observations concerning other fish species (Obatake *et al.*, 1985; Suzuki *et al.*, 1987; Wilson and Poe, 1974). The high level of free imidazole compounds in white muscle may be attributable to this tissue's physiological role where the compounds act as buffers against flooding by lactic acid during emergency exertions (Suzuki *et al.*, 1987; Love, 1980).

As with the observation of a possible sexual heterogeneity in the peptide amino acids, it is not clear whether the sexually determined differences in the FAA have any physiological function.

Taurine was the major FAA in all tissues investigated, which is in accordance with the findings of some former investigations of Atlantic halibut (Partmann and Schlaszus, 1973) and other fish species (Wilson and Poe, 1974; Assem and Hanke, 1983). Taurine is often considered to be metabolically inert (King *et al.*, 1980), and its

main function in fish is thought to be concerned with the intracellular osmoregulation (Lassere and Gilles, 1971; Colley *et al.*, 1974; Fugelli and Zachariassen, 1976; Vislie, 1983). Other physiological functions have not yet been established in fish.

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