Effect of Growth on the Levels of Free Histidine and Amino Acids in White Muscle of Milkfish (*Chanos chanos*)

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Accumulation patterns of free amino acids (FAAs) in white muscle of milkfish (*Chanos chanos*) during the period of 8-month growth were investigated. Histidine, taurine, and glycine were the predominant FAAs. An increase in histidine was observed during growth. The level present in the 1-month-reared fish (mean weight = 58 g) was about 5 times higher than that in the initial fish (1.4 g). After 8 months of growth, milkfish contained 59 μmol/g, which was responsible for 72% of the total FAAs. There was a positive correlation between the histidine content and fish body weight. In contrast, taurine decreased with increasing rearing time. The glycine level was not correlated well with fish size. The total relative amounts of histidine, taurine, and glycine remained about the same, although the individual contributions varied considerably in the FAA pool during the period of cultivation.

Keywords: *Milkfish; free amino acids; histidine; growth; white muscle*

INTRODUCTION

Milkfish is an important cultured fish in the Indo-Pacific region, particularly in the Philippines, Indonesia, and Taiwan (Chen, 1990). Technologies of artificial propagation, intensive culture, and formulated feeds for this fish have been successfully developed in these countries (Chen, 1990; Liao, 1991). However, little information has been reported on the biochemical characteristics of the milkfish muscle. Our previous study (Shiau et al., 1996) showed that the most prominent free amino acid (FAA) in the white muscle of milkfish was histidine, which accounted for 63% of the total FAAs. As compared to other fish and shellfish (Konosu and Yamaguchi, 1982; Suzuki et al., 1987), milkfish has a FAA pattern similar to that of migratory high-speed swimmers, such as mackerel, tuna, and skipjack, which also possess a very high level of histidine in their white muscle. The living environment, swimming activity, and feeding diet for pond-reared milkfish are apparently different from those of migratory marine fish. Therefore, studies on the factors influencing the contents of FAAs in milkfish were conducted by our laboratory.

FAAs have been implicated as being responsible for the characteristic taste of seafoods (Konosu and Yamaguchi, 1982; Nishimura and Kato, 1988; Komata, 1990; Fuke, 1994). In addition, FAAs play important roles in physiological functions such as osmoregulation and buffer capacity in the tissues of aquatic animals (Schoffeniels and Gilles, 1972; Wright et al., 1986; Van Waarde, 1988; Abe, 1995). Free histidine, for instance, functions as an intracellular buffer as fish move vigorously, resulting in accumulation of acidic end products during the period of anaerobic metabolism (Castellini and Somero, 1981; Abe et al., 1985; Suyama et al., 1986; Van Waarde, 1988; Abe, 1995). Besides this, physiological functions of the large amount of free histidine in pelagic red-fleshed fish have not been satisfactorily eluciated (Abe, 1995). No study has been done on FAA constituents of cultured milkfish in relation to their growth. The objective of this study was to monitor the changes in levels of free histidine and other amino acids in the white muscle of milkfish during the course of growth.

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MATERIALS AND METHODS

Fish. Milkfish (*Chanos chanos* Forsskal) were cultured and collected from a fish farm in Kaohsiung County, in the southern area of Taiwan. At the start of rearing, fish fingerlings with a mean weight of 1.39 ± 0.24 g were stocked in a growout pond [90 m (l) × 90 m (w) × 2 m (h)]. The pond water used was underground water with a 2 ppt salinity. Water temperature varied from 19 to 29 °C during the experimental period. Fish were fed daily with an amount of 4% fish weight of a commercial dry pellet feed (moisture, 11%; protein, 29%; lipid, 8%; and ash, 9%) by using an automatic disk feeder. Rearing began in July 1994 and ended in February 1995 when fish reached market size (587 ± 85 g). The fish were captured at approximate time intervals by using a trap net, and 12 fish were randomly sampled for measuring body weight and length. The fish were held frozen with dry ice until brought to the laboratory in Keelung. On arrival at the laboratory within 7 h after collection, they were stored in a deep freezer (−40 °C) until use. Six individual fish from each growth group were sampled for chemical analyses in six replicates. After partial thawing, each fish was decapitated and filleted individually to obtain the white muscle (ordinary muscle) for the analyses of proximate composition and FAAs. The initial fish fingerlings were too small to obtain tissue sample sufficient for chemical analyses; therefore, more than 30 fish were combined as one fish sample. Similarly, the analyses were done in six replicates.

Proximate Composition. Moisture, crude protein, lipid, and ash were determined according to the AOAC (1990) method. Protein was estimated by the total nitrogen multiplied by 6.25.

Extraction of Free Amino Acids. The extract of FAAs in white muscle were prepared according to the method described by Konosu et al. (1974). A 10 g tissue sample was homogenized for 2 min in 20 mL of 7% cold trichloroacetic acid (TCA) using a Polytron homogenizer. The homogenate was centrifuged at 4000*g* (4 °C) for 20 min. The precipitate was extracted twice according to the same method. The supernatants were combined and made up to 100 mL with 7% TCA. A

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Table 1. Body Weight (Grams),[a] Body Length (Centimeters),[a] and Proximate Composition (Percent)[b] in White Muscle of Milkfish during the Period of 8-Month Growth

month	weight	length	moisture	crude fat	crude protein	ash
0	1.39 ± 0.24	4.72 ± 0.37	80.78 ± 1.49^{a}	1.56 ± 0.10^{c}	16.31 ± 0.91^{b}	1.29 ± 0.07^{a}
1.0	57.53 ± 4.66	14.60 ± 0.58	75.03 ± 1.09^{b}	2.45 ± 0.18^{b}	20.89 ± 0.67^{a}	1.28 ± 0.08^{a}
2.5	194.44 ± 37.14	22.72 ± 1.18	74.99 ± 0.72^{b}	2.83 ± 0.27^{ab}	19.81 ± 1.53^{a}	1.25 ± 0.09^{a}
4.5	422.36 ± 50.69	28.58 ± 1.20	74.38 ± 0.78^{b}	3.21 ± 0.33^{a}	20.98 ± 0.71^{a}	1.22 ± 0.11^{b}
6.5	477.82 ± 70.62	30.32 ± 1.21	74.10 ± 0.64^{b}	3.40 ± 0.24^{a}	19.94 ± 0.87^{a}	1.25 ± 0.07^{ab}
8.0	587.32 ± 85.26	32.38 ± 0.37	74.63 ± 0.66^{b}	3.43 ± 0.14^{a}	21.04 ± 0.66^{a}	1.21 ± 0.08^{b}

[a] Expressed as mean ± standard deviation ($n = 12$). [b] Expressed as mean ± standard deviation ($n = 6$). Means followed by the same letter within each column are not significantly different at $P = 0.05$.

Table 2. Free Amino Acids (Micromoles per Gram of Wet Weight)[a] in White Muscle of Milkfish during the Period of 8-Month Growth

	month					
	0	1.0	2.5	4.5	6.5	8.0
phosphoserine	0.25 ± 0.01^{a}	0.08 ± 0.01^{b}	0.07 ± 0.02^{b}	0.07 ± 0.01^{b}	0.05 ± 0.00^{c}	0.05 ± 0.01^{c}
taurine	8.45 ± 1.99^{bc}	15.81 ± 1.10^{a}	12.84 ± 1.60^{b}	7.54 ± 1.93^{c}	6.37 ± 2.75^{c}	2.80 ± 0.83^{d}
aspartic acid	1.81 ± 0.23^{a}	0.19 ± 0.07^{b}	0.19 ± 0.06^{b}	0.23 ± 0.08^{b}	0.20 ± 0.22^{b}	0.06 ± 0.02^{b}
threonine	1.72 ± 0.17^{a}	0.74 ± 0.09^{c}	0.56 ± 0.07^{cd}	0.49 ± 0.11^{d}	0.60 ± 0.28^{cd}	0.98 ± 0.24^{b}
serine	2.64 ± 0.25^{a}	1.61 ± 0.29^{b}	0.94 ± 0.15^{cd}	1.09 ± 0.31^{c}	0.63 ± 0.13^{e}	0.74 ± 0.16^{de}
asparagine	0.82 ± 0.08^{a}	0.35 ± 0.05^{bc}	0.44 ± 0.15^{b}	0.40 ± 0.09^{bc}	0.27 ± 0.18^{c}	0.36 ± 0.03^{bc}
glutamic acid	6.27 ± 1.05^{a}	2.88 ± 0.64^{b}	1.63 ± 0.10^{c}	1.07 ± 0.15^{c}	1.10 ± 0.40^{c}	1.41 ± 0.82^{c}
glutamine	0.92 ± 0.06^{a}	0.98 ± 0.11^{a}	0.42 ± 0.10^{b}	0.39 ± 0.10^{b}	0.51 ± 0.11^{b}	0.48 ± 0.21^{b}
proline	3.46 ± 0.96^{a}	3.00 ± 0.67^{ab}	2.18 ± 2.03^{bc}	1.24 ± 0.41^{cd}	1.18 ± 0.29^{cd}	0.83 ± 0.14^{d}
glycine	6.92 ± 1.80^{a}	4.16 ± 1.08^{b}	2.95 ± 0.75^{b}	3.52 ± 1.44^{b}	8.39 ± 2.27^{a}	7.88 ± 1.91^{a}
alanine	6.53 ± 0.90^{a}	1.83 ± 0.09^{c}	2.11 ± 0.50^{c}	1.64 ± 0.13^{c}	1.58 ± 0.50^{c}	3.77 ± 1.54^{b}
valine	1.69 ± 0.60^{a}	0.76 ± 0.18^{b}	0.65 ± 0.10^{bc}	0.40 ± 0.15^{cd}	0.46 ± 0.06^{bcd}	0.27 ± 0.13^{d}
methionine	0.84 ± 0.13^{a}	0.19 ± 0.11^{b}	0.12 ± 0.07^{bc}	0.08 ± 0.04^{c}	0.10 ± 0.03^{c}	0.09 ± 0.02^{c}
isoleucine	1.49 ± 0.10^{a}	0.36 ± 0.07^{b}	0.28 ± 0.08^{c}	0.17 ± 0.03^{d}	0.17 ± 0.03^{d}	0.13 ± 0.03^{d}
leucine	2.84 ± 0.21^{a}	0.71 ± 0.08^{b}	0.48 ± 0.13^{c}	0.31 ± 0.09^{d}	0.27 ± 0.04^{d}	0.21 ± 0.03^{d}
tyrosine	1.10 ± 0.17^{a}	0.27 ± 0.02^{b}	0.22 ± 0.06^{bc}	0.17 ± 0.03^{c}	0.13 ± 0.05^{c}	0.12 ± 0.02^{c}
phenylalanine	1.20 ± 0.15^{a}	0.30 ± 0.03^{b}	0.19 ± 0.06^{c}	0.13 ± 0.04^{cd}	0.10 ± 0.03^{d}	0.06 ± 0.03^{d}
β-alanine	0.24 ± 0.05^{a}	0.05 ± 0.06^{c}	0.13 ± 0.05^{b}	0.06 ± 0.02^{c}	0.06 ± 0.01^{c}	0.02 ± 0.02^{c}
ornithine	0.32 ± 0.02^{b}	1.16 ± 0.12^{a}	0.40 ± 0.13^{b}	0.21 ± 0.07^{c}	0.12 ± 0.01^{cd}	0.09 ± 0.05^{d}
lysine	5.13 ± 0.29^{a}	1.87 ± 0.16^{cd}	1.78 ± 0.45^{cd}	1.41 ± 0.41^{d}	2.76 ± 1.82^{bc}	3.09 ± 0.66^{b}
histidine	7.16 ± 1.54^{e}	37.38 ± 3.15^{d}	40.41 ± 6.15^{cd}	46.17 ± 3.76^{bc}	47.90 ± 2.68^{b}	59.19 ± 8.73^{a}
arginine	2.12 ± 0.29^{a}	2.25 ± 0.14^{a}	0.75 ± 0.30^{b}	0.94 ± 0.38^{b}	0.30 ± 0.08^{c}	0.23 ± 0.05^{c}
total	63.23 ± 7.49^{d}	76.76 ± 4.38^{ab}	69.74 ± 4.38^{bcd}	67.74 ± 3.79^{cd}	73.24 ± 4.39^{bc}	82.68 ± 9.42^{a}

[a] Expressed as mean ± standard deviation ($n = 6$). Means followed by the same letter within each row are not significantly different at $P = 0.05$.

20 mL TCA-extracted supernatant was mixed with an equal amount of ether to remove the TCA. This procedure was repeated successively five times for removal of TCA. The aqueous solution was evaporated to dryness in a vacuum evaporator at a temperature below 40 °C. The dried matter was diluted with water and made up to 25 mL for FAA analysis.

Analysis of Free Amino Acids. As described in a previous paper (Shiau et al., 1996), FAAs were separated by ion exchange chromatography and analyzed by a Hitachi L-8500 high-speed amino acid analyzer with a Hitachi 2622 SC packed column (4.6 mm × 60 mm). The buffers used were the standard lithium citrate buffers. Postcolumn derivatization with ninhydrin yielded amino acid derivatives which were measured by the absorbance at 570 and 440 nm. Analytical conditions and procedures were performed according to the manual provided by the manufacturer (Hitachi, Ltd., Tokyo, Japan). The levels of FAAs were estimated on the basis of peak areas of known concentrations of the standards (Wako, Ltd., Osaka, Japan) by using a Hitachi D-2850 Chromato data processor.

Statistical Analyses. Data were analyzed using analysis of variance (ANOVA) through the general linear model (GLM) procedure (SAS Institute, Cary, NC). Duncan's multiple-range test was applied to determine the significance of differences between means.

RESULTS

Changes in Proximate Composition. Table 1 shows the changes in fish body weight and length and the proximate composition in the white muscle of milkfish during the course of growth. Milkfish increased their body weight from 1.39 ± 0.24 to 587 ± 85 g (mean ± SD of 12 fish) and body length from 4.72 ± 0.37 to 32.4 ± 0.4 cm during the 8-month growth period. The changes in moisture, protein, and fat were significant ($P = 0.05$) after the first month of growth. Moisture decreased while protein and fat increased with growth. The fat gradually increased with rearing time, but its amount throughout the growth period was <4%. Milkfish is a lean fish, that is, low in fat (5%) from the viewpoint of white muscle. This result generally agreed with that of our previous study for the white muscle of milkfish (Shiau et al., 1996).

Changes in Free Amino Acids. Table 2 shows the constituents of FAAs (expressed as micromoles per gram of wet weight) of the milkfish white muscle and their changes in levels during the growth period of 8 months. The total concentration of FAAs in the initial specimens (1.39 ± 0.24 g, mean of 12 fish) was lower than that of 1-month-reared fish with weight of 57.5 ± 4.7 g. Taurine, histidine, glycine, alanine, glutamic acid, and lysine were the major FAAs in the initial test fish. These six FAAs, with the exception of histidine and taurine, decreased significantly ($P = 0.05$) after fish were reared for 1 month, and a fluctuation in level was found during the elongated rearing period.

Histidine and taurine were the predominant FAAs in the reared fish. The levels of taurine and histidine in the fingerling fish were 7.2 and 8.5 μmol/g, accounting for 11 and 13% of the total FAAs, respectively. After the first month of growth, the histidine and taurine levels increased to as much as 15.8 and 37.4 μmol/g, which amounted to 21 and 49% of the total FAAs, respectively. Further increase in histidine was observed during the elongated growth. After 8 months of rearing, the milkfish white muscle contained free histidine at a concentration of 59 μmol/g, which was responsible for 72% of the total FAAs. In contrast to histidine, taurine gradually decreased with the increasing rearing time. The level of taurine in 8-month-reared fish was 2.8 μmol/g, accounting for only 3% of the total FAAs. Glycine, another abundant FAA in the reared fish, decreased during the first 2.5 months of growth and then increased thereafter. The level of this FAA was even higher than that of taurine after fish were reared for 6.5 months.

The amount of histidine, taurine, and glycine together in the initial test fish accounted for about 36% of the total FAAs. This ratio increased to as much as 75% in 1-month-reared fish. After 4.5 months of fish cultivation, these three FAAs reached 85% and remained relatively constant at this value thereafter. An increase in histidine concentration was counteracted by a decrease in taurine and/or glycine.

DISCUSSION

Marine pelagic red-fleshed fishes such as tuna, skipjack, and mackerel contain copious amounts of free histidine in their white muscle. This is an extraordinary feature absent in other animals (Konosu and Yamaguchi, 1982; Suzuki et al., 1987; Van Waarde, 1988; Abe, 1995). The histidine content in the white muscle ranged from 15 to 94 μmol/g in red-fleshed fishes, from 4 to 20 μmol/g in "intermediate" fishes, and from 0.07 to 1 μmol/g in white-fleshed species (Abe, 1995). Milkfish examined in this study mostly contained histidine in levels ranging from 20 to 60 μmol/g. Larger fish contained more histidine. From the viewpoint of histidine, milkfish seemed to be analogous to red-fleshed fish. Free histidine, an imidazole amino acid, has been reported to serve as a pH buffer in migratory red-fleshed fish muscle during the anaerobic burst locomotion of the animals (Castellini and Somero, 1981; Abe et al., 1985; Suyama et al., 1986; Van Waarde, 1988; Abe, 1995). Milkfish is a very active and easily scared fish (Gordon and Hong, 1986; Chen, 1990). The high level of histidine in the white muscle of milkfish may play a role in buffering capacity for maintaining the intracellular pH of skeletal muscle during burst swimming.

The accumulation of taurine in the oyster has been reported as a function of the main cellular osmoeffector (Powell et al., 1982; Heavers and Hammen, 1985). Abo Hegab and Hanke (1983) also demonstrated that free histidine together with glycine in the white muscle of goldfish played a role in maintaining osmotic homeostasis. The milkfish with the higher concentration of histidine generally contained lower levels of taurine and glycine. An increase in histidine concentration offset the decrease in taurine and/or glycine. The total relative amounts of these three FAAs remained about the samee, although the individual contributions varied considerably in the FAA pool during the period of cultivation. This result suggests that free histidine may have a compensation effect for osmoregulation in milkfish. We also found that the histidine concentration was significantly decreased by 46% after fish were starved for 40 days (data not shown). In addition to functioning as buffering agent and osmoeffector, free histidine may serve as an energy source for milkfish during prolonged starvation.

Histidine can give rise to a toxic level of histamine through decarboxylation by microbiological deterioration (Sakaguchi et al., 1984; Russell and Maretic, 1986; Taylor, 1989). No official report of illness caused by the consumption of milkfish has been reported in Taiwan. However, our preliminary data showed that the histamine levels in some dried milkfish products were over 500 ppm, which was condsidered a hazard action level (Russell and Maretic, 1986; Taylor, 1989). Because the formation of histamine in milkfish products relates to seafood safety, an investigation is necessary for further relevant information in this regard.

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