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AMINO ACID AND FATTY ACID PROFILES OF PEARL SPOTTED RABBITFISH SIGANUS CANALICULATUS (PARK) MUSCLES (PISCES : SIGANIDAE).

By

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ABSTRACT

Pearl spotted rabbitfish **Siganus canaliculatus** *(Park) hold particular promise for mariculture in many countries of the world. Seventeen amino acids were separated from their muscles and were quantitatively measured. Essential amino acids represented 33% of the total. Lysine, leucine, glutamic acid and aspartic acid were detected in considerable quantities (7.4% 6.7%, 10.7% & 7% respectively). The relative abundance of sixteen individual fatty acids of muscle lipids were also determined by Gas Liquid Chromatography. Saturated fatty acids represented about 36% of the total. Oleic and eicosenic acids constituted about 22% & 21% respectively of the total monoenoic fatty acids (54%). These figures could be considered as indicators for the nutritional requirements of the species.*

In addition, crude protein, lipids, ash and moisture contents were monthly estimated for separate sexes for the whole year 1995. The average annual estimates were 14%, 3.4%, 3.8% & 79% (on fresh weight basis respectively). Lipids content showed the highest degree of variability particularly for females.

INTRODUCTION

Siganids (F : Siganidae) or rabbitfish are marine fish species widely distributed in tropical and subtropical waters. They are primarily herbivorous by nature, but are potentially omnivorous and may turn to other diets readily. Pearl spotted ***Siganus canaliculatus*** (Park) are considered as excellent food fish and among the highly prized in the Eastern Mediterranean and Indo-Pacific regions (Lam, 1974). The marine culture potential of these fishes is rapidly growing in many parts of the world *e.g* Philippines (Baga and Sacayanan, 1980); Tanzania (Bwathondi, 1982); Singaphore (Foo ***et al***, 1985); Indonesia (Tacon ***et al***, 1990) & Guam (Nelson ***et al***, 1990) and Arabian Peninsula (Wassef and Abdel Hady, 2000 & El-Dakar, 2000). Much interest has recently arisen in their culture in the Arabian Gulf region as well (Al Thobaity ***et al***, 1984 ; Al Aradi ***et al***, 1985 & Al Ghais, 1993). Successful commercial farming of the species, and closely related siganids, would relay on the availability of balanced formulated feeds. Meanwhile, specific studies on their nutrient requirements are lacking. Basically it could be inferred that the amino acid composition of fish protein may be taken as a good indication for the quantitative requirements in the fish diet (Rumsey and Ketola, 1975 & Ogino and Nanri, 1980). This is also supported by the studies of Ogata ***et al***, (1983). Nevertheless, Wilson (1985) considered the total body composition to be the best guide. The present work was undertaken to provide information about the quantitative individual amino acids as well as fatty acids in ***S. canaliculatus*** muscles, particularly those considered to be essential for the species. It is hoped that this information would be beneficial for the development of artificial diets for the species and other siganids as well. From the available literature previous investigation relevant to the subject are limited to those of Tacon ***et al***, (1990) and Kugavankiji ***et al***, (1990).

MATERIAL & METHODS

Sampling

Live ***S. canaliculatus*** samples were obtained from the commercial fishery of Saudi Arabia waters of the Arabian Gulf landed at Dammam Center. They are locally known as *"Safi"*. Sampling extended for the whole year 1995, from January to December, at monthly intervals. To avoid size variability, fish were selected of more or less uniform size (20-25 cm) which represents the most dominant market size in the area. In the laboratory, fish were measured (mm), weighed (g), sexed, carefully skinned and filleted. Each muscle sample, before being analyzed, was homoginized (using Edmond Buhner HD 4 Homoginizer). All analyses were carried out in duplicates for separate sexes according to the standard AOAC (1980) methods. Amino acid and fatty acid profiles, were performed for two female fish samples (each 25 cm length) captured in May 5, 1995.

Analytical Procedures

Moisture content, was determined by oven drying at 60 °C until constant weight (about 24h).

Total lipids, were extracted from fresh muscles using a mixture of chloroform and methanol (2 : 1, v / v) in a Soxhelt apparatus as described by Floch ***et al*****,** (1957).

Estrification of fatty acids was carried out on the extracted lipids with the method of (Christie, 1973). Fatty acid methyl esters were separated and quantified by Gas-Liquid Chromatography (GLC, Pye Unicum , UC Mshimedzu model). Peak identification on fatty acid profile chart was performed by comparing the relative retention time (RRT) of each compound with those of standard materials.

Crude protein content was determined as total nitrogen content (in dried muscles) using the micro-Kjeldahl apparatus. Protein content was calculated by multiplying total nitrogen by 6.25 .Protein content was then converted to wet weight basis.

Amino acid profile was obtained following acid hydrolysis of a dried muscle sample (24 h in 6 N Hcl at 110 °C) , under nitrogen and chromatographic separation on Beckman Amino Acid Analyzer 119 CL. Seventeen individual amino acids were quantitatively estimated (as mg AA/100 g sample), and percentage of each in 100 g protein was then calculated (as g AA / 100 g protein).

Ash content was estimated by ignition of dried muscle samples into a muffle furnace at 600 °C for about 6 hours.

Statistical analysis :0

Mean value (X) and standard deviation (s.d) of protein, lipids, moisture and ash contents were calculated monthly for separate sexes. One-way analysis of variance (ANOVA) was used to test significance variability of data (Snedecor and Cochran, 1980).

RESULTS

Biochemical composition

The four major constituents of ***S. canalicualtus*** muscles, namely : protein, lipids, moisture and ash were determined monthly for males and females separately. Monthly variations of the average values are represented in Fig (1).

Crude protein content

The average percentage of protein varied between 12.35 & 17.7% (mean 14.26%) for males and 11.15 & 18.16 % (mean 13.81%) for females. Monthly variations of protein percent (Fig 1 A) revealed that both sexes exhibited the same trend. Maximum values were attained in July for males and August for females, whereas the minimum were shown in February and March for males and females respectively. Sex variations were tested monthly to be insignificant ($P < 0.01$), Therefore sexes were combined and the overall crude protein content was estimated to be 14.03 %.

On the other hand, seasonal variations of protein content for males, females and sexes combined (Fig 2 D) showed that the highest values were in summer (17.02 %) while the lowest were in spring (12.46 %) and winter (12.7 %).

Total lipids content

In contrast to percentage protein, average of lipids percent in any month was noticed to vary within broader limits: 0.19-6.11 % (mean 3.17 %) for males and 0.6- 6.86 % (mean 3.58 %) for females. Likewise, sex variations were proved to be insignificant ($P<0.05$).Accordingly, the average annual total lipids content was calculated to be 3.37 % for sexes combined.

Monthly fluctuations of lipids content showed a peak value in March (about 5% for males & 7% for females) followed by a remarkable drop in April and May which was more obvious for females. Another noticeable reduction in lipids content was traced during the period from June to August (Fig 1 B).

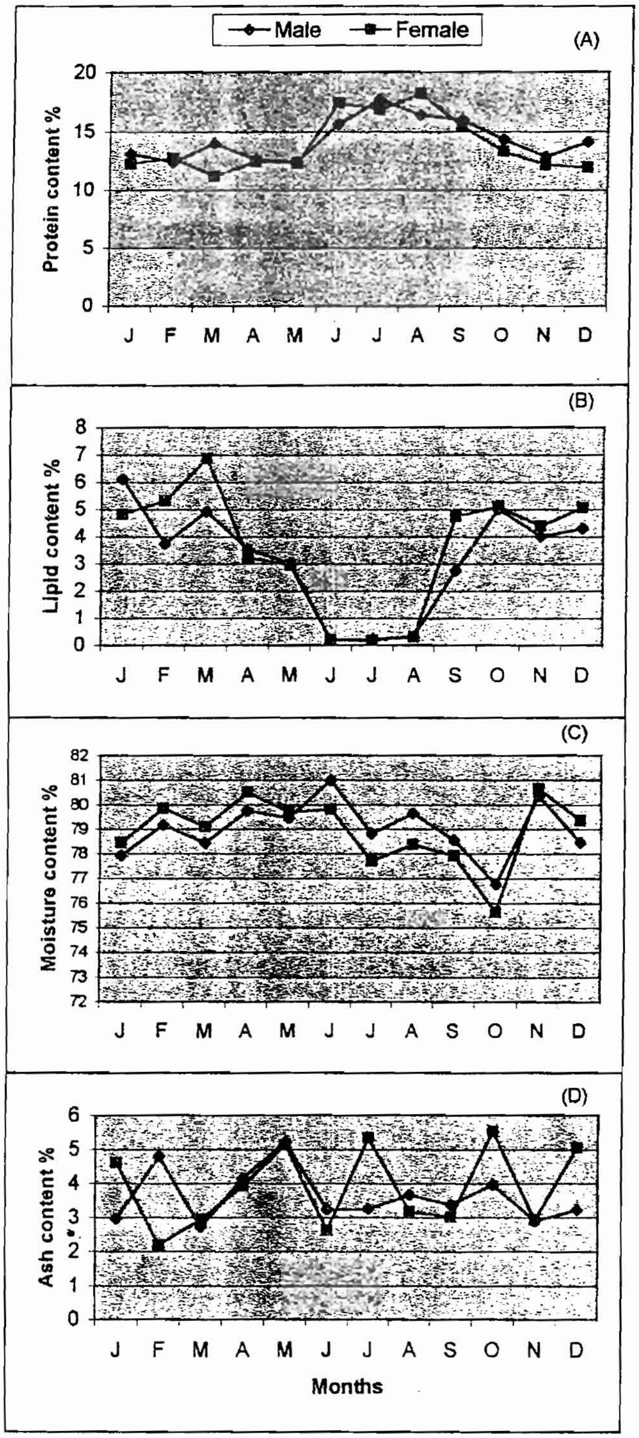

Fig (1)- Monthly variations of protein, lipid, moisture and ash contents of *Siganus canaliculatus* muscles.

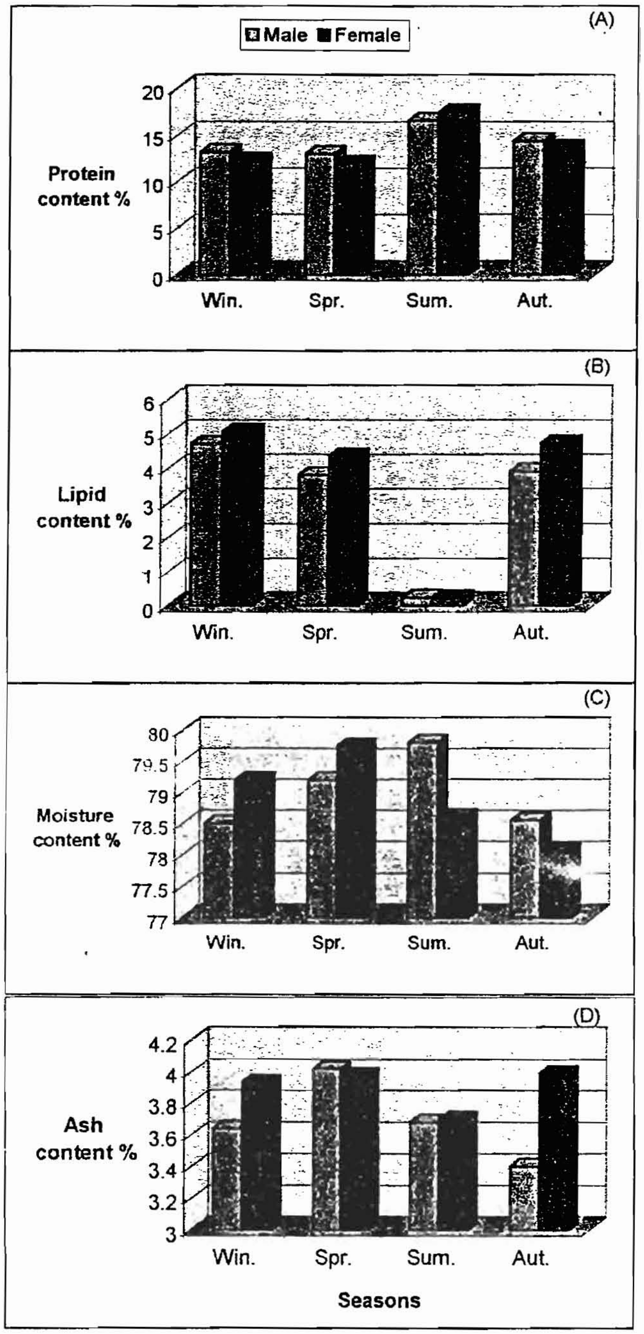

Fig (2)- Seasonal variations of protein, lipid, moisture and ash contents of *S. canaliculatus* muscles

Marked seasonal changes in muscle lipids were identified, particularly for females (Fig 2 C). The highest values were attained in winter (4.88 %), autumn (4.32 %) and spring (4.07%) and the lowest (0.23%) were in summer.

Moisture content

Percentage of water varied from 76.76 to 80.98 % (mean 79 %) for males and from 75.61 to 80.65 % (mean 78.91 %) for females. Differences due to sex variation were found to be insignificant ($P< 0.05$).Grand mean water percent was estimated to be about 79% for sexes combined. The highest records were distinguished in November (80.5%) while the lowest were in October (76.19%) for both sexes (Fig 1 C).Comparatively higher water contents were detected in spring and summer (79.5 & 79.2 % respectively) than in winter (78.9%) or autumn (78.3%) (Fig 2 B).

Ash content

Percentage of ash showed limited degree of variability and ranged between 2.71& 5.19% (mean 3.61%) for males and 2.17-5.52% (mean 3.9%) for females.

Insignificant sex variations were evidenced ($P<0.01$) .Mean annual percent was 3.76 % for both sexes. The pattern of monthly changes of ash content is given in Fig (1 D) and showed similar trend of variation among sexes, except for February where a slight reduction was noted for females. Almost constant ash percents were recorded for sexes combined in summer and autumn (3.7 %), winter (3.8 %) and spring (4 %) (Fig 2 A).

Amino acids Profile

Seventeen individual amino acids were separated from the muscular protein of *S. canaliculatus* and quantitatively estimated in Table (1). Among the nine essential amino acids (without tryptophan) which represented only about 33 % of the total, the most abundant are : lysine (7.36 %), leucine (6.72%), valine (4.36%) & phenylalanine (3.59%) .The concentration of cystine was the lowest (0.22%).

Adequate amounts of each of the nonessential amino acids were also detected .Glutamic acid showed the highest concentration (10.84%), followed by aspartic acid (6.96%) then alanine (4.82%), whereas histidine had the lowest amount (0.95%).

Fatty acids

Sixteen fatty acid components of muscle lipids were quantitatively determined (Table 2) .Unsaturated fatty acids constituted the major part (about 64.15%) of the total, of which the monoenoic fatty acids are present in significant proportions (54%).

The major saturated fatty acids were oleic acid (C 18 : 1 w 9, 22.15 %) & eicosenic (C 20:1w9, 20.56%) , followed by capric acid (C10 : 0, 15.87%) then palmitolic (C 16 : 1 w 9, 6.95%).The other fatty acids were detected in relatively smaller amounts (Table 2).

Table (1) : Amino acid (AA) profile in a femal(25 cm TL*) *Siganus canaliculatus* muscles .

Amino acid	g/100 sample	gAA/100g protein
Essential		
Isoleucine	2.29	2.95
Leucine	6.72	8.65
Lysine	7.36	9.48
Methionine	2.51	2.23
Pheylalanine	3.59	4.62
Cystine	0.22	0.28
Threonine	2.9	3.73
Tyrosine	3.09	3.89
Valine	4.36	5.61
Total	33.04	
Nonessential		
Alanine	4.82	6.21
Arginine	3.10	3.99
Aspartic acid	6.96	8.96
Glutamic acid	10.84	13.96
Glycine	3.03	3.90
Histidine	0.95	1.22
Proline	1.55	2.0
Serine	2.61	3.36
Total	66.96	

* TL = Total length

Table (2)- Fatty acid composition of muscles lipids of a female (25 cm TL*) *Siganus canaliculatus* .

Fatty acid group	Carbon No.	Common name	Conc.(%)
Saturated	8: 0	Caprylic acid	2.1
	10: 0	Capric acid	15.87
	12: 0	Lauric acid	1.76
	14: 0	Myristic acid	1. 46
	16: 0	Palmitic acid	2.06
	20: 0	Arcahidic acid	3.94
	22: 0	Behnic acid	3.35
	24: 0	Lignoceric acid	5.32
	Total		35.85
Monoenoic	12: 1		2.99
	16: 1(9)	Pamitolic acid	6.95
	18: 1(9)	Oleic acid	22.15
	20: 1	Eicosenic acid	20.56
	24: 1	Nervonic acid	1.36
	Total		54.00
Polyenoic	18: 3 (3)	Linolenic acid	1.68
	20: 2	Eicosadienic acid	3.19
	Total		4.87
Unknown			5.58

* TL = Total length

DISCUSSION

Information on the nutritive value of siganids as food fish is relatively scarce (Peiris and Grero, 1972, for ***Siganus javus*** *and* Mohamed 1991 for ***S. rivulatus****)*.The present work results showed that ***S. canaliculatus*** has a high mutritive value, being rich in protein (about 14 %) and low in fat (3.4 %). The general muscle composition is very comparable for the three siganid species and can serve as a valuable index for their nutrient requirements.

The present study has revealed monthly as well as seasonal variations in muscle constituents, mainly total lipids and crude protein (Figs 1 & 2). The marked drop in total lipids noticed in the period from June to August (summer) indicates the utilization of muscle lipid reserves during the spawning season (April and May) (Wassef and Abdel Hady, 1997) and reflects the increased energy requirement for gonad formation. This explanation can be also applicable for the relatively decreased protein content observed in spring coincided with the breeding activity. However, the slightly lower values of muscle nutrients (protein & lipids) recorded in winter might be a result of decreased feeding intensity (gut- filling indices were minimal, as stated by Wassef and Abdel Hady, 1997).These results of chemical composition could shed some light on ***S. canaliculatus*** nutrient requirement throughout the whole year. In cultivated fish, provision of proper feed during the prespawning period minimizes the need for mobilizing reserves.

Fish like other animals, do not have a true protein requirement but have requirements for a well balanced mixture of essential and non essential amino acids. Several investigators have used various methods to estimate the amino acid needs of fish, whose quantitative requirements have not been established yet, in order to design and improve their test diets (Wilson, 1985). A very good correlation between the essential amino acid requirements as determined by growth studies and the pattern of the same amino acids in the whole body protein (Cowey and Tacon, 1983 & Ogata ***et al***, 1983). Essential amino acid levels in isolated fish protein have used successfully for the initial design of test diets for various fish species (Ogata ***et al***, 1983 ; Cowey and Luquet, 1983 ; Wilson and Poe, 1985). Accordingly, amino acid figures determined in the present work could serve as a valuable index to formulate diets for ***S. canaliculatus***. Amino acid requirements when expressed as a percent of dietary protein should not change dramatically with increasing size of the fish (Wilson, 1985).

The sparing of dietary protein by lipids has been examined in many species of fish to find optimal levels of dietary lipids that allow protein to be used optimally for growth without depositing excessive lipid. The subject of essential fatty acid in fish nutrition has been well studied and has been reviewed by several authors (Watanabe, 1982). More recently, increasing attention has been paid to the relative importance of each fatty acid in fish diets (Bell ***et al***, 1986 , Hardy ***et al***, 1990 ; Izequierdo and Fernandez, 1997). Numerous studies eventually established the requirement of fish in general for the n- 3 polyunsaturated fatty acids (PUFA) but the extent to which the n-6 series are essential fatty acids (EFA) remains to be established (Izequierdo and Fernandez, 1997). Similar to amino acids, fatty acid profile of fish muscle reflect dietary fatty acid needs. Bell ***et al***, (1986) and Rodriguez ***et al***, (1993) have used fatty acid composition of fish oil successfully in the preparation of test diets for fish.

In conclusion, the commercial culture of siganids necessitates the availability of suitable feeds. Basically, it may be inferred that the figures determined in the present work for amino acid and fatty acid levels in ***S. canliculatus*** muscles could be taken as a good indication for the quantitative requirement in siganids diets.

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