Influence of season and feeding intensity on the fatty acid composition of wild cobia (*Rachycentron canadum*, Linnaeus, 1766) in the Dungun coast, Malaysia

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Cobia, *Rachycentron canadum*, is an important recreational marine fish of growing popularity in the aquaculture industry. Knowledge of the impact of environment on their fatty acids (FAs) utilization may contribute to the understanding their feeding in culture condition especially as cobia is been considered for low salinity culture. This study investigates the variations in the fatty acid contents of cobia from Dungun coast, Malaysia with respect to the changes in seasons and feeding intensity. Saturated fatty acids (FAs) comprised the majority of FAs in muscle, followed by monoenes, total poly-unsaturated fatty acids (PUFAs) (n-3) and then total PUFAs (n-6) with no seasonal variation in the quantity. A similar trend was observed in liver but total saturated FAs was significantly higher during the inter-monsoon while total monoenes significantly accumulate during monsoon. During low feeding intensity, there was a significant accumulation of PUFAs (n-6) in the muscle tissue ($P < 0.05$). Gut content analyses showed that cobia significantly increased the consumption of mollusks during the inter-monsoon ($P < 0.05$), although bony fishes dominated their diet throughout the year with no significant seasonal differences ($P > 0.05$). Our results suggest that cobia lipids are composed of a significant quantity of omega-3 and omega-6 FAs, which are considered to have important health benefits. While the environmental variability especially salinity fluctuations and prey abundance that accompany seasonal changes have a significant impact on the nutritional composition of cobia in Malaysian waters, their nutritional quality is maintained.

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Influence of season and feeding intensity on the fatty acid composition of wild cobia (*Rachycentron canadum*, Linnaeus, 1766) in the Dungun coast, Malaysia

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Abstract

Cobia, *Rachycentron canadum*, is an important recreational marine fish of growing popularity in the aquaculture industry. Knowledge of the impact of environment on their fatty acids (FAs) utilization may contribute to the understanding their feeding in culture condition especially as cobia is been considered for low salinity culture. This study investigates the variations in the fatty acid contents of cobia from Dungun coast, Malaysia with respect to the changes in seasons and feeding intensity. Saturated fatty acids (FAs) comprised the majority of FAs in muscle, followed by monoenes, total poly-unsaturated fatty acids (PUFAs) (n-3) and then total PUFAs (n-6) with no seasonal variation in the quantity. A similar trend was observed in liver but total saturated FAs was significantly higher during the inter-monsoon while total monoenes significantly accumulate during monsoon. During low feeding intensity, there was a significant accumulation of PUFAs (n-6) in the muscle tissue ($P < 0.05$). Gut content analyses showed that cobia significantly increased the consumption of mollusks during the inter-monsoon ($P < 0.05$), although bony fishes dominated their diet throughout the year with no significant seasonal differences (P > 0.05). Our results suggest that cobia lipids are composed of a significant quantity of omega-3 and omega-6 FAs, which are considered to have important health benefits. While the environmental variability especially salinity fluctuations and prey abundance that accompany seasonal changes have a significant impact on the nutritional composition of cobia in Malaysian waters, their nutritional quality is maintained.

Keywords: Season, feeding intensity, fatty acid, cobia, *Rachycentron canadum*.

Introduction

It is widely accepted that fish is an important source of protein and unique fats that provide omega-3 and omega-6 long-chain poly-unsaturated fatty acids (LC-PUFAs) (eicosapentaenoic [EPA], docosahexaenoic [DHA], and arachidonic [ARA] acids), which are not synthesized in the human body but are supplied by the diet (Schmidt et al., 2005). Fish fats are also an important source of fat-soluble vitamins A, D, E, and K and are important for regulating cholesterol metabolism (Kris-Etherton et al., 2002). Hence, commercial fisheries continue to be of significant importance to human. The quantity of lipids and types of fatty acids (FAs) present in fish have been found to differ considerably depending on the species, habitat, diet, season, and fishing period (Winston &, Di Giulio, 1999; Erkan & O-zden, 2007). Although LC-PUFAs play important physiological roles in fish, marine fish, like other vertebrate species, have a limited ability to biosynthesize LC-PUFAs from C_{18} precursors (Tocher et al., 2005). Therefore, the accumulation of PUFAs in marine fish is thought to originate from planktonic sources (Mazorra et al., 2003; Montero et al., 2001).

The cobia is a pelagic marine fish of the family Rachycentridae and is a highly prized recreational fish. This species is gaining increasing popularity in the aquaculture industry and is experiencing growing market demand, most notably in Europe. Aquaculture production of cobia improved 7000 folds from 1995 to 2005. It estimated global production in 2012 and 2014 it was estimated to be 51000 and 40000 tons (FAO, 2014). Year to year variation in cobia production in different countries was varies drastically, as the trends are increasing in some countries like China while it is fading in other countries due to production bottleneck (Global Aquaculture Advocate, 2014). Among the identified problem in cobia culture is nutrition and their adaptations to low salinity environment. Denson et al. (2003) reported that the growth rate of cobia was reduced significantly as salinity falls to 15 ppt. while another finding showed that cobia can tolerate salinity as low as 5ppt. (Resley et al., 2006). Thus, investigating their utilization of individual FAs during seasonal changes and low salinity condition would be useful for understanding their low salinity culture for possible feed optimization.

Cobia natural prey includes fish, crustaceans, and mollusks (Arendt et al., 2001). It has been demonstrated that cobia in captivity require high levels of dietary protein for effective biomass yields (Faulk & Holt, 2003; Craig et al., 2006; Zhou et al., 2007; Chou et al., 2001). Dietary sources of lipids provide not only essential FAs, but are also a source of energy for fish (Sargent et al., 1989). A study investigating the potential nutritive value of cobia reported them to be composed of essential amino acids, and that cobia are considered to be of significant nutritive value to humans (Li et al., 2002). However, quantitative assessments of the FA composition of commercially important species of fish are essential for human dietary regulation, to paint a complete picture of the nutritional quality of various fish species and their potential health benefits. Despite that cobia make up a large portion of total catches in the Malaysia (exceeding 1000 metric tons annually), cobia is not favored for consumption in Malaysia and the cobia aquaculture industry remains underdeveloped FAO, 2009). Therefore, we investigated the relationship of season and prey availability with the fatty acid compositions of lipids from wild cobia caught off the coast of Dungun, Malaysia in other to provide information on the cobia as a potential food source, and gauge the potential for the development of cobia as a commercially important species in Malaysia. Also, we evaluated their utilization of some FAs during salinity shift that accompanied the season changes, as the culture of cobia in low salinity yet to be fully understood.

Materials and Methods

Sampling

The samples of cobia were collected from commercial fish land centre at Dungun, Terengganu, Malaysia (Fig 1) for gut content and FA analyses. Cobia was not a target species in any fisheries in the area and it was fisheries-independent, hence samples used in this study were purely bycatch.

As a result of low abundance and inconsistent availability, a total of 53 specimens were collected during the study period. These samples were collected during the southwest monsoon [May (n=10) and September (n=10)], inter-monsoon [October (n=10)], northwest monsoon [November (n=5) and March (n=10),], and second inter-monsoon [April (n=8)]. Specimens were kept on ice while transported to the laboratory. Fork length (from the tip of the snout to the end of the middle caudal fin rays) (cm) and wet weight (kg) were recorded. Specimens were immediately dissected and sex was determined. The stomach was dissected and the weight (g) of food items was recorded. Gut contents were preserved in 5% formalin until further identification. Stomachs with food (full, half full, and trace) were grouped as “stomachs with food” (SWF) while those without food were grouped as “empty stomachs” (ESM). Food items were identified and categorized as fish, mollusks, and crustacean. Mean percent weight of the individual food classes was reported as the average for the seasons. Muscle and liver samples were kept at −20°C for fatty acid analysis.

Analysis of FA composition

The FA composition of the liver and muscle tissue samples were determined by extracting lipids using chloroform: methanol (2:1, v/v) mixture followed by the preparation of fatty acid methyl esters (FAMEs) according to the method proposed by Folch et al., (1957) and modified by Ebrahimi et al. (2014). Heneicosanoic acid (C21:0) was added to each sample as an internal standard (Sigma-Aldrich, Inc., St. Louis, MO, USA) prior to trans-methylation. The trans-methylation to FAMEs was carried out using 0.66 N KOH in methanol and 14% methanolic boron trifluoride (BF_3) (Sigma-Aldrich Inc.) according to the method of the AOAC (1990). The FAMEs were separated in a gas chromatograph (Model 6890A Agilent Technologies, Santa Clara, USA) equipped with a flame ionization detector and a splitless injector with the aid of Supelco SP-2560 capillary column (L × I.D. 30 m × 0.25 mm, df 0.20 μm). High purity nitrogen was used as the carrier gas at 40 mL/min. Compressed air and high purity hydrogen were used for the flame ionization detector in the chromatograph. To facilitate optimal separation, the oven temperature was set at 100°C for 2 min and warmed to 170°C at 10°C/min, held for 2 min, warmed to 230°C at 5°C/min, and then held for 20 min. Identification of individual FAs was performed by comparing the resulting peaks with the relative FAME peak retention times of the heneicosanoic acid standards used. Results of FA composition were expressed as percentages of the total FAs determined. A reference standard (mix C4–C24 methyl esters; Sigma-Aldrich, Inc.,) and CLA standard mix (CLA cis-9 trans-11 and CLA trans-10, cis-12; Sigma-Aldrich) were used to determine the recoveries and correction factors for the determination of individual FA composition. All reagents were analytical grade.

Water quality parameters

The physical water quality parameters (salinity, temperature, DO and pH) were measured *in situ* using an YSI multi parameter (model 6600, YSI, United States) and calibrated according to the manufacturer's recommendations before field sampling. The water parameters were measure10 km off-shore at 2m, 4m and 6m depth and the average was recorded.

Statistical analyses

Quantities of each FA and their respective classes (saturated, mono-, and poly-unsaturated fatty acids) were reported as mean ± standard error (SE) to show variation between season and feeding status, while an independent t-test was used to determine the significant differences between these factors. An independent t-test was also used to determine the significant seasonal differences in the weight of each food class and water parameters. Analyses were performed using SPSS version 16.0 (IBM; Chicago, IL, USA). The significance level was set at $P < 0.05$, while outcomes of $P < 0.01$ were considered to be highly significant.

Results

Water quality parameters

The water quality parameters measured showed higher mean values of salinity and temperature during the monsoon, while DO and pH were higher during inter-monsoon, although the differences were not significant ($P> 0.05$) (Figure 2).

Seasonal variations in FA composition

In this study, we measured a total of 15 fatty acids: 5 saturated (myristic acid, pentadecanoic acid, palmitic acid, margaric acid, and stearic acid), 3 monoenes (palmitoleic acid, cis-10-heptadecanoic acid, and oleic acid), 3 PUFAs n-6 (linoleic acid, γ-linoleic acid, and

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arachidonic acid), and 4 PUFAs n-3 (α-Linoleic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid). Measurements of mean FA profiles for cobia and corresponding seasonal changes in the muscle and liver showed that palmitic acid, stearic acid, and margaric acid, were the dominant FAs (in order of descending quantity) among the saturated FAs in both the liver and muscle tissues during all seasons (Table 1).

Among the saturated FAs, pentadecanoic acid was significantly higher ($P < 0.05$) in the muscle during the monsoon season with a value of 1.23 ± 0.10% and lower during the inter-monsoon period with a value of 0.74 ± 0.08% while margaric acid was significantly higher during inter-monsoon ($P < 0.05$). In the liver, only palmitic acid showed significantly seasonal difference among the saturated FAs during as it value increased from 29.82 ± 0.59% during the monsoon season to 32.12±0.23% in the inter-monsoon.

Among the monoenes, oleic acid was the dominant FA in this class, while of the polyenoic acids, docosahexaenoic (DHA represented the highest proportion from both liver and muscle samples, followed by arachidonic acid (ARA) and eicosapentaenoic acid (EPA, C20:5 n-3) (Table 1). In the current study, palmitoleic acid and cis-10-heptadecanoic acid were significantly higher in the muscle during the monsoon season, while in the liver, oleic acid showed significantly higher seasonal abundances during periods of high rainfall. Among the polyenoic FAs, linoleic acid and α-linoleic acid were significantly higher in the muscle during the monsoon. Conversely, DHA was significantly reduced. However in the liver, ARA was higher during the inter-monsoon period while a significant reduction in α-linoleic acid was observed at the same time.

Variation in FA composition based on feeding intensity

The FA composition of muscle tissue samples of cobia collected during their active feeding period (SWF) showed a significant higher palmitoleic acid (Table 2), whereas, ARA was significantly higher during low feeding intensity in the muscle. Among all the FA classes, only total PUFA n-6 was significantly accumulated in the cobia muscle despite low of feeding activity. However, in the liver, total saturated FAs was significantly higher in SWF samples while total PUFA n-3 was significantly retained in those with empty stomach. In the present study, EPA and DHA were significantly higher in the livers of SWF specimens.

Food composition of cobia and their seasonal changes

On the Dungun coast, cobia prefers to feed on bony fish, which dominated the gut content throughout the year. No significant seasonal difference ($P > 0.05$) was observed in the weight of fish consumed, while a significant increase in the consumption of mollusks was recorded during the inter-monsoon period ($P < 0.05$) (Figure 3).

Discussion

The major saturated FA found in cobia in the present study was palmitic acid in both liver and muscle samples. Not only was palmitic acid the dominant saturated FA in cobia in the present study, the dominance of palmitic acid has also been well reported in several other marine species (e.g., the Baltic herring, *Clupea harengus* (Szlinder-Richert et al., 2010) and the Baltic sprat, *Sprattus sprattus balticus* (Usydus et al., 2012). Additionally, a fairly recent study examining the chemical compositions and FA profiles of three freshwater fish species from Pakistan found that palmitic acid was also the most abundant FA in *Cyprinus carpio*, *Labeo rohita*, and *Oreochromis mossambicus* (Jabeen & Chaudhry, 2011). The dominant FAs in cobia muscle tissue were also found to be dominant in the liver in both seasons. Even though, their seasonal variation followed a different pattern in the liver and muscle (Table 1).

The minimum value of salinity in the coastal water of Dungun during this study was 30.30 ppt. in monsoon and the maximum was 32.90 ppt. in the inter-monsoon, while the temperature ranged from 29.30 °C to 31.50 °C. These two parameters were not significantly vary across the season, and are within the optimal range for cobia (Shaffer and Nakamura, 1989). Hence, the higher value of pentadecanoic, palmitoleic, cis-10-heptadecanoic, linoleic, and α-linoleic FA in the muscle during the monsoon season may be attributed to increased feeding activities (Figure 3). The nutritional composition of the diet has direct effects on an organism's nutritional makeup. In a study on cobia, a positive correlation was reported between dietary DHA and the DHA content of different body tissues of larval cobia (Faulk & Holt, 2005). Lipid concentrations in the cobia muscle were found to increase significantly as dietary lipids increased (Chou et al., 2001). Moreso, in commercial cobia broodstock, dietary n-3, highly unsaturated fatty acids (HUFAs), and egg quality were all found to exhibit a high degree of association (Faulk & Holt, 2008). Additionally, Nguyen et al. (2010) reported that increases in ARA in the diet of cobia broodstock and its subsequent accumulation in the egg might possibly cause low fertilization success. This is especially relevant as reproductive activity is one of the factors known to affect the accumulation and distribution of PUFAs (Sushchik et al., 2007), where reproductive success is dependent on sources of energy such as the nutritional composition of broodstock diets (Tandler et al., 1995). Therefore, if ARA is not well utilized for reproduction in cobia, it may be accumulating in the body, possibly explaining the accumulation of ARA in cobia which was significantly higher in the liver observed during the inter-monsoon period. Also, during low feeding, ARA tends to accumulate in the muscle.

In teleosts, the content of various FAs in lipids varies from species to species, as does its distribution in the body of each organism and the metabolic physiology associated with individual FAs may vary from species to species (Liu et al., 2009; Szlinder-Richert et al., 2010). Cobias in Dungun water showed no significant difference in muscle FA classes with changes in rainfall abundance and salinity shift. Therefore, from aquaculture point of view, cobia could be considered to be for low salinity environment if dietary requirement for such condition is met.

Carnivorous fish species such as cobia require much higher dietary protein than do most omnivorous or herbivorous species. This difference likely result from the fact that carnivorous species do not rely on carbohydrates as an energy source to the same extent as omnivorous and herbivorous species. The significant quantities of n-3 and n-6 FAs in cobia suggests that this species has the ability to accumulate and retain these FAs from the herbivorous constituents of its diet. Likewise, other species known to consume phytoplankton have been shown to be rich in PUFAs (Mazorra et al., 2003; Montero et al., 2001). During low feeding, the cobia analyzed in this study tended to utilize FAs from the saturated class and monoenes faster than those in the PUFA n-6 which was significantly higher in muscle samples from specimens with empty stomachs compared to those caught in the active feeding condition. The highly emphasized medicinal value of DHA and EPA, and the high abundance of these two FAs in cobia suggest that cobia would be a highly nutritious addition to the human diet.

The overall n6: n-3: FA ratios recorded for cobia liver and muscle in the current study were 0.36 and 0.52, respectively. As the n-6: n-3 FA ratio is suggested to be an indicator when comparing the nutritional values of fish oils, where values $\geq$ 0.25 are considered desirable (Jabeen & Chaudhry, 2001), our results imply that cobia is highly nutritionally beneficial for human consumption. In addition, the high levels of the PUFA n-6 arachidonic acid further supports the potential health benefits of cobia, as PUFAs are renowned for reducing plasma cholesterol, thereby imparting cardiological benefits by lowering the risk of coronary heart disease (Kolanowski et al., 2006; Harris et al., 2006).

Conclusion

This study provides novel information on the proximate composition and nutritional quality of cobia in Malaysian waters. Our finding showed that the nutritional quality of cobia is not seasonally affected, and that cobia could play an important role for humans in the acquisition of essential FAs. In addition, this study highlights the enormous potential of the development of cobia as a commercially important species in Malaysia, where it is currently considered bycatch by commercial fishermen. For low salinity culture of cobia, farmers need to combine efforts to manipulate the nutritional quality of these species to enhance their n-3 HUFAs concentrations especially when these species are reared in captivity system. The wash-out strategy may provide an adequate description of the changes in the fillet lipid fatty acid profiles of fatty fish.

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Author Contributions

S. M. Nurul Amin and Taofik A. Babatunde conceived and designed the experiment.

Taofik A. Babatunde and Mahdi Ebrahimi performed the experiments and analyzed the data.

S. M. Nurul Amin and Mahdi Ebrahimi contributed reagents.

Fatimah Md. Yusoff, Arshad A. and Yuzine B. Esa reviewed draft of the manuscript.

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Legends of Figures

Figure 1. Map of peninsular Malaysia showing sampling location.

Figure 2. Seasonal changes in water quality parameters of Dungun coast.

Figure 3. Mean monthly percentage weight (g) of food classes in cobia gut by season.

Legends of Tables

Table 1. Fatty acid composition (% of identified fatty acids) of cobia muscle and liver from Dungun coast, Malaysia by season.

Table 2: Fatty acid composition (% of identified fatty acids) of cobia muscle and liver from Dungun coast, Malaysia with respect to feeding status


THAILAND
SOUTH CHINA SEA
0 100 km
PERLIS
KEDAH
George Town
PENANG
KELANTAN
TERENGGANU
PERAK
DUNGUN
MALAYSIA
Sitiawan
PAHANG
SELANGOR
Kuala Lumpur
NEGERI SEMBILAN
MELAKA
Melaka
JOHOR
INDONESIA
Singapore
Dungun coast
DUNGUN

Fig 1

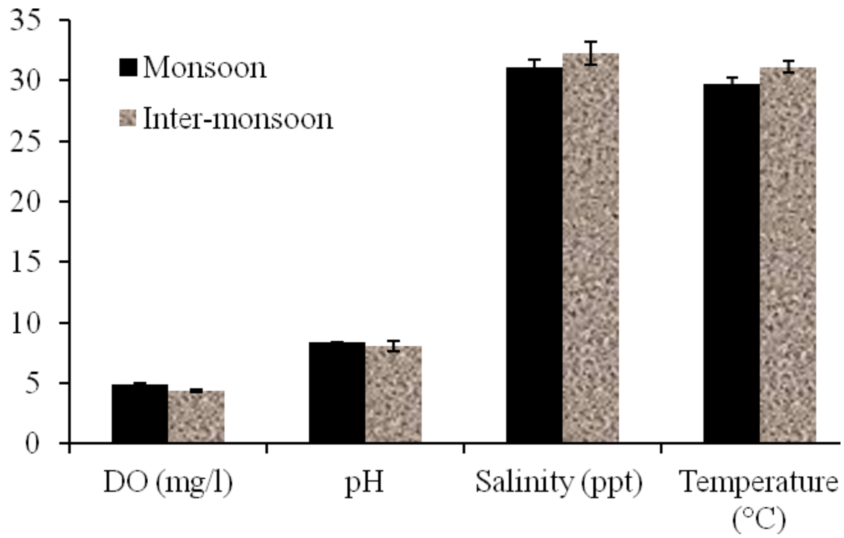

Fig 2

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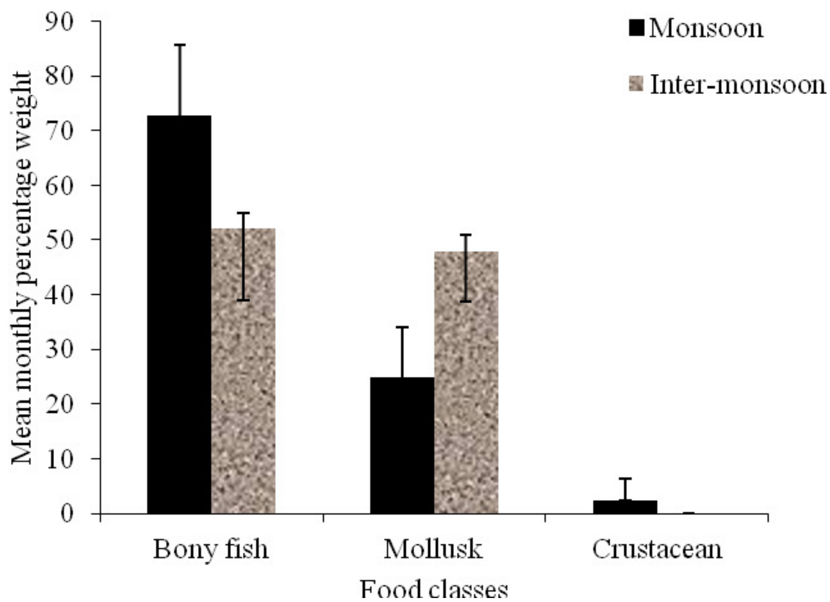

Fig 3

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Table 1. Fatty acid composition (% of identified fatty acids) of cobia muscle and liver from Dungun coast, Malaysia by season

% Fatty acid	Muscle		Liver	
	Inter-monsoon (N = 18)	Monsoon (N = 35)	Inter-monsoon (N = 18)	Monsoon (N = 35)
14:0 (Myristic acid)	1.94±0.10	2.06±0.22	0.81±0.03	0.87±0.03
15:0 (Pentadecanoic acid)	0.74±0.08[a]	1.23±0.10[b]	1.48±0.07	1.37±0.05
16:0 (Palmitic acid)	26.73±0.50	26.57±0.66	32.12±0.23[a]	28.82±0.59[b]
17:0 (Margaric acid)	3.37±0.29[a]	2.07±0.15[b]	1.76±0.10	2.02±0.12
18:0 (Stearic acid)	11.60±0.33	12.68±0.63	11.51±0.45	11.69±0.40
Total Saturated	44.11±0.48	44.45±0.77	47.68±0.35[a]	44.77±0.63[b]
16:1 (Palmitoleic acid)	3.88±0.21[a]	4.93±0.29[b]	3.64±0.15	3.88±0.22
17:1 (Cis-10-heptadecanoic acid)	1.00±0.08[a]	1.84±0.13[b]	0.72±0.03	0.80±0.05
18:1 n-9 (Oleic acid)	21.20±0.70	20.29±0.56	23.73±0.56[a]	27.31±0.64[b]
Total Monoenes	26.07±0.78	27.07±0.72	28.09±0.65[a]	31.99±0.78[b]
18:2 n-6 (Linoleic acid)	1.53±0.26[a]	2.43±0.21[b]	1.38±0.29	1.77±0.20
18:3 n-6 (γ-linolenic acid)	0.62±0.08	0.81±0.10	0.45±0.03	0.40±0.02
20:4 n-6 (Arachidonic acid)	5.30±0.38	5.26±0.40	5.48±0.34[a]	4.20±0.29[b]
Total PUFA n-6 (%)	7.46±0.54	8.50±0.49	7.31±0.45	6.38±0.38
18:3n-3 (α-Linoleic acid)	1.18±0.12[a]	2.35±0.18[b]	0.50±0.04[a]	0.65±0.04[a]
20:5n-3 (Eicosapentaenoic acid)	3.07±0.20	3.12±0.12	2.02±0.14	1.83±0.11
22:5n-3 (Docosapentaenoic acid)	2.68±0.23	2.52±0.14	1.86±0.09	2.17±0.16
22:6n-3 (Docosahexaenoic acid)	15.42±1.18[a]	11.78±0.80[b]	12.70±0.33	12.25±0.75
Total PUFA n-3 (%)	22.35±1.18	19.76±0.79	17.08±0.41	16.90±0.91
n-6:n-3 Ratio	0.34±0.03[a]	0.61±0.12[b]	0.33±0.03	0.38±0.02
Unsaturated:Saturated	1.24±0.03	1.65±0.29	1.27±0.04	1.21±0.03
Total PUFA	29.80±1.16	28.27±1.12	24.39±0.79	23.28±1.09
Poly:Sat Ratio	0.68±0.03	0.65±0.03	0.51±0.02	0.53±0.03

Different superscripted letters between pairs in the row indicate significant differences (($P < 0.05$)
Total saturated: sum of C14:0+C15:0+C16:0+C17:0+C18:0
Total Monoenes: sum of C16:1+C17:1+C18:1(n-9)
Total PUFA n-6: sum of C18:2n-6+ C18:3n-6+ C20:4n-6
Total PUFA n-3: sum of C18:3n-3+ C20:5n-3+ C22:5n-3+ C22:6n-3

Table 2: Fatty acid composition (% of identified fatty acids) of cobia muscle and liver from Dungun coast, Malaysia with respect to feeding status

	Muscle		Liver	
	SWF (n=25)	ESM (n=28)	SWF(n=25)	ESM(n=28)
14:0 (Myristic acid)	1.88±0.17	2.14±0.24	0.87±0.03	0.83±0.03
15:0 (Pentadecanoic acid)	1.03±0.13	1.10±0.09	1.32±0.07[a]	1.49±0.05[b]
16:0 (Palmitic acid)	27.54±0.67	25.82±0.60	31.10±0.50[a]	28.90±0.67[b]
17:0 (Margaric acid)	2.56±0.27	2.47±0.20	1.83±0.11	2.02±0.13
18:0 (Stearic acid)	11.96±0.62	12.46±0.63	11.61±0.42	11.64±0.44
Total Saturated	44.97±0.93	43.77±0.55	46.74±0.59[a]	44.89±0.69[b]
16:1 (Palmitoleic acid)	5.34±0.29[a]	3.89±0.25[b]	4.02±0.26	3.60±0.16
17:1 (Cis-10-heptadecanoic acid)	1.66±0.17	1.46±0.13	0.75±0.03	0.79±0.06
18:1 n-9 (Oleic acid)	20.72±0.71	20.50±0.54	26.62±0.79	25.63±0.67
Total Monoenes	27.71±0.80	25.85±0.71	31.39±0.96	30.02±0.78
18:2 n-6 (Linoleic acid)	2.02±0.26	2.22±0.24	1.76±0.26	1.53±0.21
18:3 n-6 (γ-linolenic acid)	0.71±0.10	0.78±0.10	0.43±0.02	0.41±0.03
20:4 n-6 (Arachidonic acid)	4.59±0.40[a]	5.89±0.40[b]	4.24±0.33	4.98±0.32
Total PUFA n-6 (%)	7.32±0.60[a]	8.89±0.44[b]	6.44±0.39	6.93±0.45
18:3n-3 (α-Linoleic acid)	1.79±0.18	2.10±0.22	0.60±0.05	0.60±0.03
20:5n-3 (Eicosapentaenoic acid)	3.13±0.16	3.07±0.13	1.69±0.09[a]	2.07±0.13[b]
22:5n-3 (Docosapentaenoic acid)	2.45±0.21	2.69±0.13	1.87±0.16	2.23±0.16
22:6n-3 (Docosahexaenoic acid)	12.56±0.95	13.42±1.03	11.30±0.68[a]	13.39±0.70[b]
Total PUFA n-3 (%)	19.93±0.96	21.28±0.95	15.47±0.79[a]	18.30±0.85[b]
n-6:n-3 Ratio	0.59±0.17	0.44±0.03	0.38±0.03	0.35±0.02
Unsaturated:Saturated	1.79±0.41	1.25±0.03	1.21±0.04	1.26±0.03
Total PUFA	27.25±1.25	30.17±1.07	21.90±0.97[a]	25.22±1.09[b]
Poly:Sat Ratio	0.62±0.04	0.70±0.03	0.47±0.02[a]	0.57±0.03[b]

Different superscripted letters between pairs in the row indicate significant differences (($P < 0.05$)
Total saturated: sum of C14:0+C15:0+C16:0+C17:0+C18:0
Total Monoenes: sum of C16:1+C17:1+C18:1(n-9)
Total PUFA n-6: sum of C18:2n-6+ C18:3n-6+ C20:4n-6

Total PUFA n-3: sum of C18:3n-3+ C20:5n-3+ C22:5n-3+ C22:6n-3