Feed type regulates the fatty acid profiles of golden pompano *Trachinotus ovatus* (Linnaeus 1758)

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ABSTRACT
The aim of the present study was to evaluate the effect of different feed types on the fatty acid profiles of golden pompano (*Trachinotus ovatus*). Three feed types (pelleted feed, frozen squid and frozen fish) were assigned to triplicate groups of fish (146.22 ± 3.26 g) in a total of 9 floating cages (50 fish per cage). Analysis of the fatty acid profiles of the three feed types revealed that frozen squid had the highest levels of long-chain polyunsaturated fatty acids (LC-PUFAs), while the pelleted feed and frozen fish had lower levels of LC-PUFA, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). After three months of being fed a specific feed type, the fatty acid profiles of golden pompano muscle tissues were analysed. The highest levels of LC-PUFA were detected in fish fed with frozen squid. Golden pompano fed with frozen fish had intermediate levels of LC-PUFA content. The results suggest that the fatty acid composition of prey items can significantly affect the muscle fatty acid composition of the consumer. Muscle fatty acid composition could also be used as an indicator of diet type for golden pompano in the aquaculture industry.

1. Introduction
Long-chain polyunsaturated fatty acids (LC-PUFAs), such as arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are essential nutrients which play a number of crucial structural, functional and signalling roles (Ruxton et al. 2007; Morais et al. 2015). These LC-PUFAs are components of biomembranes, important precursors for eicosanoids, ligands of transcription factors, bioactive molecules and also function as messengers in cellular pathways (Jump 2002; Marsh 2008; Morais et al. 2015). The inclusion of LC-PUFA in human diets has played an effective role in preventing cardiovascular disease, protecting against oxidative stress and reducing cardiovascular mortality (Neuringer et al. 1988; Ruxton et al. 2007), making it an increasingly important research area.

LC-PUFA cannot be synthesized *de novo* by vertebrates including humans as they lack the fatty acid desaturase enzymes required for the production of linoleate (LA; 18:2n-6) and linolenate (ALA; 18:3n-3) from oleic acid (18:1n-9), which must be obtained from the diet (Wallis et al. 2002; Zheng et al. 2004). Fish are the primary source of n-3 LC-PUFA in the human diet. Changes in the fatty acid composition of tissue are known to be controlled by various regulatory mechanisms. The fatty acid composition of fish can be regulated by species, environment (salinity, temperature, season and habitat) (Leaver et al. 2008; Kuah et al. 2015; Taşbozan et al. 2015) and by the fatty acid content and composition of their prey (Li et al. 2008; Monroig et al. 2010; Santigosa et al. 2011).

Golden pompano, *Trachinotus ovatus* (Carangidae, *Trachinotus*) (Linnaeus 1758) is an economically important marine teleost species. It is found in tropical and subtropical waters of the eastern Atlantic Ocean, the Indian Ocean, the west coast of the Pacific, in the Mediterranean and along the African coast, including offshore islands (Xie et al. 2014). This species exhibits fast growth and efficient feed conversion, making it a good candidate for intensive farming in these regions. Additionally, it has been found that there are high levels of LC-PUFA (particularly DHA) in golden pompano muscle (Zhang et al. 2010). In recent years, large-scale marine cages have been established for golden pompano culture in the Asian-Pacific region, including China and Southeast Asian countries. Currently, there are no studies addressing the effect of diet on the nutritional values of golden pompano. The present study investigated the effect of different feed types on the fatty acid profile of golden pompano in floating sea cage.

2. Materials and methods

2.1. Experimental setup and sampling

Healthy juvenile golden pompano with an average body weight of 146.22 ± 3.26 g were obtained from a 4 × 4 × 4 m³...
were homogenized using a meat grinder and stored at −80°C. The boneless golden pompano fillets were cut into small pieces which suitable for mouth (about 10–20 mm) by machine before feeding. The fish were fed 5–8% of their total biomass twice a day (at 09:00 and 17:00 hours) for 3 months. During the experimental period, the seawater temperature was 29.0 ± 1.5°C (mean ± SD) and the salinity was 32.5 ± 2.0‰. There was no difference in the survival rate of fish in different feed type groups (P > .05).

After the experimental period, three individual fish were obtained from each cage and anesthetized with eugenol. These fish were dissected to separate the visceral tissues. Muscle tissue was extracted and washed with tap water to remove adhering blood. The boneless golden pompano fillets were homogenized using a meat grinder and stored at −80°C.

### 2.2. Fatty acid composition analysis

Fatty acid methyl esters (FAMEs) from fatty acids of freeze-dried muscle from golden pompano in each experimental treatment were prepared and quantified using Agilent HP6890 gas chromatography. Total lipids of the experimental feed types and muscle from golden pompano in each experimental treatment (n = 3 per treatment) were extracted by chloroform/methanol (2:1, v/v) containing 0.1% butylated hydroxytoluene as an antioxidant following Folch et al. (1957). Total lipids were quantified gravimetrically after evaporation of the solvent under nitrogen flow, followed by vacuum desiccation overnight (Morais et al. 2015). Subsequently, total lipids were subjected to acid-catalyzed transmethylation with 21:0 internal standard at 50°C for 2 hours (Navarro-Guillén et al. 2014). FAMEs were extracted using isohexane/diethyl ether (1:1, v/v), and purified by thin-layer chromatography (Christie 1982). FAMEs were separated and quantified using HP6890 gas chromatography equipment with a fused silica capillary column (007-CW) and a flame ionization detector. The procedures for analysing the fatty acid profiles were based on those described by Metcalfe et al. (1966) with minor modifications (Ai et al. 2008). The column temperature was programmed to rise from 150°C to 200°C at a rate of 15°C/min and from 200°C to 250°C at a rate of 2°C/min. Injector and detector temperatures were 220°C and 250°C, respectively. Fatty acids were identified by comparing the retention times of fatty acids between experimental groups and standard fatty acid methyl esters (Sigma, USA).

### Table 1. Fatty acid compositions (% of total fatty acids) of three feed types.

| Fatty acids | Pelleted feed | Frozen squid | Frozen fish |
|-------------|---------------|---------------|-------------|
| C14:0       | 2.37 ± 0.12   | 2.47 ± 0.38   | 3.13 ± 0.87  |
| C14:1n-5    | ND            | ND            | ND          |
| C15:0       | 0.73 ± 0.06   | 0.70 ± 0.00   | ND          |
| C16:0       | 24.03 ± 0.32  | 29.40 ± 0.95  | 26.23 ± 1.34 |
| C16:1n-7    | 3.43 ± 0.32   | 0.53 ± 0.06   | 2.90 ± 1.22  |
| C17:0       | ND            | 1.37 ± 0.12   | 0.97 ± 0.06  |
| C18:0       | 4.70 ± 0.17   | 7.80 ± 0.50   | 11.8 ± 0.70  |
| C18:1n-9c   | 28.40 ± 0.10  | 3.03 ± 0.61   | 14.0 ± 5.12  |
| C18:2n-6    | 28.63 ± 1.50  | 0.33 ± 0.23   | 12.3 ± 0.12  |
| C18:3n-6    | ND            | 0.20 ± 0.00   | 0.40 ± 0.00  |
| C18:3n-3    | 3.13 ± 0.12   | ND            | 0.43 ± 0.12  |
| C20:0       | ND            | 0.37 ± 0.06   | 0.43 ± 0.15  |
| C20:1       | ND            | 1.87 ± 0.06   | 0.50 ± 0.17  |
| C20:2       | ND            | 0.23 ± 0.06   | 0.30 ± 0.00  |
| C20:3n-3    | ND            | 0.13 ± 0.06   | ND          |
| C20:4n-6(ARA)| ND           | 3.70 ± 0.52   | 2.67 ± 0.23  |
| C20:5n-3(EPA)| ND          | 10.77 ± 0.21  | 5.20 ± 0.79  |
| C22:0       | ND            | 0.47 ± 0.06   | ND          |
| C22:1n-9    | ND            | 1.10 ± 0.30   | 1.50 ± 1.13  |
| C22:6n-3(DHA)| ND         | 33.33 ± 1.07  | 26.20 ± 7.10 |
| C23:0       | ND            | 0.20 ± 0.00   | ND          |
| C24:0       | ND            | 0.90 ± 0.10   | 2.73 ± 0.15  |
| C24:1n-9    | ND            | 1.20 ± 0.46   | 1.77 ± 0.32  |
| ∑SFA        | 31.30 ± 0.84  | 43.61 ± 0.88  | 42.39 ± 2.80 |
| ∑UFA        | 63.50 ± 6.59  | 56.42 ± 1.75  | 57.10 ± 2.72 |
| ∑MUFA       | 31.74 ± 6.22  | 7.73 ± 1.11   | 20.67 ± 5.54 |
| ∑PUFA       | 31.76 ± 0.93  | 48.69 ± 0.66  | 36.43 ± 8.18 |
| EPA + DHA   | 0.00 ± 0.58   | 44.10 ± 0.58  | 31.40 ± 7.89 |
| ∑n-3        | 3.13 ± 0.58   | 44.23 ± 0.45  | 31.83 ± 8.47 |
| ∑n-6        | 28.63 ± 0.31  | 4.23 ± 0.75   | 4.30 ± 0.17  |
| ∑3n-3/∑n-6  | 0.11 ± 0.02   | 10.46 ± 1.70  | 7.40 ± 1.67  |

Notes: Data are expressed as mean ± SD of triplicate, n = 3. ND, not detected; SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-3 PUFA, n-3 polyunsaturated fatty acid; n-6 PUFA, n-6 polyunsaturated fatty acid.
2.3. Statistical analysis

Mean and standard deviation values were calculated for each treatment. Data from each treatment were subjected to one-way analysis of variance (ANOVA) using SPSS 19.0 for Windows. Tukey’s multiple range test was chosen as a multiple comparison test and the significance level of $P \leq 0.05$ was used. All the data were tested for normality, homogeneity and independence before ANOVA.

3. Results and discussion

The fatty acid profiles of the three feed types and of the golden pompano in each of the treatments are listed in Tables 1 and 2. The fatty acid profiles of these diets suited the objectives of the feeding experiment (Table 1). Frozen squid was found to contain the highest levels of all LC-PUFA, in particular EPA and DHA, but had low levels of LA and ALA. Highest levels of LA and ALA were found in the pelleted feed, but EPA and DHA levels were almost zero for this feed type. The frozen fish showed intermediate levels of LA, ALA and LC-PUFA. During the experimental period all feed types were consumed by the experimental fish.

The fatty acid composition of golden pompano muscle revealed that the fatty acid composition of the feed type had a significant effect on fatty acid composition of golden pompano muscle (Table 2). The content of saturated fatty acid and unsaturated fatty acid were not significantly different in the muscle of golden pompano from different groups ($P > 0.05$), however, monounsaturated fatty acid (MUFA) and PUFA levels were significantly affected by the different feed types ($P < 0.05$). Fish consuming the pelleted feed had the highest percentage of MUFA, while the frozen squid group had the lowest content of MUFA. The predominant fatty acid in MUFAs for all groups was C18:1n-9. Highest levels of PUFA were detected in the muscle tissues of fish fed the frozen squid. The most abundant fatty acids in PUFAs for all groups were LA (C18:2n-6), ALA (C18:3n-3), ARA (C20:4n-6), EPA (C20:5n-3) and DHA (C22:6n-3), respectively. These results are in keeping with previous studies by Fallah et al. (2011), Zakipour et al. (2012), Ehsani et al. (2013) and Taşbozan et al. (2015). In the present study, golden pompano consuming pelleted feed exhibited higher proportions of 18:2n-6 and total n-6 PUFA, and lower proportions of EPA and DHA compared to those fed frozen fish and frozen squid ($P < 0.05$). Higher levels of EPA and DHA were found in the muscle samples from the frozen squid group in comparison with the pelleted feed group.

The high content of monoenic fatty acids in the commercial pellets led to higher levels of MUFA in the muscle of the pelleted feed group. Low levels of MUFA in the frozen squid group can be attributed to frozen squid having a lower content of MUFA than the other feed types. EPA and DHA are desirable properties in fish because they play an active role in promoting human health. In the present study, DHA/EPA levels were significantly higher for fish fed frozen squid and frozen fish than fish fed with pellets ($P < 0.05$). Frozen squid were found to be rich in n-3 highly unsaturated fatty acid (HUFAs), especially EPA and DHA, similar to other reports (Wilson and Moreau 1996; Luo et al. 2007). The n-3/n-6 ratio is a good index for comparing relative nutritional value of fish oils (Pigott and Tucker 1987; Taşbozan et al. 2015). Studies examining nutrient intake of wild animals and of humans over evolutionary time have suggested an optimal intake of n-3/n-6 at a 1:1 ratio (Simolopoulos 1989). In the present study, the ratio of n-6/n-3 values were 0.29, 1.76 and 1.51 for the pelleted feed, frozen squid and frozen fish groups, respectively. The high n-3/n-6 ratio of frozen squid, in addition to its fatty acid composition, make this a nutritious food source for golden pompano. The results of this study indicate that differences in diet can significantly influence the fatty acid composition of the muscle of the consumer. As expected, high levels of PUFAs in the diet of golden pompano in the present study lead to increased deposition of body PUFAs. Further, lipid composition of golden pompano muscle tissues were reflective of the lipid levels present in the different feed types used in this study. Similar effects have been noted in other marine fishes (Nematipour et al. 1992; Kirsch et al. 1998; Turner and Rooker 2005) but this is the first study examining the effect on golden pompano.

4. Conclusion

This study has shown that the fatty acid composition of feed type can significantly influence the muscle fatty acid composition of golden pompano. Comparing three different feed types, frozen squid was found to have higher nutritional values than frozen fish and pelleted feed. Frozen squid were abundant in n-3 PUFA, especially EPA and DHA, so it could preferentially conserve n-3 HUFAs, helping juvenile golden pompano to maintain normal membrane function in the absence of this essential dietary fatty acid. In conclusion, PUFAs act as a good indicator of diet in golden pompano and may be useful dietary markers. This could be of particular use for culturing golden pompano and may prove useful for retrospectively examining trophic relationships of fishes in aquaculture.

Disclosure statement

No potential conflict of interest was reported by the authors.

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