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Original Research Article

Dietary n-3/n-6 polyunsaturated fatty acid ratio modulates growth performance in spotted seabass (*Lateolabrax maculatus*) through regulating lipid metabolism, hepatic antioxidant capacity and intestinal health

Yanzou Dong [a], Yu Wei [a], Ling Wang [a], Kai Song [a], Chunxiao Zhang [a], Kangle Lu [a, *], Samad Rahimnejad [b, c]

[a] *Key Laboratory of Healthy Mariculture for the East China Sea, Ministry of Agriculture and Rural Affairs, Fisheries College, Jimei University, Xiamen 361021, China*

[b] *Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Zátiší 728, Vodňany 389 25, Czech Republic*

[c] *Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia 30100, Spain*

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ABSTRACT

An 8-week feeding experiment was carried out to explore the effects of dietary n-3/n-6 polyunsaturated fatty acid (PUFA) ratio on growth performance, lipid metabolism, hepatic antioxidant status, and gut flora of spotted seabass (*Lateolabrax maculatus*). Six experimental diets were formulated to contain different levels of two purified oil sources including docosahexaenoic and eicosapentaenoic acids enriched oil (n-3) and linoleic acid-enriched oil (n-6) leading to n-3/n-6 PUFA ratios of 0.04, 0.35, 0.66, 1.35, 2.45 and 16.17. Each diet was fed to triplicate groups of juvenile *L. maculatus* (11.06 ± 0.20 g, 30 fish/tank). Final body weight (FBW), weight gain (WG), specific growth rates (SGR), protein efficiency ratio (PER) and feed utilization efficiency increased as n-3/n-6 PUFA ratio increased up to a certain level, and then decreased thereafter. Fish fed the diet with n-3/n-6 PUFA ratio of 0.66 exhibited the highest FBW, WG, SGR and PER and the lowest feed conversion ratio. Lower n-3/n-6 PUFA ratios induced up-regulated expression of lipid synthesis-related genes (*fas*, *acc2* and *srebp-1c*) and down-regulated expression of lipolysis related genes (*atgl*, *pparα*, *cpt-1* and *aox*). Higher expression of lipolysis-related genes (*atgl*, *pparα* and *cpt-1*) was recorded at moderate n-3/n-6 PUFA ratios (0.66 to 1.35). Moreover, inappropriate n-3/n-6 PUFA ratios triggered up-regulation of pro-inflammatory genes (*il-6* and *tnf-α*) and down-regulation of anti-inflammatory genes (*il-4* and *il-10*) in the intestine. The diet with n-3/n-6 PUFA ratio of 0.66 inhibited intestine inflammation, improved intestinal flora richness, increased the abundance of beneficial bacteria such as *Lactobacillus, Alloprevotella* and *Ruminococcus*, and reduced the abundance of harmful bacteria including *Escherichia-Shigella* and *Enterococcus*. In summary, it could be suggested that a dietary n-3/n-6 PUFA ratio of 0.66 can improve growth performance and feed utilization in *L. maculatus*, as is deemed to be mediated through regulation of lipid metabolism and intestinal flora.

* Corresponding author.
E-mail address: lukangle@jmu.edu.cn (K. Lu).

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1. Introduction

Fats play essential roles in vertebrates by serving as an energy source and participating in key metabolic processes (Watanabe, 1982; Zechner et al., 2012; Wang et al., 2022). Notably, it has been demonstrated that the fat requirement of animals is determined to a great extent by its fatty acids (Glencross, 2009). Most

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limiting points of de novo synthesis of fatty acids (Dong et al., 2020). Transcription factor SREBP-1C is an important regulator of the expression of *acc* and *fas*, and some other genes involved in triglyceride (TAG) and cholesterol formation (Horton et al., 2002). Previous studies have demonstrated that the expression of *srebp-1c* could be affected by PUFA (Worgall et al., 1998). In the present study, fish fed Diet 1 had the highest expression level of *acc2*, *fas* and *srebp-1c*, indicating that a low dietary n-3/n-6 PUFA ratio enhances lipid synthesis, which agrees with previous reports in other fish species (He et al., 2021; Jin et al., 2019). ATGL is the rate-limiting enzyme for TAG hydrolysis (Yang et al., 2010). As the main product of TAG hydrolysis, fatty acids are further broken down by β-oxidation (Den Broeder et al., 2015). The β-oxidation of fatty acids with a carbon number ≥20 occurs in peroxisome, and other fatty acids are β-oxidated in mitochondria. The transfer of fatty acids into peroxisome or mitochondria is regulated by the key enzymes CPT-1 and AOX, respectively (Tocher, 2003). Moreover, the expression of *cpt-1* and *aox* can be activated by the transcription factor PPARα (Pyper et al., 2010). In this study, the fish fed Diet 3 or/and Diet 4 showed high expression levels of *atgl*, *pparα* and *cpt-1*, indicating that inappropriate dietary n-3/n-6 PUFA ratios inhibit lipolysis, as is in parallel with previous studies (Ayala et al., 2014; Zhu et al., 2017). Increased expression of *aox* by increasing the n-3/n-6 PUFA ratio corresponded with enhanced AFP and serum TG and TC concentrations. These results indicate that spotted seabass may have a limited catabolic capacity of n-6 PUFA.

The intestine is a vital digestive organ which also plays key roles in the immune and metabolic functions in fish (Cai et al., 2020; Liu et al., 2021). Intestinal inflammation is a common phenomenon in cultured fish, and could be induced by unsuitable nutritional conditions (Liu et al., 2021). Fish suffering from intestinal inflammation often imply an inefficient utilization of nutrients and impaired health status (Venold et al., 2012; Wang et al., 2020). Several authors have found that inappropriate dietary fat level and fatty acid composition can activate inflammatory responses (Dai et al., 2021; Xie et al., 2021). In the current study, we determined the transcriptional levels of pro-inflammatory cytokines (IL-6 and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10). Fish fed the diet with the n-3/n-6 PUFA ratio of 0.66 (Diet 3) showed the lowest expression levels of *il-6* and *tnf-α* and the highest expression levels of *il-4* and *il-10*, indicating that a suitable dietary n-3/n-6 ratio can inhibit intestinal inflammation. The extreme n-3/n-6 PUFA ratios (Diet 1 or 6) triggered intestinal inflammation, as was evidenced by the up-regulated expression of *il-6* and *tnf-α*, and down-regulated expression of *il-4* and *il-10*. Furthermore, the fish fed Diet 1 displayed the highest expression levels of *il-6* and *tnf-α*, and the lowest expression levels of *il-4* and *il-10*. Interestingly, there are several reports indicating that excessive dietary n-6 PUFA can activate inflammation (Li et al., 2021; Tan et al., 2022), and that supplementation of n-3 PUFA can ameliorate the inflammatory response (Zhu et al., 2022), as are in agreement with the results of the current study.

Studies in mammalian models have demonstrated that the interactions between dietary fatty acid composition and intestinal flora play a significant role in regulation of intestinal inflammation (Ye et al., 2021). In the present study, the fish fed the diet with n-3/n-6 PUFA ratio of 0.66 and the extreme ratios (0.04 and 16.17) displayed large differences in intestinal inflammatory responses. Accordingly, we further analyzed the intestinal flora composition of these 3 groups. The results of the PCoA analysis showed that the intestinal flora composition of *L. maculatus* was clearly modified by dietary n-3/n-6 PUFA ratio. At the same time, the value of observed species, Chao1 index and ACE index indicated that at extreme dietary n-3/n-6 PUFA ratios (0.04 and 16.17), particularly at a high ratio (16.17), the richness of intestinal flora decreases. Recently, the link between intestinal flora and inflammation has been widely reported. Many members of the *Lactobacillus* species have anti-inflammatory activity (Jiang et al., 2018; Mohamadzadeh et al., 2011). Also, *Alloprevotella* and *Ruminococcus* play a role in anti-inflammatory processes through producing short-chain fatty acids (Clemente et al., 2018; Yang et al., 2019; Zhong et al., 2021). Moreover, the abundance of *Escherichia-Shigella* and *Enterococcus* has been shown to increase with the activation of the inflammatory response, thus serving as a biomarker of inflammation (Cattaneo et al., 2017; Grant et al., 2021). In the present study, the abundance of *Lactobacillus*, *Alloprevotella* and *Ruminococcus* increased at dietary n-3/n-6 PUFA ratio of 0.66 compared to the ratios of 0.04 and 16.17. Furthermore, the abundance of *Escherichia-Shigella* and *Enterococcus* increased at dietary n-3/n-6 PUFA ratios of 16.17 and 0.04, respectively. These results indicate that dietary n-3/n-6 PUFA ratio may affect intestinal inflammation through modulating the microbial flora. It has been well documented that EPA and DHA could directly promote the growth of beneficial bacteria or indirectly through increasing the production of short-chain fatty acids or short-chain fatty acid salts (Fu et al., 2021). In this study, at both low and high extremes of dietary n-3/n-6 PUFA ratio, the intestinal flora was negatively influenced indicating that n-6 PUFA is also an essential nutrient for probiotics. Accumulated evidence reveals that the intestinal flora is involved in the inflammatory response, digestive process and metabolic regulation of the host (Liu et al., 2019, 2021; Murakami et al., 2016). In the current study, the diet with the n-3/n-6 PUFA ratio of 0.66 enhanced the abundance of Gram-positive bacteria (Phylum Firmicutes) and reduced the relative abundance of Gram-negative bacteria (Phylum Proteobacteria). This phenomenon was seen as a positive modulation of the intestinal flora, which was associated with better growth performance and innate immunity (Liu et al., 2021).

In summary, results of the present study indicated that dietary n-3/n-6 PUFA ratio influences the growth performance of *L. maculatus* and that maximum growth was obtained at ratio of 0.66. Furthermore, an optimal n-3/n-6 PUFA ratio improved liver health and lipid metabolism. A sub-optimal n-3/n-6 PUFA ratio induced intestinal inflammation, which is speculated to be caused by the disturbance in intestinal flora. The diet with the n-3/n-6 PUFA ratio of 0.66 could inhibit intestinal inflammation through positively modulating the intestinal flora.

Author contributions

Yanzou Dong: conceptualization, data curation, writing-original draft preparation. **Yu Wei:** conceptualization, data curation. **Ling Wang:** methodology. **Kai Song:** methodology. **Chunxiao Zhang:** methodology, resources. **Kangle Lu:** conceptualization, supervision, writing-review & editing. **Samad Rahimnejad**: writing-review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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