Aquaculture 464 (2016) 497–504

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# Dietary hydroxyproline improves the growth and muscle quality of large yellow croaker *Larimichthys crocea*

Zehong Wei [a], Jun Ma [a,b], Xiaoyi Pan [a,b], Hua Mu [a,b], Jun Li [a,b], Jikang Shentu [c], Wenbing Zhang [a,b,*], Kangsen Mai [a,b]

[a] The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, Ocean University of China, Qingdao 266003, China
[b] The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China
[c] Ningbo Ocean and Fisheries Research Institute, Ningbo 315010, China

ARTICLE INFO

*Article history:*
Received 29 March 2016
Received in revised form 12 July 2016
Accepted 13 July 2016
Available online 15 July 2016

*Keywords:*
Large yellow croaker
Hydroxyproline
Growth
Muscle quality
Collagen

ABSTRACT

The present study was conducted to estimate the effect of dietary hydroxyproline (Hyp) on growth, muscle texture and collagen content of large yellow croaker. Six isonitrogenous and isolipidic practical diets were formulated to contain graded levels of Hyp (0.17%, 0.26%, 0.33%, 0.50%, 0.69% and 0.86% of dry matter). Fish with similar size (initial body weight, 189.87 ± 0.89 g) were distributed into 18 floating net-cages (1.5 m × 1.5 m × 2.0 m). Each diet was hand-fed to triplicate groups of large yellow croaker for 82 days. The results showed that the specific growth rate (SGR) of fish fed diet with 0.33% of Hyp was significantly higher than those fed with the basal diet (0.17% Hyp) ($P < 0.05$). No significant differences were found in survival rate, viscerosomatic index, hepatosomatic index, condition factor and feed intake among all treatments ($P > 0.05$). Protein efficiency ratio and feed efficiency significantly increased with increasing levels of dietary Hyp up to 0.33% ($P < 0.05$). Moisture and crude lipid contents in muscle showed no significant difference among all treatments ($P > 0.05$), while crude protein was significantly improved by dietary Hyp ($P < 0.05$). Significant decreases were observed in muscle liquid loss and water loss with increasing levels of dietary Hyp ($P < 0.05$), while little variation was detected in lipid loss ($P > 0.05$). A statistically significant difference was observed in muscle pH with dietary Hyp ($P < 0.05$). Except for the cohesiveness and adhesiveness, all other analyzed texture properties including hardness, springiness, chewiness in muscle were significantly affected by dietary Hyp. Alkaline-soluble Hyp reached to the highest value in muscle when fish were fed diet with 0.69% of Hyp. The highest value of the total Hyp content in muscle was found in the treatment with 0.86% of dietary Hyp ($P < 0.05$). No significant difference was detected in muscle alkaline-insoluble Hyp, salt-soluble protein and water-soluble protein ($P > 0.05$) among all treatments. Pyridinium crosslink (PYD) in muscle increased with increasing dietary Hyp content up to 0.69% ($P < 0.05$). Hardness, springiness and chewiness showed a high and positive correlation with alkaline-soluble Hyp, total Hyp and PYD, and negative correlation with liquid loss and water loss. It was concluded that dietary Hyp could benefit fish growth, promote the formation of collagen, and thereafter influence muscle quality of large yellow croaker. Using the broken-line models based on SGR and the total Hyp content in muscle, the optimal dietary Hyp content for large yellow croaker were estimated to be 0.33% and 0.61%, respectively.

*Statement of relevance:* This study is not a test of commercial aquaculture.



## 1. Introduction

Large yellow croaker (*Larimichthys crocea*) is a very popular and commercial farmed fish species, which has been cultured widely especially in south of China because of its delicious meat and high market value. It is now the first major mariculture fish species with 127,917 metric tons produced in 2014 in China (China Fishery Statistical Yearbook, 2015). Previous studies have been reported to estimate the nutrition value of large yellow croaker in farmed (Duan et al., 2001) and wild (Lin et al., 2006). Farmed large yellow croaker tends to have a softer texture, less robust flavor and colour than wild one (Yi et al., 2014b). Compared with farmed fish, consumers in China prefer wild caught fish due to their firmer texture and superior organoleptic qualities (Yi et al., 2014a). It is urgent to find an efficient way to improve the quality of the farmed large yellow croaker.

Flesh quality is a complex concept and has been defined as 'a combination of such characteristics as wholesomeness, integrity and freshness' (Martin, 1988). It is affected by endogenous factors such as genetic background (Larsson et al., 2012), colour, strains (Johnston,

* Corresponding author.
*E-mail address:* wzhang@ouc.edu.cn (W. Zhang).

http://dx.doi.org/10.1016/j.aquaculture.2016.07.015

1999), texture and fat content (Periago et al., 2005). Meanwhile, it is also influenced by exogenous factors such as environmental factors (Johnston, 2008), feed and feeding (Johansson et al., 2000). The main edible portion and quality value of fish flesh is the skeletal muscle tissue, which consists of muscle fiber and intramuscular connective tissue (IMCT). The IMCT is correlated with muscle firmness (Johnston et al., 2000). It principally consists of fibres of the proteins collagen and elastin, surrounded by a proteoglycan matrix (Purslow, 2005). The collagen is a major structural element (Shearer et al., 1994) contributing to the tissues stability and maintaining their integrity of structure (Liu et al., 1996). Collagen content, type and structure are important factors influencing the muscle texture of fish (Cheng et al., 2014; Periago et al. 2005).

Approximately 99.8% of the Hyp in body's stores are found in collagen (Barbul, 2008). Hyp is essential for the formation of triple helical molecules in vivo and is indispensable for intramolecular hydrogen bonds, which maintains the integrity of the collagen fibrils and contributes to the thermal stability of the triple helical structure (Gelse et al., 2003; Myllyharju and Kivirikko, 2004). It is also recognized as a substrate for the synthesis of glycine, pyruvate, glucose and glutamate (Wang et al., 2013; Wu et al., 2010) and now considered as a conditionally essential amino acid in aquatic animals (Li et al., 2009). Aksnes et al. (2008) reported that the growth and Hyp in vertebra of salmon (*Salmo salar* L.) were significantly improved by dietary Hyp addition. In contrast, Zhang et al. (2013) showed that Hyp did not affect growth but improved collagen content in muscle of juvenile turbot (*Scophthalmus maximus* L.).

In the biosynthesis of collagens, the pyridinium crosslink (PYD) is able to connect collagen molecules to make tissue stable (Li et al., 2005). Significant positive correlation was observed between PYD concentration and fillet firmness in Atlantic salmon (*Salmo salar* L.) (Johnston et al., 2006; Li, et al., 2005). Moreover, Johnsen et al. (2011) showed that PYD was the only factor that significantly influenced fillet firmness. Lysyl hydroxylase (LOX) is traditionally known to catalyze the oxidative deamination of the ε-amino group of lysines and hydroxylysines to promote crosslinking, which is the only enzymatic step involved in the formation of the collagen crosslinking (Gelse et al., 2003; Wang et al., 1996). Few studies have evaluated the effects of dietary Hyp on PYD content and LOX activity, and their contribution to muscle texture in fish. It therefore requires further investigation.

The present study selected Hyp as a nutrient added to diet and fed the large yellow croakers for 82 days to analyze muscle collagen content, related enzyme activities, and PYD content in the process of collagen biosynthesis. Muscle quality indexes were also detected. The main purpose of this study is to investigate whether dietary Hyp can influence the growth and muscle quality of large yellow croaker.

## 2. Materials and methods

### 2.1. Experimental diets

The ingredients and compositions of the experimental diets are presented in Table 1. L-Hyp (>99% pure) was obtained from Huayang Chemical Co., Ltd. (China). Six isonitrogenous and isolipidic diets were formulated to supplement graded levels of Hyp: 0% (the basal diet), 0.1%, 0.2%, 0.4%, 0.6% and 0.8%, respectively. The analyzed dietary Hyp contents were 0.17%, 0.26%, 0.33%, 0.50%, 0.69% and 0.86%, respectively. The basal diet was formulated to contain fish meal, soybean meal and wheat meal as the intact protein sources. It contained about 43% of crude protein and 12% of crude lipid. The diet was supplemented with lysine-$H_2SO_4$, DL-methionine, L-threonine, L-arginine, L-isoleucine, L-leucine, L-valine, and L-phenylalanine (crystalline amino acids) as premix to simulate the whole body amino acid pattern of large yellow croaker. The amino acid compositions of the experimental diets are shown in Table 2.

**Table 1**
Formulation and proximate composition of the experimental diets (% dry matter).

| | Dietary Hyp levels | | | | | |
|---|---|---|---|---|---|---|
| Ingredients | 0% | 0.1% | 0.2% | 0.4% | 0.6% | 0.8% |
| Fish meal[a] | 25 | 25 | 25 | 25 | 25 | 25 |
| Soybean meal[a] | 25 | 25 | 25 | 25 | 25 | 25 |
| Wheat meal[a] | 26 | 26 | 26 | 26 | 26 | 26 |
| Fish oil | 6 | 6 | 6 | 6 | 6 | 6 |
| Soybean lecithin | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Mineral premix[b] | 2 | 2 | 2 | 2 | 2 | 2 |
| Vitamin premix[c] | 2 | 2 | 2 | 2 | 2 | 2 |
| Choline Chloride | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Attractant[d] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Mold inhibitor[e] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Ethoxyquine | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Amino acid premix[f] | 5.70 | 5.70 | 5.70 | 5.70 | 5.70 | 5.70 |
| Microcrystalline cellulose | 3.15 | 3.15 | 3.15 | 3.15 | 3.15 | 3.15 |
| Alanine | 0.8 | 0.7 | 0.6 | 0.4 | 0.2 | 0 |
| Hydroxyproline | 0 | 0.1 | 0.2 | 0.4 | 0.6 | 0.8 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| *Proximate analysis* | | | | | | |
| Crude protein | 43.56 | 44.20 | 43.90 | 43.89 | 43.42 | 43.85 |
| Crude lipid | 12.82 | 13.43 | 12.71 | 13.35 | 12.34 | 12.95 |
| Moisture (% wet weight) | 5.32 | 5.09 | 5.04 | 6.41 | 5.36 | 5.04 |
| Hydroxyproline | 0.17 | 0.26 | 0.33 | 0.50 | 0.69 | 0.86 |

[a] All of these ingredients were supplied by Qingdao Great Seven Biotechnology Co., Ltd., China. Fish meal, crude protein: 74.31%, crude lipid: 8.98%; Soybean meal, crude protein: 57.40%, crude lipid, 1.70%; Wheat meal, crude protein: 17.39%, crude lipid: 1.47%.
[b] Mineral premix (mg/kg diet): $Na_2SeO_3$ (1%), 20, $Ca(IO_3)_2 \cdot 6H_2O$ (1%), 60; $CoCl_2 \cdot 6H_2O$ (1%), 50; $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $MnSO_4 \cdot H_2O$, 45; $MgSO_4 \cdot 7H_2O$, 1200; Ca $(H_2PO_4)_2 \cdot H_2O$, 10,000; microcrystalline cellulose, 8485.
[c] Vitamin premix (mg/kg diet): thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin $B_{12}$, 10; vitamin K3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin, $Ca(IO_3)_2$, 60; retinol acetate, 32; cholecalciferol, 5; α-tocopherol, 240; ascorbic acid, 2000; wheat middling, 16,473.
[d] Attractant: glycine: betaine = 1:2.
[e] Mold inhibitor: 50% calcium propionic acid and 50% fumaric acid.
[f] Lysine-$H_2SO_4$, DL-methionine, L-threonine, L-arginine, L-isoleucine, L-leucine, and L-valine, and L-phenylalanine.

### 2.2. Experimental procedure

The present study was carried out strictly according to the recommendations in the Guide for the Use of Experimental Animals of Ocean University of China.

The feeding trial was conducted at Xiangshan Harbor of Ningbo, Zhejiang Province, China. Large yellow croaker juveniles were obtained from a commercial hatchery. Prior to the feeding trial, the fish were

**Table 2**
Amino acid composition of the experimental diets (% dry matter).

| | Dietary Hyp levels (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0.17 | 0.26 | 0.33 | 0.50 | 0.69 | 0.86 |
| Aspartic acid | 3.57 | 3.37 | 3.51 | 3.41 | 3.39 | 3.59 |
| Threonine | 1.99 | 1.87 | 1.96 | 1.85 | 1.88 | 2.08 |
| Serine | 1.80 | 1.70 | 1.76 | 1.70 | 1.70 | 1.81 |
| Glutamic acid | 7.11 | 6.67 | 7.02 | 6.92 | 6.85 | 7.19 |
| Glycine | 2.27 | 2.13 | 2.21 | 2.22 | 2.23 | 2.19 |
| Alanine | 2.85 | 2.81 | 2.69 | 2.29 | 2.23 | 2.11 |
| Cysteine | 0.43 | 0.46 | 0.45 | 0.45 | 0.48 | 0.47 |
| Valine | 2.23 | 2.17 | 2.18 | 2.18 | 2.18 | 2.28 |
| Methionine | 1.23 | 1.27 | 1.25 | 1.32 | 1.36 | 1.21 |
| Isoleucine | 2.07 | 1.95 | 2.03 | 2.04 | 1.99 | 2.07 |
| Leucine | 3.87 | 3.70 | 3.81 | 3.80 | 3.78 | 3.90 |
| Tyrosine | 1.39 | 1.30 | 1.35 | 1.36 | 1.32 | 1.34 |
| Phenylalanine | 2.17 | 2.06 | 2.09 | 2.14 | 2.04 | 2.15 |
| Lysine | 3.68 | 3.52 | 3.66 | 3.62 | 3.60 | 3.73 |
| Histidine | 1.09 | 1.04 | 1.09 | 1.09 | 1.06 | 1.12 |
| Arginine | 3.80 | 3.63 | 3.71 | 3.71 | 3.73 | 3.84 |
| Proline | 1.90 | 1.93 | 1.97 | 1.94 | 1.98 | 1.96 |