Comparative Study on the Effects of Different Protein Sources on Flesh Quality of Grass Carp and Japanese Seabass (Postprint)

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Abstract

This experiment aimed to compare the effects of different protein sources on body composition, muscle free amino acid composition, and texture properties of raw and cooked fillets in grass carp (Ctenopharyngodon idellus) and Japanese sea bass (Lateolabrax japonicas). Three experimental diets were formulated for each species, respectively, which were isonitrogenous and isoenergetic. The three grass carp experimental diets were: CI-FM group with fish meal as the sole protein source (control group), CI-PPB80 group with mixed plant proteins (soybean meal and soy protein concentrate) replacing 80% of fish meal, and CI-PPB100 group with mixed plant proteins replacing 100% of fish meal. The three Japanese sea bass experimental diets were: LJ-FM group with fish meal as the sole protein source (control group), LJ-PPB50 group with mixed plant proteins (cottonseed protein concentrate and soy protein concentrate) replacing 50% of fish meal, and LJ-PPB100 group with mixed plant proteins replacing 100% of fish meal. The initial body weight of grass carp was (153.40$±0.30)g, randomlydividedinto3groupswith3replicatespergroupand20fishperreplicate; theinitialbody g, randomly divided into 3 groups with 4 replicates per group and 25 fish per replicate. After 8 weeks of feeding, body composition, muscle free amino acid content, fillet texture properties, shear force, drip loss, and collagen content of the two experimental fish were determined. The results showed: compared with the CI-FM group, the CI-PPB100 group of grass carp exhibited significantly decreased muscle crude lipid content ($P<0.05$) and significantly increased crude ash content ($P<0.05$); whereas the muscle crude lipid content of Japanese sea bass showed a trend of first increasing then decreasing with increasing replacement levels, with the LJ-PPB50 group being significantly higher than the LJ-PPB100 group ($P<0.05$). No significant differences were observed among grass carp groups in total muscle free amino acids and biogenic amine precursor contents ($P>0.05$); as the replacement level increased, Japanese sea bass showed significantly decreased total muscle free amino acids and flavor amino acid contents (P<0.05), and significantly increased biogenic amine precursor content (P<0.05). Specific components existed in the muscle of each of the two experimental fish species. The texture properties of raw and cooked fillets differed substantially between the two species. The hardness, adhesiveness, chewiness, springiness, and shear force of raw fillets in the CI-FM group of grass carp and the LJ-FM group of Japanese sea bass were significantly higher than those in the other two groups of the same species (P<0.05). For cooked fillets, the cohesiveness of the CI-PPB80 group of grass carp was significantly higher than the other two groups (P<0.05), while no significant differences were observed among groups for other indices (P>0.05); the hardness and adhesiveness of the LJ-PPB100 group of Japanese sea bass were significantly higher than the LJ-FM group (P<0.05), while no significant differences were observed among groups for other indices (P>0.05). Based on these results, mixed plant protein replacement of fish meal reduced fat accumulation in grass carp and Japanese sea bass, had no significant effect on total muscle free amino acid content in grass carp, but resulted in decreased flavor amino acid content and essential amino acid content while increasing biogenic amine precursor content and shortening shelf life in Japanese sea bass; high plant protein diets caused significant deterioration in meat quality of both grass carp and Japanese sea bass, whereas high fish meal diets could ensure higher meat quality in both experimental fish species.

Full Text

Comparative Study on the Effects of Different Protein Sources on Flesh Quality of Grass Carp (Ctenopharyngodon idellus) and Japanese Seabass (Lateolabrax japonicus)

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Abstract: This study aimed to compare the effects of different protein sources on body composition, muscle free amino acid composition, and texture characteristics of raw and cooked fillets in grass carp (Ctenopharyngodon idellus) and Japanese seabass (Lateolabrax japonicus). Three isoenergetic and isonitrogenous experimental diets were formulated for each species. For grass carp, the control diet (CI-FM group) used fish meal as the sole protein source, while the CI-PPB80 and CI-PPB100 groups used blended plant protein (soybean meal and soybean protein concentrate) to replace 80% and 100% of fish meal, respectively. For Japanese seabass, the control diet (LJ-FM group) used fish meal as the sole protein source, while the LJ-PPB50 and LJ-PPB100 groups used blended plant protein (cottonseed protein concentrate and soybean protein concentrate) to replace 50% and 100% of fish meal, respectively. Grass carp with an initial body weight of (153.40$\$\pm 0.30) gwererandomlydividedinto3groupswith3replicatesof20fisheach, whileJapaneseseabasswitha$ g were randomly divided into 3 groups with 4 replicates of 25 fish each. After an 8-week feeding trial, body composition, muscle free amino acid content, fillet texture characteristics, shear force, drip loss, and collagen content were determined. The results showed that compared with the CI-FM group, the CI-PPB100 group exhibited significantly lower muscle crude lipid content ($P<0.05$) and significantly higher crude ash content ($P<0.05$) in grass carp. In Japanese seabass, muscle crude lipid content showed a trend of first increasing then decreasing with substitution level, with the LJ-PPB50 group being significantly higher than the LJ-PPB100 group ($P<0.05$). No significant differences were observed in total free amino acids or biogenic amine precursors among grass carp groups ($P>0.05$). However, in Japanese seabass, total free amino acids and flavor amino acids decreased significantly ($P<0.05$), while biogenic amine precursors increased significantly ($P<0.05$) as substitution level increased. Both species contained specific components unique to each. The texture characteristics of raw and cooked fillets differed substantially between the two species. For raw fillets, the CI-FM group for grass carp and LJ-FM group for Japanese seabass showed significantly higher hardness, gumminess, chewiness, resilience, and shear force compared to the other two groups within each species ($P<0.05$). For cooked fillets, the CI-PPB80 group of grass carp showed significantly higher cohesiveness than the other two groups ($P<0.05$), with no significant differences in other indices ($P>0.05$). In Japanese seabass, the LJ-PPB100 group showed significantly higher hardness and adhesiveness than the LJ-FM group ($P<0.05$), with no significant differences in other indices ($P>0.05$). These results indicate that replacing fish meal with blended plant protein reduced lipid accumulation in both species, did not affect total free amino acid content in grass carp, but decreased flavor amino acids and essential amino acids while increasing biogenic amine precursors in Japanese seabass, thereby shortening shelf life. High plant protein diets significantly reduced flesh quality in both species, while high fish meal diets maintained superior flesh quality.

Keywords: grass carp; Japanese seabass; protein source; fish meal; blended plant protein; free amino acid; flesh quality

Introduction

With economic development and improving living standards, consumer demand for aquatic products has increased, and attention to fish flesh quality has grown accordingly. Consequently, the effects of different protein source diets on cultured fish flesh quality have become a focal point in alternative protein source research. Cabral et al. [?] reported that Senegalese sole (*Solea senegalensis*) could effectively utilize high plant protein diets with fish meal replacement levels up to 75% while maintaining high levels of n-3 PUFA and DHA in muscle. De Francesco et al. [?] found that replacing 75% of fish meal with blended plant protein (corn gluten meal and wheat flour) in gilthead sea bream (*Sparus aurata*) resulted in significantly higher total amino acid content in muscle after 11 days. Zhu [?] observed that feeding broad beans to channel catfish (*Ictalurus punctatus*) significantly improved muscle hardness and chewiness compared to formulated feed groups. Jiang et al. [?] reported that 100% fish meal replacement with blended plant protein (cottonseed meal, rapeseed meal, corn gluten meal, and broad beans) significantly reduced flavor amino acid content in tilapia muscle. However, some studies have shown no effect of plant protein sources on fish flesh quality. For example, Matos et al. [?] found that replacing 90% of fish meal with plant protein sources such as soybean meal, wheat gluten, and soybean protein concentrate did not significantly affect muscle composition, free amino acid content, or texture characteristics in gilthead sea bream. Most research on fish meal replacement has focused on carnivorous fish, seeking optimal substitution ratios to maximize benefits. However, few studies have investigated the mechanisms by which different protein sources, particularly fish meal versus plant proteins, affect flesh quality across different feeding guilds. Therefore, this study selected typical herbivorous fish grass carp (*Ctenopharyngodon idellus*) and typical carnivorous fish Japanese seabass (*Lateolabrax japonicus*) for comparative analysis of protein source effects on flesh quality.

Both grass carp and Japanese seabass are economically important species in China, though taxonomically distant. Grass carp belongs to Cyprinidae, Cypriniformes, and is one of China' s four major domestic fish and the most important freshwater aquaculture species. In 2012, grass carp accounted for nearly 20% of China' s 23.34 million tonnes of freshwater aquaculture production. Japanese seabass belongs to Serranidae, Perciformes, and is a eurythermal and euryhaline carnivorous fish valued for its delicious taste, high market value, and short culture cycle, representing the highest-yielding marine aquaculture species in China. This study investigated the effects of fish meal versus blended plant protein as sole protein sources on body composition, muscle free amino acid content, and raw and cooked fillet texture characteristics in both species through an 8-week feeding trial, providing theoretical basis for rational utilization of plant protein sources.

1.1 Experimental Diets

Three isoenergetic and isonitrogenous experimental diets were formulated for each species. For grass carp, the control diet used fish meal as the sole protein source (CI-FM group), while the CI-PPB80 and CI-PPB100 groups used blended plant protein (soybean meal and soybean protein concentrate) to replace 80% and 100% of fish meal, respectively. For Japanese seabass, the control diet used fish meal as the sole protein source (LJ-FM group), while the LJ-PPB50 and LJ-PPB100 groups used blended plant protein (cottonseed protein concentrate and soybean protein concentrate) to replace 50% and 100% of fish meal, respectively. Feed ingredients were mixed sequentially in order of increasing addition amount. Grass carp diets were processed into 3 mm extruded floating pellets, while Japanese seabass diets were processed into 2 mm extruded sinking pellets (TSE65 type, Beijing Xiandai Yanggong Machinery Technology Development Co., Ltd.), air-dried and stored at room temperature in a dry, ventilated place. The composition and nutrient levels of experimental diets are shown in Table 1 .

1.2 Experimental Fish and Rearing Management

Two-year-old grass carp were obtained from Beijing Tongzhou Xiaowu Fishery, while Japanese seabass juveniles were obtained from Shandong Weihai Yulong Aquatic Development Co., Ltd. and gradually acclimated from seawater to freshwater. Fish were domesticated for 4 weeks before the trial to adapt to experimental conditions. Healthy, uniform-sized grass carp [average body weight (153.40$±0.30)*g*]*andJapaneseseabass*[*averagebodyweight*(12.97±$0.03) g] were randomly allocated into 3 groups for an 8-week feeding trial. Fish were fed to apparent satiation twice daily at 09:00 and 21:00. Grass carp were stocked at 20 fish per replicate with 3 replicates per group, while Japanese seabass were stocked at 25 fish per replicate with 4 replicates per group.

The feeding trial was conducted in an indoor recirculating aquaculture system at the National Aquatic Feed Safety Assessment Center (located in Nankou Town, Changping District, Beijing). Fish were reared in 256 L tanks with aerated well water at a flow rate of approximately 0.4 L/min. Water temperature was measured daily, and water quality parameters were measured weekly. Dissolved oxygen concentration was >7 mg/L, pH was 7.5-8.5, ammonia nitrogen concentration was <0.3 mg/L, and nitrite concentration was <0.1 mg/L. Water temperature was maintained at 22-25°C for grass carp and 23-26°C for Japanese seabass.

1.3 Sample Collection

At the end of the feeding trial, fish were fasted for 24 h before sampling. Grass carp and Japanese seabass were sampled and analyzed using the same methods, following the procedures described by Zhang et al. [**?**].

1.4 Analytical Methods

1.4.1 Proximate Composition of Diets and Whole Fish For whole fish analysis, one grass carp and three Japanese seabass were randomly selected from each tank, homogenized (Retsch GM300), and dried at 60°C. Proximate composition of experimental diets and whole fish was determined using standard methods: moisture by 105°C oven drying (GB/T 6435-2006), crude protein by Kjeldahl method (GB/T 6432-94), crude lipid by Soxhlet extraction (GB/T 6433-2006), crude ash by combustion at 550°C (GB/T 6438-2007), and gross energy by bomb calorimetry.

1.4.2 Muscle Free Amino Acid Composition Two fish were randomly selected from each tank for both species. After skin removal, muscle from both sides was collected, freeze-dried, and pulverized. Muscle samples were treated with 8% sulfosalicylic acid, diluted with distilled water, and defatted with n-hexane. After centrifugation, the lower clear layer was collected, purified through a column, and analyzed for free amino acid content using an amino acid analyzer (Sykam S433D; column: LCAK07/Li; mobile phases: lithium citrate buffers at pH 2.9, 4.2, and 8.0 for phases A, B, and C, respectively).

1.4.3 Fillet Texture Characteristics, Shear Force, Drip Loss, and Collagen Content Determination of fillet texture characteristics, shear force, drip loss, and collagen content followed the methods described by Zhang et al. [?].

Statistical Analysis

Data for each species were expressed as mean±SE and analyzed by one-way ANOVA using SPSS 22.0 software. Duncan' s multiple range test was used for post-hoc comparisons. Independent samples t-test was used to compare means between the two species. Significance was set at $P<0.05$ for both tests.

Results

Final body weight and body composition of the two species are shown in Table 2. Grass carp showed significantly lower whole-body crude protein content ($P<0.05$) but significantly higher crude lipid, crude ash, gross energy, and final body weight ($P<0.05$) compared to Japanese seabass. Among grass carp, final body weight in the CI-FM group was significantly lower than in the other two groups ($P<0.05$). In Japanese seabass, final body weight in the LJ-PPB100 group was significantly lower than in the other two groups ($P<0.05$). For grass carp, whole-body crude lipid content in the CI-FM group was significantly higher than in the CI-PPB100 group ($P<0.05$), while crude ash content was significantly lower than in the CI-PPB100 group ($P<0.05$). No significant differences were observed in crude protein, moisture, or gross energy among grass carp groups (P>0.05). In Japanese seabass, whole-body crude lipid content in the LJ-PPB50 group was significantly higher than in the LJ-PPB100 group (P<0.05), with no significant differences in other body composition indices (P>0.05).

Muscle free amino acid composition of the two species is shown in Table 3. Grass carp and Japanese seabass showed substantial differences in flavor amino acids, biogenic amine precursors, and essential amino acids relative to total free amino acids. Flavor amino acids accounted for 31.13%-34.92% of total free amino acids in grass carp muscle, compared to 68.16%-78.58% in Japanese seabass. Biogenic amine precursors accounted for 47.77%-50.66% in grass carp versus 12.02%-20.17% in Japanese seabass. Essential amino acids accounted for 55.51%-56.48% in grass carp versus 8.52%-18.83% in Japanese seabass. Grass carp showed significantly higher contents of biogenic amine precursors, essential amino acids, and total free amino acids than Japanese seabass (P<0.05).

For flavor amino acids in muscle, the CI-FM group of grass carp was significantly higher than the CI-PPB100 group (P<0.05) but not significantly different from the CI-PPB80 group (P>0.05). Similarly, flavor amino acid content in Japanese seabass muscle decreased significantly with increasing substitution level (P<0.05). Regarding individual flavor-related amino acids, grass carp showed significantly lower glutamic acid in the CI-PPB80 group (P<0.05), significantly higher alanine in the CI-PPB100 group (P<0.05), significantly higher glycine in the CI-FM group (P<0.05), and no significant differences in taurine among groups (P>0.05). In Japanese seabass, glutamic acid and alanine were significantly lower in the LJ-FM group than in the LJ-PPB100 group (P<0.05), while glycine and aspartic acid were significantly higher in the LJ-FM and LJ-PPB50 groups than in the LJ-PPB100 group (P<0.05). Taurine content decreased significantly with increasing substitution level (P<0.05).

For biogenic amine precursors, no significant differences were observed among grass carp groups (P>0.05), while the LJ-PPB100 group of Japanese seabass was significantly higher than the other two groups (P<0.05). Regarding individual precursor amino acids, grass carp showed significantly lower histidine in the CI-FM group (P<0.05), significantly higher tyrosine and lysine in the CI-FM group compared to the CI-PPB100 group (P<0.05), and no significant differences in arginine or ornithine among groups (P>0.05). In Japanese seabass, histidine and lysine increased significantly with substitution level (P<0.05), while ornithine, tryptophan, and arginine decreased significantly (P<0.05).

For essential amino acids, no significant differences were observed among grass carp groups (P>0.05), while Japanese seabass showed a significant increase with substitution level (P<0.05). Regarding limiting essential amino acids, grass carp showed significantly higher methionine and lysine in the CI-FM group (P<0.05). In Japanese seabass, no significant differences were observed in methionine among groups (P<0.05), while lysine decreased significantly with substitution level (P<0.05).

Analysis revealed specific components unique to each species. Japanese seabass muscle contained free amino acids aspartic acid and tryptophan, the essential amino acid derivative γ-aminobutyric acid, and the nitrogenous compound urea. Grass carp muscle contained carnosine and tyrosine.

Fillet texture characteristics, shear force, collagen content, and drip loss are shown in Table 4. Raw fillet texture characteristics differed substantially between grass carp and Japanese seabass. Grass carp raw fillets showed significantly lower hardness, cohesiveness, gumminess, chewiness, resilience, and shear force than Japanese seabass raw fillets ($P<0.05$). Cooked fillets of grass carp showed significantly higher adhesiveness but significantly lower cohesiveness, gumminess, and chewiness than Japanese seabass ($P<0.05$).

For raw fillet texture characteristics, both species showed consistent results for hardness, gumminess, chewiness, and resilience, with full fish meal groups (CI-FM and LJ-FM) being significantly higher than the other two groups within each species ($P<0.05$). Adhesiveness showed opposite trends: the CI-PPB80 group of grass carp was significantly higher than the other two groups ($P<0.05$), while the LJ-PPB50 group of Japanese seabass was significantly lower than the LJ-FM group ($P<0.05$) but not significantly different from the LJ-PPB100 group ($P>0.05$). For cohesiveness, the CI-FM group of grass carp was significantly higher than the CI-PPB80 group ($P<0.05$) but not significantly different from the CI-PPB100 group ($P>0.05$), while no significant differences were observed among Japanese seabass groups ($P>0.05$). For springiness, the CI-PPB100 group of grass carp was significantly higher than the other two groups ($P<0.05$), while the LJ-PPB50 group of Japanese seabass was significantly higher than the other two groups ($P<0.05$).

For cooked fillet texture characteristics, the CI-PPB80 group of grass carp showed significantly higher cohesiveness than the other two groups ($P<0.05$), with no significant differences in hardness, adhesiveness, springiness, gumminess, chewiness, or resilience ($P>0.05$). In Japanese seabass, the LJ-PPB100 group showed significantly higher hardness than the other two groups ($P<0.05$), while the LJ-FM group showed significantly lower adhesiveness than the other two groups ($P<0.05$). No significant differences were observed in springiness, cohesiveness, gumminess, chewiness, or resilience among Japanese seabass groups ($P>0.05$).

Shear force of raw fillets differed substantially between the two species, with Japanese seabass showing much higher values than grass carp ($P<0.05$). Both species exhibited significantly higher shear force in full fish meal groups compared to the other groups within each species ($P<0.05$).

For raw fillet collagen content, no significant difference was observed between grass carp and Japanese seabass ($P>0.05$). The CI-FM group of grass carp was significantly higher than the CI-PPB100 group ($P<0.05$) but not significantly different from the CI-PPB80 group ($P>0.05$). A similar pattern was observed in Japanese seabass. For raw fillet drip loss, no significant difference was observed between species (P>0.05). The CI-FM group of grass carp was significantly higher than the other two groups (P<0.05), while the LJ-FM group of Japanese seabass was significantly higher than the LJ-PPB50 group (P<0.05) but not significantly different from the LJ-PPB100 group (P>0.05).

Discussion

3.1 Effects of Different Protein Sources on Body Composition of Grass Carp and Japanese Seabass

In grass carp, different protein sources significantly altered whole-body crude lipid and ash contents, while in Japanese seabass, only crude lipid content showed significant changes. De Francesco et al. [?] replaced 75% of fish meal with blended plant protein in gilthead sea bream and observed no significant changes in whole-body crude lipid or crude protein content. Liu [?] replaced 40%, 50%, and 60% of fish meal with blended plant protein (wheat flour, soybean meal, corn gluten meal, etc.) in turbot and found no significant effects on moisture, ash, crude protein, or crude lipid content. Gómez-Requeni et al. [?] reported that replacing fish meal with blended plant protein in gilthead sea bream significantly decreased whole-body crude lipid content and increased ash content, consistent with the changes observed in grass carp in this study. These findings suggest that body composition may be influenced by multiple factors including species, size, and diet composition. The substantial difference in final body weight between grass carp and Japanese seabass in this study indicates that lipid deposition efficiency varies across growth stages. Additionally, soybean oil was used as the lipid source in grass carp diets to balance energy, while Japanese seabass diets contained appropriate fish oil to meet requirements for n-3 highly unsaturated fatty acids (HUFA) [?] before balancing energy with soybean oil. When fish meal was replaced by blended plant protein, dietary fatty acid composition changed substantially, and different lipid sources significantly affected lipid deposition [?]. The LJ-PPB50 group showed no significant difference in final body weight or whole-body crude lipid content compared to the LJ-FM group, while the LJ-PPB100 group showed significantly reduced crude lipid content, likely related to its significantly reduced growth performance.

3.2 Effects of Different Protein Sources on Muscle Free Amino Acid Composition of Grass Carp and Japanese Seabass

Dietary protein digestion in animals is a dynamic process of peptide and amino acid release and absorption [?]. Most small peptides are hydrolyzed by intestinal epithelial cells into free amino acids, which are transported into circulation together with absorbed amino acids [?]. Amino acids absorbed from the intestine combine with those from tissue protein degradation to form an amino acid metabolic pool for protein synthesis and amino acid metabolism. Grass carp is a typical herbivorous fish, while Japanese seabass is a typical carnivorous fish. Due to differences in feeding habits, their digestive tracts have different capacities for digesting various protein sources [?]. Gómez-Requeni et al. [?] reported that replacing fish meal with blended plant protein in gilthead sea bream significantly increased muscle total free amino acid content. When non-fish-meal protein sources replaced 45% of dietary fish meal protein, muscle total amino acids and essential amino acids decreased significantly compared to the fish meal group, regardless of exogenous amino acid supplementation [?]. In this study, replacing fish meal with blended plant protein in Japanese seabass resulted in a trend of first increasing then decreasing muscle total free amino acid content with substitution level, differing from previous results [?]. Research on the effects of different protein sources on free amino acid content in herbivorous fish muscle remains limited.

Fillet flavor is primarily determined by flavor amino acids with umami taste in muscle free amino acids, mainly including alanine, glutamic acid, aspartic acid, and glycine [?]. Glycine and alanine are characteristic sweet-tasting amino acids, while glutamic acid and aspartic acid are characteristic umami-tasting amino acids. Glutamic acid is generally considered the most important contributor to flavor, followed by glycine [?]. Complete replacement of fish meal with soybean meal in extruded feed reduced glutamic acid and glycine contents in Jian carp muscle [?]. Zhang et al. [?] found that flavor amino acid content in Japanese seabass muscle showed a trend of first increasing then decreasing with increasing substitution level. In grass carp, flavor amino acid content first decreased then increased with plant protein substitution level, while in Japanese seabass it decreased consistently with substitution level. This result was not consistent with final body weight data, indicating that flavor amino acid content is not entirely affected by growth performance. Dietary flavor amino acid content was lower in full fish meal diets than in the other two diets for both species, yet muscle flavor amino acid content was highest in full fish meal groups, suggesting efficient transport of flavor-related amino acids from fish meal in both grass carp and Japanese seabass. This study found that glycine content in Japanese seabass muscle was much higher than in grass carp, substantially enhancing the sweet taste and improving palatability. Using fish meal as the sole protein source increased glutamic acid and glycine contents in grass carp muscle, thereby improving fillet flavor. For Japanese seabass, glycine made a substantial contribution to flesh flavor. Additionally, taurine is an important flavor amino acid. Taurine is abundant in marine animals but deficient in plant protein sources. Both mixed plant protein groups showed lower taurine content than full fish meal groups in both species, indicating that dietary taurine content affects muscle taurine levels. In conclusion, fish meal ensures good flavor quality in both carnivorous and herbivorous fish.

Biogenic amines are low-molecular-weight nitrogenous organic compounds formed from free amino acids after fish and shellfish death, including histamine, tyramine, cadaverine, putrescine, spermidine, spermine, and tryptamine. Their precursors are histidine, tyrosine, lysine, ornithine, arginine, and tryptophan, respectively [?]. Excessive biogenic amines pose health risks [?] and cause food safety issues, with histamine being the most hazardous [?]. Studies have shown that complete fish meal replacement with soybean meal reduced free histidine content in Jian carp muscle [?]. In this study, histidine content in grass carp muscle was much higher than in Japanese seabass, indicating higher risk in long-term stored grass carp. Histidine content in both species increased significantly with substitution level, suggesting that high plant protein diets shorten the shelf life of both grass carp and Japanese seabass, consistent with results reported by Zhang et al. [?].

Methionine and lysine are the most limiting amino acids in non-fish-meal protein sources. Studies have shown that in carnivorous fish such as European eel (*Anguilla anguilla*) [?], masu salmon (*Oncorhynchus masou*) [?], and rainbow trout (*Oncorhynchus mykiss*) [?], increased plant protein intake significantly reduced methionine and lysine contents in muscle free amino acids. In this study, both mixed plant protein groups in grass carp showed significantly reduced methionine and lysine contents. This may be attributed to: (1) lower biological value of plant protein sources, with severe methionine deficiency in soybean meal and lysine as the second limiting amino acid; even with crystalline amino acid supplementation after fish meal replacement, effective transport into muscle is limited; and (2) inherent amino acid imbalance in blended plant protein sources. Research indicates that the central nervous system responds to amino acid imbalance through amino acid response (AAR), promoting catabolism. In contrast, lysine content in Japanese seabass muscle showed the opposite trend, with the full plant protein group showing the highest lysine content, suggesting species-specific differences in amino acid transport mechanisms requiring further investigation.

Specific amino acids were detected in both species. Japanese seabass muscle contained aspartic acid, γ-aminobutyric acid, and tryptophan, while grass carp muscle contained carnosine and tyrosine. Additionally, urea was detected in Japanese seabass muscle. Analysis revealed that marine fish regulate osmotic pressure by retaining urea and small amounts of other nitrogenous compounds, while freshwater fish primarily excrete nitrogenous waste as ammonia [?], explaining the presence of urea in Japanese seabass. Grass carp and Japanese seabass represent typical freshwater herbivorous and marine carnivorous fish, respectively. Differences in species, feeding habits, and native habitats result in specific differences in muscle free amino acid and nitrogenous compound composition, though no related reports have been found and further research is needed.

3.3 Effects of Different Protein Sources on Fillet Texture Characteristics, Shear Force, Collagen Content, and Drip Loss of Grass Carp and Japanese Seabass

Texture profile analysis simulates two chewing cycles, recording force-time relationships to identify parameters corresponding to sensory evaluation, including hardness, chewiness, cohesiveness, springiness, adhesiveness, gumminess, and resilience [?]. Compared to raw fillets, cooked fillets of both species showed significantly reduced hardness due to denaturation of myofibrillar and sarcoplasmic proteins upon heating, disruption of hydrogen and hydrophobic bonds maintaining muscle structure, destruction of protein secondary structure, and loosening of muscle fibers. Raw fillet texture characteristics differed substantially between grass carp and Japanese seabass, with grass carp showing significantly lower hardness, gumminess, and chewiness than Japanese seabass, while differences in cooked fillets were smaller. Since higher hardness and chewiness indicate better flesh quality [?], Japanese seabass raw fillets had superior palatability. This study demonstrated that fish meal as a protein source significantly improved hardness and chewiness in both species.

Cohesiveness and adhesiveness reflect opposite physical properties, with increased cohesiveness corresponding to decreased adhesiveness [?]. In grass carp fed full fish meal diets, muscle cohesiveness and resilience increased significantly, while springiness and adhesiveness decreased significantly, indicating enhanced intercellular binding capacity, improved integrity maintenance, and better texture during chewing. In Japanese seabass fed full plant protein diets, resilience, springiness, and adhesiveness decreased significantly, demonstrating that fish meal effectively improves flesh quality in both herbivorous and carnivorous fish.

Collagen is an important muscle tissue component that maintains muscle structure, flexibility, strength, and texture [?]. Studies have shown that collagen content is negatively correlated with meat tenderness [?]. Hydroxyproline content is relatively stable at 13%-14% of collagen, so hydroxyproline is commonly used to reflect collagen content [?]. Zhang et al. [?] found that collagen content in turbot muscle increased with dietary hydroxyproline content. Albrektsen et al. [?] reported that dietary hydroxyproline supplementation significantly increased Atlantic salmon muscle firmness by 5%-10%. Similarly, Periago et al. [?] found that European sea bass muscle collagen content was positively correlated with hardness, chewiness, and springiness. In this study, full fish meal groups showed significantly higher collagen content in raw fillets than the other two groups in both species, accompanied by significantly higher hardness, chewiness, and resilience. Fish have limited capacity to synthesize hydroxyproline, and fish meal contains hydroxyproline that is absent in plant protein sources. Using blended plant protein as the protein source significantly reduced muscle hydroxyproline content, thereby decreasing flesh hardness in both grass carp and Japanese seabass.

Shear force is the most common instrumental method for objectively measuring meat hardness [?], simulating tooth cutting of muscle fibers through single-cut analysis [?]. This study showed that replacing fish meal with blended plant protein significantly reduced shear force of raw fillets in both species, indicating decreased flesh hardness and inferior texture.

Drip loss indicates water-holding capacity and reflects the degree of water retention in muscle. High water-holding capacity maintains muscle moisture content, reduces excessive water loss, and prevents loss of freshness and inherent flavor [?]. Drip loss is related to shelf life, with increased drip loss indicating shortened shelf life and greater economic loss [?]. In this study, the highest drip loss in raw fillets occurred in full fish meal groups for both species, suggesting that fish meal as a protein source may shorten shelf life and increase economic loss risk.

Conclusions

1. Grass carp and Japanese seabass showed substantial differences in body composition, muscle free amino acid composition, and flesh quality indices. Both species contained specific components: grass carp contained carnosine and tyrosine, while Japanese seabass contained aspartic acid, γ-aminobutyric acid, tryptophan, and urea.
2. Using blended plant protein as the protein source significantly affected crude lipid content in both species, reducing lipid accumulation.
3. Evaluation based on muscle free amino acid content showed that plant protein replacement did not significantly affect total free amino acid content in grass carp but reduced flavor amino acids and essential amino acids while increasing biogenic amine precursors in Japanese seabass, thereby shortening shelf life.
4. Evaluation based on flesh quality showed that plant protein replacement significantly reduced flesh quality in both species. High fish meal diets before market could improve flesh quality and increase product value.

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