Skin characteristic of Pangasius Catfish in Indonesia

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Abstract. Pollution of the freshwater ecosystem can cause adverse effects on aquatic organisms, especially fish. Fish skin is a multi-purpose tissue that serves as the first barrier to protect the fish from aquatic pollution and defense system against pathogens. Pangasiidae is an economically important riverine catfishes that generally exist in freshwater from the Indian subcontinent to the Indonesian Archipelago. Among genera in Pangasiidae, the genus Pangasius has numerous species. Indonesia has 14 strains of this fish and 3 of them, Patin siam (Pangasianodon hypophthalmus), Patin Jambal (Pangasius djambal), and Patin Pasupati (Pangasius sp.), are commonly cultured. This study aimed to characterize the skin of three Pangasius strains commonly found in Indonesia. The study was conducted at the Aquaculture facilities of the Research Center of Limnology. Three strains of Pangasius catfish were obtained from aquaculture farms. The fish were measured for the total length and body weight. The proportion of head, skin, bone, and meat was calculated. Collagen was extracted from the skin and was analyzed for type I, by ELISA methods. The thickness of the skin collagen was measured under a microscope after stained by the Masson Trichrome technique. ANOVA was conducted to evaluate the skin collagen content and skin collagen thickness. The result showed that there was no significant difference in collagen content among the fish strain, however, the collagen thickness was different.

1. Introduction

Pollution of the freshwater ecosystem can occur in several ways such as natural occurrences such as algal blooms, domestic wastes agricultural practices for pesticides and fertilizer applications, industrial wastes, mining activities, and accidents such as oil spills. That pollution can cause adverse effects on aquatic organisms, especially fish. The presence of pollutants in the aquatic ecosystem besides causes acute effect can also cause stress in fish which leads to greater susceptibility to attack by pathogens [1].

Fish skin is a multi-purpose tissue that serves as the first barrier to protect the fish from aquatic pollution and defense system against pathogens. Its vital functions including chemical and physical protection, sensory activity, behavioral purposes, or hormone metabolisms [2]. Several skin diseases have been reported to be associated with water pollution, include epidermal papilloma, parasitic disease, and skin ulceration. These diseases are associated with various contaminants, such as heavy metals, hydrocarbons, nitrogenous compounds, sewage, and unspecified pollutants that enter the aquatic ecosystem [1].
Catfishes form a significant part of inland fisheries, where several species have been introduced in fish culture. Numerous species are of interest to the aquarium industry where they represent a substantial portion of the world trade [3]. Pangasiidae is an economically important riverine catfishes that generally exist in freshwater from the Indian subcontinent to the Indonesian Archipelago. Among genera in Pangasiidae, the genus Pangasius, which their local name in Indonesia was Patin, has numerous species. There are 22 strains of Pangasius catfish in the world while Indonesia has 14 of them [3].

Patin Siam (Pangasianodon hypophthalmus), Patin Jambal (Pangasius djambal), and Patin Pasupati (Pangasius sp.) are three Pangasius strains among them which are commonly cultured by farmers in Indonesia. Pangasius is a freshwater aquaculture commodity that has a very large market share both domestic and abroad [4].

High water temperature condition in tropical aquaculture is common. It varies between 25° C and 30° C and sometimes can reach 34° C in the surface layers during sunny days. This condition is usually coupled with low dissolved oxygen levels (hypoxia), because of lowering of oxygen solubility as temperature and the oxygen demand of both fishes and microorganisms rise [5].

The family of Pangasiidae is air breather fish, either facultative, which normally breath aquatically and only take aerial oxygen during hypoxia, or obligate, which means the fish depend on and require access to aerial oxygen and, even if they are in well-oxygenated water, they will have a reduced aerobic scope or even suffocated if prevented from air-breathing. They use their swim bladder as an air-breathing organ (ABO) [6]. With their ability to access the abundant oxygen in the air, it has been considered that air-breathing fishes can grow faster than non-air-breathing species [5].

Pangasius fish is a scaleless type of fish. Their skin is uncovered by scale therefore it will directly contact the external environment. The collagen content of Pangasius fish is 2.75 mg/Kg fish skin or equal to 85.3 mg/Kg fish [7]. Collagen is a fibrous protein that determines the strength and flexibility of tissues and bones [8]. Fish collagen is made up of mostly type I collagen. Because fish collagen is indeed a Type I collagen, it is rich in two particular amino acids: glycine and proline. Glycine is foundational to DNA and RNA strand creation, while proline is foundational to the body’s ability to naturally produce its collagen. Considering glycine is vital to DNA and RNA, it holds many significant functions for the body, including blocking endotoxin and transporting nutrients for body cells to utilize for energy. While proline can act as an antioxidant for the body and can prevent cell damage from free radicals [9].

This study aimed to characterize the skin of the three Pangasius strains, Patin Siam (Pangasianodon hypophthalmus), Patin Jambal (Pangasius djambal), and Patin Pasupati (Pangasius sp.).

2. Materials and Methods
This study was conducted October – December 2018 at Aquaculture facilities of the Research Center of Limnology. Three Pangasius strains, Patin Siam (Pangasianodon hypophthalmus), Patin Jambal (Pangasius djambal), and Patin Pasupati (Pangasius sp.) were used in this study because those Pangasius strains were commonly found in Indonesia, especially Java (Figure 1). All the fish used in this study were obtained from aquaculture farms in Bogor and Karawang. The body weight and length of fish samples were ranged between 637 – 1170 g and 43 – 48 cm, respectively. Three fish of each strain were sampled for analysis.
2.1. Body Proportion
Body proportion was measured by calculating head, skin, meat, tail, and viscera weight, compared to the fish total body weight.

2.2. Amino acids Composition
The amino acid composition of fish skin was analyzed by liquid chromatography methods [5] using HPLC with Thermo Scientific ODS-2 Hyersil column and Fluorescence detector. The total protein content of fish skin was determined using the Kjeldahl method.

2.3. Collagen Layer Thickness
The fish skin was used to analyze collagen layer thickness. A histological method with Masson Trichrome staining was applied. Collagen thickness was measured using a micrometer.

2.4. Type I Collagen Concentration
Type I collagen concentration was conducted through the Enzyme-linked immunosorbent assay (ELISA) method [10, 11]. The skin and flesh tissue of the fish were homogenized in a Phosphate buffer Saline (PBS) solution and followed by centrifugation of the samples at 1500 x g for 15 minutes. Supernatants were collected for ELISA analysis. ELISA was performed using the Fish Collagen Type I (COL1) ELISA kit (MyBioSource) and was read by ELISA reader (EPOCH 2) at wavelength 570 nm (540-580 nm).

2.5. Statistical Analysis
One-way ANOVA was performed to analyze the difference in the measured parameters among fish strains.

3. Results and Discussion
The skin proportion in Pangasius Patin fish was ranged between 3.8% up to 5.3%. The highest amount of skin proportion was found in Patin Pasupati followed by Patin Jambal and Patin Siam, respectively. The proportion of meat, head, and tail in Patin Pasupati was the highest among the three fish. The
highest viscera proportion was found in Patin Siam, while the highest proportion of bone and others was found in Patin Jambal (Figure 2).

Figure 2. Body Proportion of Pangasius Catfish.

Pangasius is a scaleless fish. Compared to eel, all of the Pangasius species used in this study had a much lower skin proportion [12]. In their study, Nafsiyah et al found that skin proportion in eel Anguilla bicolor was 21% while in Anguilla marmorata was 22%. On the other hand, the skin proportions of Yellowfin Tuna (Thunnus albacares) were almost the same as Patin, which was equal to 3.25% [13]. In our study, there was an indication that fish species can affect skin proportion. This phenomenon was also found in previous research [11]. Fish skin or integument consists of two layers namely an outer epidermis and an inner dermis or corium which differ in origin, structure, and function [14]. The outer layer, the epidermis, is comprised of a multilayered epithelium that usually includes specialized cells. The epidermis of the skin is equipped with different types of cells which are involved in the secretion of surface mucus-mucous cells, which are known to contain various biologically active macromolecules, predominantly glycoproteins. Glycoproteins are implicated in important biological functions at immune reactions. While the dermis, consisting of a layer of dense vascular connective tissue, is composed of a complicated network of cells (fibroblasts, monocytes, lymphocytes, mastocytes), fibers (i.e., collagen and elastin), and macromolecules synthesized by the cells (proteoglycans, glycosaminoglycans). Fish dermis connects directly onto myosepta protein of the muscle and the caudal fins.

Protein content in the skin of Patin Siam was the highest (24.71%) compared to Patin Jambal (23.30%) and Patin Pasupati (21.03%). In Patin Pasupati, glycine was the highest amino acid residue found in the skin. Even though glycine content was high in Patin Siam and Patin Jambal, it was not clear whether glycine also the highest amino acid residue in those fish. Its because the analysis methods used in this study could not detect the other residue (others: tryptophan, cysteine, glutamine, proline, asparagine, and selenocysteine) while the sum of those amino acids was found to be the highest in Patin Siam and Jambal (Figure 3).
Figure 3. Amino acids composition in the skin of Pangasius Catfish.

Figure 4 presented the photograph of the skin collagen layer colored by the Masson Trichrome dying method in Patin Jambal, Pasupati, and Siam. The Blue layer indicated by the arrow was the collagen in dermal skin. The average thickness of the collagen layer was ranged between 0.48 – 0.68 mm. Collagen in Patin Pasupati was the thinnest among the three strains. Patin Jambal had the same thickness of collagen dermal as Patin Siam (Figure 5). Collagen in fish skin occurs both as fibrous protein and protein sheet [14]. Collagen is a fibrous protein that determines the strength and flexibility of tissues and bones including scales. While collagen sheets are called myosepta, it separate layers of the muscle fibers along the fish body.

Figure 4. Collagen layer (blue color, indicated by arrow) in the skin of three Pangasius Catfish strains (40x magnification).
ANOVA study in the thickness of the collagen layer in the fish dermal found that there was a significant difference with the P-value $5 \times 10^{-7}$ ($P < 0.05$) (Table 1). Post hoc analysis revealed that the dermal collagen layer in Patin Jambal was not different significantly from Patin Siam. In contrast, Patin Pasupati was significantly different from both Patin Jambal and Patin Siam ($P < 0.05$).

**Figure 5.** The Thickness of Skin Collagen Layer in Pangasius Catfish.

**Table 1.** Analysis of Variance of the thickness skin collagen layer in Pangasius Catfish.

| Groups   | Count | Sum   | Average | Variance |
|----------|-------|-------|---------|----------|
| Jambal   | 23    | 15.7  | 0.68    | 0.01     |
| Siam     | 11    | 7.5   | 0.68    | 0.00     |
| Pasupati | 19    | 9.1   | 0.48    | 0.02     |

| Source of Variation | SS  | df | MS  | F   | P-value | F crit |
|---------------------|-----|----|-----|-----|---------|--------|
| Between Groups      | 0.50| 2  | 0.25| 19.67| 5E-07   | 3.18   |
| Within Groups       | 0.64| 50 | 0.01|      |         |        |
| Total               | 1.15| 52 |     |      |         |        |
Type I collagen in the Patin fish skin was analyzed using the ELISA method. The results showed that Patin Siam had the highest content of type I collagen in the skin as much as 29.69 ng/g, followed by Patin Jambal (29.06 ng/g) and Patin Pasupati (28.12 ng/g) respectively (Figure 6). Even though there were differences in type I collagen content, ANOVA results showed that these differences were not significant indicating a P-value of more than 0.05 (P=0.52) (Table 2).

![Figure 6. Type 1 collagen content in the skin of Pangasius Catfish.](image)

| Groups    | Count | Sum   | Average | Variance |
|-----------|-------|-------|---------|----------|
| Jambal    | 3     | 87.17 | 29.06   | 2.18     |
| Pasupati  | 3     | 84.35 | 28.12   | 1.07     |
| Siam      | 3     | 89.06 | 29.69   | 4.56     |

Source of Variation | SS  | df | MS  | F    | P-value | F crit |
---------------------|-----|----|-----|------|---------|--------|
Between Groups       | 3.74| 2  | 1.87| 0.72 | 0.52    | 5.14   |
Within Groups        | 15.62| 6  | 2.60|      |         |        |
Total                | 19.36| 8  |     |      |         |        |

Total protein and collagen content in Patin Pasupati was the lowest among those three species of Patin. This finding indicated that Patin Pasupati might have the lowest survival rate compared to Patin Siam and Patin Jambal. The thickness of the epidermis and the mucus secretion influence the susceptibility of the skin to damage [15]. Fish species is one of the factors that affect those factors. Atlantic menhaden fish which has an extremely thin epidermis was reported to have a highly susceptible nature to skin ulceration. In Patin fish, our previous study also found that Patin Pasupati had a low survival rate compared to Patin Siam [16]. Abedi et al. based on their study in heavy metals toxicity to common Carp and Sutchi catfish hypothesized that thicker epidermis could effectively
shield the fish against extra permeation of toxicants [17]. Their conclusions were following the widely accepted studies that skin thickness was the determinant in the percutaneous (via skin) uptake rate of toxicants in fish.

Although Patin Pasupati has the greatest risk among the three pangasius species for its skin vulnerability, the fish still has the economic consideration to be cultured. It requires careful handling for its successful cultivation and our other study found that biofloc technology could overcome this problem [18].

4. Conclusion
The skin proportion in Pangasius Patin fish was ranged between 3.8% up to 5.3%. The highest amount of skin proportion was found in Patin Pasupati followed by Patin Jambal and Patin Siam, respectively. Protein content in the skin of Patin Siam was the highest (24.71%) compared to Patin Jambal (23.30%) and Patin Pasupati (21.03%).

The average thickness of the collagen layer was ranged between 0.48 – 0.68 mm. Collagen in Patin Pasupati was the thinnest among the three species. Patin Jambal had the same thickness of collagen dermal as Patin Siam. The dermal collagen layer in Patin Jambal was not different significantly with Patin Siam, while Patin Pasupati was significantly different from both Patin Jambal and Patin Siam. Patin Siam had the highest content of type I collagen in the skin as much as 29.69 ng/g, followed by Patin Jambal (29.06 ng/g) and Patin Pasupati (28.12 ng/g) respectively.

Acknowledgment
This study was supported by Mininstry of Research Technology and Higher Education as part of Program Insinas Riset Pratama Kemitraan 2018 entititled “Kajian Kandungan Nutrisi Kolagen Pada Ikan Patin (Pangasius Sp.) sebagai Dasar Pengembangan Pangan Fungsional untuk Lansia”. We would also thank Agus Waluyo, Heri Suheri and Eva Nafisyah for their technical assistance during our laboratory works. Amino acids analyses were performed at Integrated Laboratory Facilities, Bogor Agricultural University.

We declare that both authors have equal contributions to this study.

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