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Proteins Characterization in The Skin Mucus of Mutiara Catfish (*Clarias Gariepinus*) by Using SDS Page Electrophoresis Method

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Abstract: Fish have a body defense system to protect themselves from various threats originating from the aquatic environment. One of the outermost defense mechanisms in the fish body is skin mucus. Mucus on fish skin is composed of water, glycoproteins, enzymes, antibodies, and other organic compounds. Through proteomic screening analysis, we can investigate the types and abundance of major proteins in fish mucosal exosomes. The aim of this study was to analyze the molecular weight of proteins in the surface mucus of mutiara catfish, and to identify the proteins detected in the mucus of mutiara catfish. The research method uses a descriptive method. The characterization process using SDS-PAGE. Analysis of protein molecular weight is obtained by measuring the displacement distance (Rf) in the sample. The molecular weight of proteins detected in mutiara catfish mucus had a molecular weight of 7.10 kDa, 13.51 kDa, 15.46 kDa, 28.78 kDa, 30.72 kDa, 42.40 kDa and 69.17 kDa. Proteins found abundantly in the mucus on the surface of catfish skin have a molecular weight ranging from 7-15 kDa, where this protein has various roles including immune defense, regulation of metabolism, oxidative stress, and the nervous system, stress regulation, regulation of cellular function, muscle contraction, and nervous system function. It can be concluded that the proteins found in the mucus on the surface of catfish skin are mostly small-sized proteins and have a major role in body defense, immune protection, and adaptation to environmental stress. These proteins are very important in maintaining fish health.
Keywords: protein characterization; SDS page; mutiara catfish; protein molecular weight

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INTORDUCTION

The aquaculture industry with high fish density has the potential to support the creation of pathogenic organisms in the aquatic environment. It can cause fish to be susceptible to diseases caused by bacteria and poor water conditions (Dash et al., 2018). However, fish have a body defense system to protect themselves from various threats originating from the aquatic environment. Mucus is a thick, porous and very sticky mass that forms a lubricating layer for the underlying mucosal tissue (Faeste et al., 2020). Mucus on fish skin is produced by mucus cells which are composed of water, glycoproteins, enzymes, antibodies, and other organic compounds. Mucus also contains various active components related to innate immunity such as proteases, lectins, immunoglobulins, antimicrobial peptides, complement, C-reactive protein, and lysozyme (Faeste et al., 2020). On the surface of the skin, mucus acts as the first barrier to various stressors received by fish (Montero et al., 2021). In addition, mucus has a role in carrying out the functions of protection, respiration, reproduction, excretion, communication, food processing, and osmotic regulation (Dash, 2018).

Biochemically, mucus on the surface of fish skin contains various defense proteins to fight infection and repair damaged fish body tissue. Physical or surface factors include scales, mucus layers, and epithelial cells lining the gills, skin, and digestive tract play an important role in fighting infection. The epidermal epithelium

prevents the entry of foreign objects, and it is also inhabited by macrophages, eosinophilic granular cells, and lymphocytes. Fish can interact with their environment through the mucosal barrier while maintaining homeostasis (Mokhtar et al., 2023). The composition of mucus in fish varies between different species and is influenced by various external and internal factors (Mohd et al., 2020).

Mutiara catfish (*Clarias gariepinus*) is one of the new strains of fish comes from a cross between four strains of African catfish found in Indonesia, namely Egyptian catfish, Paiton catfish, Sangkuriang catfish, and Dumbo catfish (Sugianti et al., 2022). Catfish have skin that is not scaly, slimy, and slippery (Prasetyo, 2021) due to the presence of a mucus layer from mucus cells (Ismail et al., 2020). The epidermal layer of catfish consists of epithelial cells, mucus cells, club cells, pigment cells (Andriani et al., 2017), and has layered epithelial cells with many malpigian cells (Zoghby et al., 2016). Many studies have been conducted on the protein profile of mucus, but there is still very little information on the protein profile of mucus in Mutiara catfish.

Proteome refers to all proteins expressed by a single genome, or a particular cell, tissue or subcellular structure, and its composition varies according to various conditions (Liu et al., 2020). Through proteomic screening analysis, we can investigate the types and abundance of major proteins in fish mucosal exosomes (Zhao et al., 2021). The proteomic technology that is currently widely used in analyzing protein profiles is electrophoresis. Electrophoresis is a technique used in the process of separating protein molecules by relying on the basic properties of proteins that have ionic charges. (Nurfaidah et al., 2020). Proteins can be positively or negatively charged depending on the pH and ionic conditions around them, so that when given an electric charge, the protein will move towards the opposite pole. (Nurfaidah et al., 2020). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is one of the electrophoresis techniques that is widely used to characterize proteins based on their molecular weight. The use of this technology can help provide information and map the protein profile in fish skin mucus as a body defense system.

The aim of this study was to analyze the molecular weight of proteins in the surface mucus of mutiara catfish, and to identify the proteins detected in the mucus of mutiara catfish. The resulting mucus protein profile can provide important information regarding the role of mucus proteins which and it also can be used to support fish conservation and habitat management efforts.

METHOD

The research method uses a descriptive method, namely describing or depicting the observed object based on the data results obtained by the researcher. This research was conducted at the Basah Laboratory, Faculty of Fisheries and Marine Sciences, Airlangga University, and the Proteomics Laboratory, Institute of Tropical Disease, Airlangga University from June to August 2024.

The sample used in this study were mutiara catfish (*C. gariepinus*) aged 70-80 days with a body weight of 115 ± 25 g. The tools used in this study were aquariums measuring 30 × 30 × 30 cm, aerators, hoses, fishing nets, 20 L plastic buckets, spatulas, microtubes, labels, SDS PAGE, cameras, notebooks, and markers. The materials used for protein analysis using the SDS-PAGE method were ammonium persulphate, Acrylamide, butanol, bis Acrylamide, tris HCl, E buffer, Sodium Dodecyl Sulphate, methanol, Comassie Brilliant Blue, acetic acid, formaldehyde, ddH_2O, and Tetra Methyl Diamine.

Skin mucus samples were collected using the method described by Guardiola et al. (2014) with slight modifications. The fish were placed on a sterile tray, then the

mucus was collected by scarping, which is by scraping the lateral surface of the fish's skin. When scraping, avoid the area around the fish's anus to avoid unwanted contamination. The mucus was centrifuged at 12,000 g at 4°C for 10 minutes and immediately stored at -80°C until further analysis. The characterization process using SDS-PAGE consists of making SDS-PAGE gel, Running Gel SDS-PAGE, Staining and Destaining Gel. 20µL of protein sample is premixed with 5µL of loading buffer in a microcentrifuge tube. The sample is heated for 5 minutes at 95ºC and the sample is spun down for 1 minute at 13,000 rpm. ~ 6µL of protein marker and ~ 10µL of protein sample are loaded into each well. The chamber is closed with a lid then the electrode points are connected to the power cable (Voltage) then electrophoresis is run in 2 steps: Step 1 - 30 minutes at 60V, Step 2 - 1 hour 30 minutes at 140V. The gel staining and destaining process was carried out using Staining Solution (500 mL Aquades; 100 mL Acetic Acid; 400 mL Ethanol; 1.5 grams Coomassie Brilliant Blue).

Analysis of protein molecular weight is obtained by measuring the displacement distance (Rf) in the sample. The Rf value is determined based on the following formula:

$$Rf = \frac{\text{Protein migration distance}}{tracking\ dye\ distance}$$

Then the Rf value is entered into the linear regression equation with the formula:

$$Y = ax^3 + bx^2 + cx + d \quad (\textit{cubic})$$

Y is molecule weight; x is RF Value; a,b,c,d is constanta value. The Y value in the formula is still in log form, so the Y value is first changed to antilog to get the molecular weight of the protein.

RESULT AND DISCUSSION

Description of Mutiara Catfish

Mutiara catfish (*Clarias gariepinus*) is a type of fish strain obtained from breeding in Indonesia which was formed through a selection program (selective breeding program) (Iswanto et al., 2015). Initially, the mutiara catfish was the result of development carried out by BPPI (Fish Breeding Research Agency) Sukamandi in West Java and was officially declared to have passed the Type/Variety Release Assessment on October 27, 2014 (Sugianti et al., 2022).

Figure 1. Mutiara catfish (*Clarias gariepinus*)

The classification of mutiara catfish is as follows: Kingdom Animalia, Phylum Chordata, Class Actinopterugii, Order Siluriformes, Family Clariidae, Genus *Clarias*, Species *Clarias gariepinus* (Burchell, 1822). Comparison of morphometric and meristic characters of Mutiara catfish with its parent fish showed no significant differences. In addition, Mutiara catfish has genetic diversity, including a higher number of alleles and

heterozygosity compared to Egyptian, Paiton, Sangkuriang, and Dumbo catfish (Iswanto et al.,2015). A systematic picture of catfish can be seen in Figure 1.

Mutiara catfish have specific characteristics including a slightly flat body shape, an elongated head shape, a pair of small eyes, a pair of nostrils, a mouth equipped with teeth and four pairs of barbels, a round and elongated body shape, a black to grayish body, a rounded tail shape, and a pair of sharp spines on the pectoral fins (Helmizuryani et al., 2022). The results of the evaluation of various zootechnical aspects show that mutiara catfish have superior characteristics that are quite comprehensive as a fishery cultivation commodity (Iswanto et al., 2015). Another advantage of mutiara catfish is its very high growth rate, which is 20-70% faster than other catfish. In addition, this fish takes about 45-50 days in a ground pond from seeds measuring 5-7 cm or 7-9 cm (Sugianti et al., 2022). Furthermore, mutiara catfish can also increase its growth by 100-150% and even 150-200% (Cahyani et al., 2022).

Characterization of Proteins in Mutiara Catfish Mucus

The molecular weight of proteins detected in mutiara catfish mucus samples has a molecular weight of 7.10 kDa, 13.51 kDa, 15.46 kDa, 28.78 kDa, 30.72 kDa, 42.40 kDa and 69.17 kDa. The molecular weight of the proteins can be seen in Table 1.

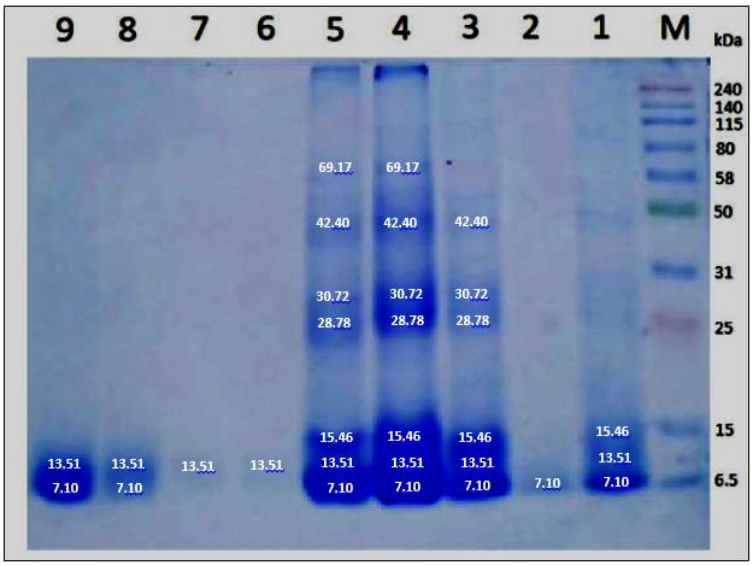

Figure 2. Characterization of proteins in mutiara catfish surface mucus. M (marker), lanes 1-9 (samples 1-9).

There are 3 major proteins, namely 7.10 kDa, 13.51 kDa, 15.46 kDa found in the sample, in addition there are 4 minor proteins, namely 28.78 kDa, 30.72 kDa, 42.40 kDa and 69.17 kDa in the sample. Strong protein bands indicate high protein concentrations in the mucus on the surface of the mutiara catfish skin. This protein is included in the category of small proteins that have a simple structure and function as hormones, signal peptides, and defense mechanisms in fish mucus. Protein characterization can be seen in Figure 2.

Table 1. Molecular weight of proteins in mutiara catfish surface mucus

Sample	Rf distance	Rf value	log BM	BM
Sample 1	12.8	0.95	0.85	7.10
	11.4	0.84	1.13	13.51
	11	0.82	1.19	15.46

Sample	Rf distance	Rf value	log BM	BM
Sample 2	12.8	0.95	0.85	7.10
Sample 3	12.8	0.95	0.85	7.10
	11.4	0.84	1.13	13.50
	11	0.81	1.19	15.46
	7.6	0.56	1.46	28.78
	7	0.52	1.49	30.72
	4.8	0.36	1.63	42.40
Sample 4	12.8	0.95	0.85	7.10
	11.4	0.84	1.13	13.51
	11	0.82	1.19	15.46
	7.6	0.56	1.46	28.78
	7	0.52	1.49	30.72
	4.8	0.36	1.63	42.39
	3.1	0.23	1.84	69.17
Sample 5	12.8	0.95	0.85	7.10
	11.4	0.84	1.13	13.51
	11	0.82	1.19	15.46
	7.6	0.56	1.46	28.78
	7	0.52	1.49	30.72
	4.8	0.36	1.63	42.39
	3.1	0.23	1.84	69.17
Sample 6	11.4	0.84	1.13	13.51
Sample 7	11.4	0.84	1.13	13.51
Sample 8	12.8	0.95	0.85	7.10
	11.4	0.84	1.13	13.51
Sample 9	12.8	0.95	0.85	7.10
	11.4	0.84	1.13	13.51

The molecular weight of the proteins that appear most in the mucus of mutiara catfish are 7.10 kDa, 13.51 kDa, and 15.46 kDa. The frequency of the appearance of proteins with a certain molecular weight in each sample repetition indicates that the protein is abundant in the mucus on the surface of the skin of mutiara catfish (Table 2). Determination of protein molecular weight is carried out by observing the position of the protein band with the position on the marker track whose molecular weight is known or commonly known as a marker (Nurfaidah et al., 2020). Several protein bands show the same band position indicating that there is the same protein molecular weight in each sample (Nurfaidah et al., 2020). The SDS Page technique also has a band thickness that indicates the concentration of a protein molecule, namely the major band and the minor band (Alberts et al., 2015). The major band explains that the protein has a high concentration in the sample, while the minor band explains that the protein has a low concentration found in the observation sample (Alberts et al., 2015).

Table 2. Frequency of Molecular Weight of Proteins on the Surface of Mutiara Catfish

Molecule Weight (kDa)	Sample									Frequency
	I	II	III	IV	V	VI	VII	VIII	IX	
7.01	√	√	√	√	√	-	-	√	√	7
13.51	√	-	√	√	√	√	√	√	√	9
15.46	√	-	√	√	√	-	-	-	-	4

Molecule Weight (kDa)	Sample									Frequency
	I	II	III	IV	V	VI	VII	VIII	IX	
28.78	-	-	√	√	√	-	-	-	-	3
30.72	-	-	√	√	√	-	-	-	-	3
42.39	-	-	√	√	√	-	-	-	-	3
69.17	-	-	-	√	√	-	-	-	-	1

Fish mucus, secreted by goblet cells, plays an important role in nonspecific immunity by acting as a chemical or physical barrier against infectious microorganisms (Dash et al., 2018). Common proteins exist as the main component proteins of red hybrid tilapia mucus under normal conditions. Their composition and rheological properties are important for mucus to maintain its functions, including respiration, osmoregulation, communication, locomotion, and disease resistance (Lai et al., 2009). It can be concluded that 14 kDa protein is a common protein in Oreochromis spp mucus (Mohd et al., 2020). In Indian scad, short-bodied mackerel, fusilier goldband, Japanese threadfin bream, white-lipped eel catfish, and milkfish, albumin protein bands are in the weight range of 25-62 kDa (Fatma et al., 2023). Based on the results of the analysis of molecular weight in gourami fish 10 kDa, 25 kDa, 20 kDa, 37 kDa, 50 kDa, 75 kDa, 150 kDa, and 250 kDa (Nurfaidah et al., 2020).

Types and Roles of Proteins

Each type of protein has a different molecular weight due to the composition of amino acids and different chemical structures. Types of small proteins that have a molecular weight approaching or ranging from 7-28 kDa are Cystatin, Small Heat Shock Proteins (sHSPs), Glutathione S-transferase (GST) Isoforms, Neuropeptide Y (NPY), Endothelin, Tachykinin, Angiotensin, S100 Proteins, Neurotensin, Cathepsin L (Isoform), Phosducin, Vimentin, Transferrin, Apolipoprotein, Heat Shock Protein 15 (Hsp15), Cytochrome c, Tropomyosin, beta-defensin, Insulin-like Growth Factor (IGF), Myosin Light Chain, Albumin, Ferroportin, Glutamine Synthetase, Phosphofructokinase (PFK), Calsequestri. Types of medium-sized proteins that have a molecular weight approaching or ranging from 30-69 kDa include Myosin Light Chain (MLC), Tropomyosin, Glutamine Synthetase, Apolipoprotein, Cytochrome c, Heat Shock Protein 30 (Hsp30), Calmodulin, Glutathione S-Transferase (GST), Vimentin, Ferroportin, Heat Shock Protein 40 (Hsp40), Cytochrome P450 (CYP), Glycogen Synthase, Phosphoglucomutase (PGM), Ribosomal Protein S6 (RPS6), Cathepsin L, Heat Shock Protein 70 (Hsp70), Myosin Heavy Chain (MHC), Collagen, Ribosomal Protein L9 (RPL9), Fibrinogen, Protein Kinase (PKC), Ferritin.

Proteins found abundantly in the mucus on the surface of catfish skin have a molecular weight ranging from 7-15 kDa, where this protein has various roles including immune defense, regulation of metabolism, oxidative stress, and the nervous system, stress regulation, regulation of cellular function, muscle contraction, and nervous system function.

Table 3. Types and roles of mutiara catfish surface mucus proteins

Molecule Weight (kDa)	Types of Protein	Role of Protein
~ 7.01	Cystatin, Small Heat Shock Proteins (sHSPs), Glutathione S-transferase (GST) Isoforms, Neuropeptida Y (NPY), Endothelin, Tachykinin, Angiotensin	Physiological regulation such as immune defense, metabolic regulation, oxidative stress, and the nervous system

Molecule Weight (kDa)	Types of Protein	Role of Protein
~13.51	S100 Proteins, Neurotensin, Glutamine Synthetase (isoform), Small Heat Shock Proteins (sHSPs), Cystatin, Cathepsin L (Isoform), Phosducin, Vimentin, Transferrin, Apolipoprotein	Immune defense, metabolism, stress regulation, and regulation of cellular function
~15.46	Cystatin, Heat Shock Protein 15 (Hsp15), Cytochrome c, Tropomyosin, Neuropeptide Y (NPY), Glutathione S-Transferase (GST), Apolipoprotein, beta-defensin, Insulin-like Growth Factor (IGF), Phosducin	Regulation of metabolism, immune defense, muscle contraction, and nervous system function
~28.78	Myosin Light Chain, Cytochrome c, S100 Protein (S100A1), Albumin, Ferroportin, Glutamine Synthetase, Phosphofructokinase (PFK), Tropomyosin, Calsequestri	Cell structure, energy metabolism, stress response
~30.72	Myosin Light Chain (MLC), Tropomyosin, Glutamine Synthetase, Apolipoprotein, Cytochrome c, Heat Shock Protein 30 (Hsp30), Calmodulin, Glutathione S-Transferase (GST), Vimentin, Ferroportin	Muscle contraction, metabolic regulation, detoxification, stress regulation, and immune system function
~42.39	Heat Shock Protein 40 (Hsp40), Cytochrome P450 (CYP), Glycogen Synthase, Phosphoglucomutase (PGM), Apolipoprotein A-I (ApoA-I), Tropomyosin, Ribosomal Protein S6 (RPS6), Ferroportin, Vimentin, Cathepsin L,	Carbohydrate metabolism, lipoprotein regulation, stress response, muscle contraction, and protein synthesis process
~69.17	Heat Shock Protein 70 (Hsp70), Myosin Heavy Chain (MHC), Collagen, Ribosomal Protein L9 (RPL9), Apolipoprotein B (ApoB), Phosphoglucomutase (PGM), Fibrinogen, Protein Kinase (PKC), Ferritin, Glycogen Synt	Metabolic process, muscle contraction, protein synthesis, and response to environmental stress

According to Reverter et al. (2018), Skin mucus in fish not only functions as a physical protector, but mucus also has biological and bioactive functions in the defense of the fish's body which can support immunity, cellular metabolism, and protection from environmental stress. Epidermal mucus in fish is very effective in fighting the threat of pathogenic bacteria because mucus contains various bioactive components to paralyze pathogens and act as a barrier so that bacteria cannot enter deeper tissues (Gobi et al., 2018). Mucus has the ability to bind organic and inorganic materials and then release them into the environment, this has an impact on reducing the number of mucosal cells (Dang et al., 2019).

Heat shock protein is a protein that plays an important role in stress such as stress from high temperatures, tissue damage, heavy metal pollution, and pathogenic bacterial infections (Ali et al., 2023). Based on its molecular weight, heat shock proteins are grouped into five, namely HSP 15-30 kDa, HSP60 (60 kDa), HSP70 (66-79 kDa), HSP90 (83-90 kDa), and HSP100 (Ali et al., 2023). Several studies have shown that there are heat shock proteins in the epidermal layer of several fish species including

Dicentrarchus labrax (Zhang et al., 2013), Sparus aurata (Jurado et al., 2015), Cyclopterus lumpus (Patel et al., 2017), and Salmo salar (Provan et al., 2013).

In the study of Jurado et al. (2015), it was found that there were several isoforms of actin, beta globin (HBB), and tropomyosin4-2 in the mucus of gilthead seabream skin. Beta globin (HBB) has a role in binding other metals in mucus (eg, lactoferrin and transferrin) to create a low-iron environment that limits microbial pathogenesis (Jurado et al., 2015). Peptides derived from ribosomal protein S30 were also found in the skin secretions of rainbow trout (Fernandes et al., 2002). Apolipoprotein-A1 (Apo-A1) type protein was found in S. aurata mucus samples (Jurado et al., 2015).

CONCLUSION

The proteins found abundantly in the mucus on the surface of the pearl catfish skin are small-sized proteins with molecular weights ranging from 7-28 kDa and medium-sized proteins with molecular weights ranging from 30-69 kDa. The protein with the highest concentration is found in the small-sized protein type which is characterized by a very thick protein band. It is estimated that there are various types of proteins with molecular weights ranging from 7-69 kDa. The proteins found mostly have a major role in body defense, immune protection and adaptation to environmental stress. This protein is very important in maintaining fish health.

RECOMENDATION

This study contains information related to mucus protein on the surface of catfish skin, it is expected that further research can deepen understanding of the variability of mucus protein profiles in different environmental conditions and types of fish. This aims to identify differences that may be useful in designing health management strategies in the field of aquaculture. It is also hoped that future research will be able to develop products in increasing mucus production in fish in order to strengthen the immunological defense of fish so as to reduce dependence on the use of antibiotics.

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REFERENCES

Ali, S., Ullah, W., Kamarulzaman, A. F. S., Hassan, M., Rauf, M., Khattak, M. N. K., Dawar, F. U. (2023). Proteomic profile of epidermal mucus from *Labeo rohita* reveals differentially abundant proteins after *Aeromonas hydrophila* infection. *Fish and Shellfish Immunology Reports*, 5.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., Walter, P. (2015). *Molecular Biology of the Cell, 6th Edition*. Garland Science, New York.

Andriani, D., Masyitha, D., Zainuddin, Z. 2017. Struktur Histologi Kulit Ikan Gabus (*Channa striata*) (The Histology of Skin's Snakehead Fish (*Channa striata*)). *Jurnal Ilmiah Mahasiswa Veteriner*, 1(3), 283-290.

Cahyani, L. R., Hafiludin. (2022). Manajemen Pemberian Pakan pada Pembesaran Ikan Lele Mutiara (*Clarias Gariepinus*) di Karamba Tancap Balai Benih Ikan Pamekasan. *Juvenil,* 3(2), 19-26.

Dang, M., Pittman, K., Bach, L., Sonne, C., Hansson, S. V., Sondergaard, J., Stride, M., Nowak, B. (2019). Mucous cell responses to contaminants and parasites in shorthorn sculpins (*Myoxocephalus scorpius*) from a former lead-zinc mine in West Greenland. *Sci. Total Environ*, 678, 207 - 216.

Dash, S., Das, S. K., Samal, J., Thatoi, H. N. (2018). Epidermal mucus, a major determinant in fish health: a review. *Iranian Journal of Veterinary Research*, 19(2), 72-81.

Faeste, C. K., Tartor, H., Moen, A., Kristoffersen, A. B., Dhanasiri, A. K. S., Anonsen, J. H., Furmanek, T., Grove, S. 2020. Proteomic Profiling of Salmon Skin Mucus for The Comparison of Sampling Methods. *Journal of Chromatography*, 121965.

Fatma, N., Metusalach, Taslim, N. A., Nurilmala, M. (2023). SDS-PAGE Protein Profile of Albumin Extracted by Steaming from Four Marine and Three Brackish-Water Fishes. *Biodiversitas,* 24(7), 4027-4033.

Guardiola, F. A., Cuesta, A., Abellan, E., Meseguer, J., Esteban, M. A. (2014). Comparative Analysis of the Humoral Immunity of Skin Mucus from Several Marine Teleost Fish. *Fish & Shellfish Immunology*, 40(1), 24-31.

Gobi, N., Vaseeharan, B., Chen, J. C., Rekha, R., Vijayakumar, S., Anjugam, M., and Iswarya, A. (2018). Dietary supplementation of probiotic *Bacillus licheniformis* Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*. *Fish Shellfish Immun*, 74, 501 - 508.

Helmizuryani, Dasir, Asiati, D.I. (2022). *Teknik Pembenihan Ikan Lele (Clarias gariepinus Burchell 1822) di Kolam Terpal.* Palembang: Penerbit Noer Fikri.

Ismail, W. A., Pangastuti, P. H., Purnama, M. T. E. 2020. *Perbandingan Struktur Kulit Ikan Lele (Clarias batrachus) dan Nila (Oreochromis niloticus) di Perairan Tawar Banyuwangi*. Prosiding Seminar Nasional Kedokteran Hewan dan Call of Paper. Fakultas Kedokteran Hewan Universitas Airlangga

Iswanto, B., Suprapto, R., Marnis, H., Imron. (2015). Karakteristik Morfologis dan Genetis Ikan Lele Afrika (*Clarias gariepinus* Burchell, 1822) Strain Mutiara. *Jurnal Riset Akuakultur,* 10 (3), 325-334.

Lai, S.K., Wang, Y.Y., Wirtz, D. and Hanes, J. 2009. Micro- and macrorheology of mucus. *Adv. Drug Deliv. Rev.,* 61(2), 86–100.

Liu, P. F., Xia, Y., Hua, X. T., Fan, K., Li, X., Zhang, Z., Liu, Y., 2020. Quantitative proteomic analysis in serum of Takifugu rubripes infected with Cryptocaryon irritans. *Fish Shellfish Immunol*. 104, 213–221.

Mohd, F., Nazifah, N., Ridzuan, S., Norazlan, W., Abdullah, A., Ramly, R. (2020). Protein Profiling of Oreochromis spp. Epidermal Mucus Subsequent to Challenge of Common Freshwater Bacteria. *International Journal of Agriculture, Environment and Biotechnology*, 13(4), 511-515.

Mokhtar, D. M., Zaccone, G., Alesci, A., Kuciel, M., Hussein, M. T., Sayed, R. K. A. (2023). Main Components of Fish Immunity: An Overview of the Fish Immune System. *Fishes*, 8(2), 1-24.

Nurfaidah, Metusalach, Mahendradatta, M., Sukarno. (2020). Analysis of Molecular Weight Albumin Concentrate on Various Types of Freshwater Fish using SDS-page Electrophoresis Method. *IOP Conference Series: Earth and Environmental Science*, 564, 1-7.

Patel, D. M., Brinchmann, M. F. (2017). Skin Mucus Proteins of Lumpsucker (*Cyclopterus lumpus*). *Biochemistry and Biophysics Reports*, 9, 217–225.

Prasetyo, A. 2021. Identifikasi Ektoparasit pada Ikan Lele Dumbo (*Clarias gariepinus* Burchell, 1822) yang Dibudidayakan di Cibubur, Jakarta Timur. *Skripsi*. Universitas Islam Negeri Syarif Hidayatullah. Jakarta

Provan, F. , Jensen, L. B., Uleberg, K. E., Larssen, E., Rajalahti, T., Mullins, J., Obach, A. (2013). Proteomic analysis of epidermal mucus from sea lice-infected to Altlantic salmon, Salmon salar L. 36(3), 311-321.

Reverter, M., Tapissier-Bontemps, N., Lecchini, D., Banaigs, B., and Sasal, P. (2018). Biological and ecological roles of external fish mucus: A review. Fishes, 3(41), 1-19.

Sugianti, E. P., Hafiludin. (2022). Manajemen Kualitas Air pada Pembenihan Ikan Lele Mutiara (*Clarias Gariepinus*) di Balai Benih Ikan (Bbi) Pamekasan. *Juvenil*, 3(2), 32-36.

Zhang, J., Li, J., Liu, B., Zhang, L., Chen, J., Lu, M. (2013). G enome-wide analysis of the *Populus Hsp90* gene family reveals differential expression patterns, localization, and heat stress responses. *BMC Genomics*, 14, 1-14.

Jurado, J., Almagro C. A. F., Guardiola, F. A, Cueste, A., Esteban M. A., Alamo, M. J. P. (2015). Proteomic profile of the skin mucus of farmed gilthead seabream (*Sparus aurata*). *Journal of Proteomics*, 120, 21-34.

Zhao, N., Jia, L., He, X., Zhang, B. 2021. Proteomics of mucosal exosomes of Cynoglossus semilaevis altered when infected by Vibrio harveyi. *Developmental and Comparative Immunology*. 119, 104045.

Zoghby, E. E., Attia, H., Emam, M., Tantawy, A2016. . Histological, Ultrastructural and Immunohistochemical Studies on The Skin of Catfish. *Benha Journal of Applied Science,* 1(2), 59-65.