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Proximate and Amino Acid Analysis of Processed Chicken Feather Meal for Fish Feed Ingredient

Desy Putri Handayani[a*], Alim Isnansetyo[a], Muhsin Al Anas[b], Senny Helmiati[a], Puan Sri Maharani[a], Amalia Yunita Ismail[a]

[a] Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia
[b]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada

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Abstract

Currently, it is critical to turn byproducts in the surrounding environment into valuable resources. These various kinds of materials can be easily found, such as chicken feathers from chicken slaughterhouses. This investigated was pointed to hydrolyze and ferment chicken feathers into feather meal and determine the nutritional content of feather meal. Chicken feathers were obtained from Rumah Pemotongan Ayam Berbah, Sleman. Several methods for chicken feather processing were conducted by probiotic, chloride acid, and natrium hydroxide. The next step was to analyze the nutrition value of chicken feather meal by proximate, amino acids (Liquid Chromatography Mass Spectrometry) analysis. The research results showed that fermentation of chicken feather meal by using probiotic improved the nutritional value namely protein content was 78.18% and essential amino acids content (isoleucine was 14,763.26 µg/g, histidine was 12,043.825 µg/g, glycine was 326.51 µg/g, methionine was 138.17 µg/g, arginine was 33,321.63 µg/g, phenylalanine was 22,815.65 µg/g and leucine was 44,511.41 µg/g). This research can be used as a basis for using fermented chicken feathers as a cheap and nutritious alternative fish feed ingredient.

Keywords: Amino Acid; Chicken Feather; Fish Feed; Protein; Probiotic

1. Introduction

Environmental degradation due to waste produced by humans increases every year. One source of waste in the surrounding environment is feathers, which are produced by the chicken slaughter industry. Data from the Directorate General of Animal Husbandry and Animal Health, Ministry of Agriculture (2022) shows that broiler chicken production in Indonesia in 2022 will reach 3.7 million tons. The percentage of chicken feathers is 4-5% or is estimated to reach 160 thousand tons. Chicken feathers are usually not utilized by humans and only become waste in the land and waters. It is currently being utilized as an alternative feed component for animals, specifically ruminants, pigs, and broiler chickens (Haryanto et al, 2017; Dalle et al., 2022).

Feathers from chickens have a lot of potential as materials for fish feed. Every year, the cost of the substances used in fish feed goes up, so we must find other sources. Even though chicken feathers have a very high protein content—nearly 75%—they are difficult for animals to digest. Keratin makes up 90% of the proteins found in chicken feathers. According to Mulia et al. (2016), the composition of chicken feathers is 73.56% crude protein, 3.37% fat, 1.37% ash, 21.61% crude fiber, and 11.69% water. Fish or animals cannot readily digest keratin, necessitating many procedures. We used IW probiotics containing *Lactococcus* and *Bacillus* spp., HCl, and NaOH in this investigation. *Aspergillus flavus, Aspergillus niger, Bacillus* sp., and *Pseudomonas* sp. have all been shown in several studies to be capable of degrading the keratin in chicken feathers through fermentation (Mondal et al., 2014; Mulia et al., 2016; Oliveira et al., 2019; Adelina et al. 2020; Huang et al. 2022; Masood et al., 2023). Alkaline and acid hydrolysis are two methods for processing chicken feathers. To weaken the strong keratin connections in chicken feathers, apply NaOH, hydrochloric acid, and formic acid (Alamsyah et al., 2015; Fitriyanto et al., 2022).

It hasn't been common practice to feed fish with chicken feather meal. There is no study that has produced different methods for breaking down chicken feathers, analyzing amino acids, or figuring out the molecular weight (MW) of the protein. Using proximate analysis and amino acid profiles, the nutritional value of hydrolyzed and fermented chicken feather meal will be determined in terms of crude protein, fiber, fat, water content, and ash. The sample which has the best result of all parameters is used as the basis for selecting procedures for processing chicken feathers into feather meal. Chicken feather meal will be mass produced to be used as a feed ingredient and tested on commercially farmed fish.

*) Corresponding author: desy.putri.h@mail.ugm.ac.id

2. Materials and Method

2.1. Time and Location of Research

This research was carried out at fish feed and nutrition laboratory, fisheries department, Universitas Gadjah Mada. The sample of chicken feather was given free from RPA (chicken slaughterhouse) Berbah, Sleman.

2.2. Sampling and Preparation

Chicken feathers are washed using soap and running water to remove dirt and fishy smell then sterilized using an autoclave (Tomy ES-215, Japan) at 121 °C for 20 minutes. Drying process was carried out using an oven at 50 °C for 7 hours and then ground into meal (Mulia et al. 2016).

2.3. Experimental Design

This research consisted of 4 treatments with 3 replications, namely: P1 (chicken feather meal), P2 (chicken feather meal + 10% HCl), P3 (chicken feather meal + 10% NaOH), and P4 (chicken feather meal + 1% IW). Acid hydrolysis of chicken feathers was carried out using a modification of the method of Fitriyanto et al. (2022). The chicken feather meal was soaked in 10% HCL (1:5, w:v) and stored for 96 h at room temperature then added with NaOH to normalize the pH. The alkaline hydrolysis method with NaOH was used a modification from Sukendro et al., (2020). Chicken feathers were washed and then sterilized at 121 °C for 20 minutes. While another sample was fermented by IW probiotic with composition of chicken feather: water: molasses: bacteria (25 g: 12.5 mL: 12.5 mL: 0.5 g). The fermentation process was carried out in a closed container, impermeable to light and water, and placed on an orbital shaker (Daihan Scientific SHO-2D, South Korea) at 120 rpm for 96 hours at room temperature. All the sample was then dried in an oven at 50 °C for 7 hours and stored at 4 °C for further investigation. The nutritional value of each sample (protein, fat, ash, water, and fiber) was analyzed with proximate method (AOAC 2012).

2.4. Amino Acid Analysis

Amino acid was analyzed by LCMS, based on Nugraha et al. (2022). A total of 1 g of sample was placed in a 50 mL screw-on test tube and added 10 mL of 6 N HCI then hydrolyzed at 110 °C for 12 h. Samples were neutralized by adding up to 100 mL of 6 N NaOH, passed through a 0.22 µm filter, diluted with H_2O 100 times, and then injected into an LCMS instrument (Xevo TQD Waters, USA) equipped with a tandem quadrupole mass detector. Exactly 2 µL of sample was injected and eluted with solution A (ddH_2O:CH_3CN, 99.5%:0.5% (v/v), with 0.1% (v/v) PDFOA, and 0.1% (v/v) formic acid) and solution B (ddH_2O:CH_3CN, 10%: 90% (v/v) with 0.1% (v/v) PDFOA, with 0, 1% (v/v) formic acid) with a flow rate of 0.6 mL/min.

2.5. Statistical Analysis

All data were evaluated with one-way ANOVA in SPSS version 25. ANOVA reached a 95% level of significance. A one-way analysis of variance was done to determine if there was a significant difference (α=0.05) between treatment means and tested using Duncan's multiple range test (DMRT) (Chor et al. 2013).

3. Results and Discussion

3.1. Result

The result of all proximate analysis was shown in Figure 1. The results showed that the crude protein content of all treatments was significantly different from that of the control. The crude protein in Probiotic chicken feather hydrolysate is the highest compared to other treatments, which was 78.18%. The lowest crude protein content was detected in the hydrolysis treatment with 10% NaOH, which was 56.40%. Chicken feather meal hydrolyzed using control and 10% HCl had crude protein of 71.79% and 63.95%.

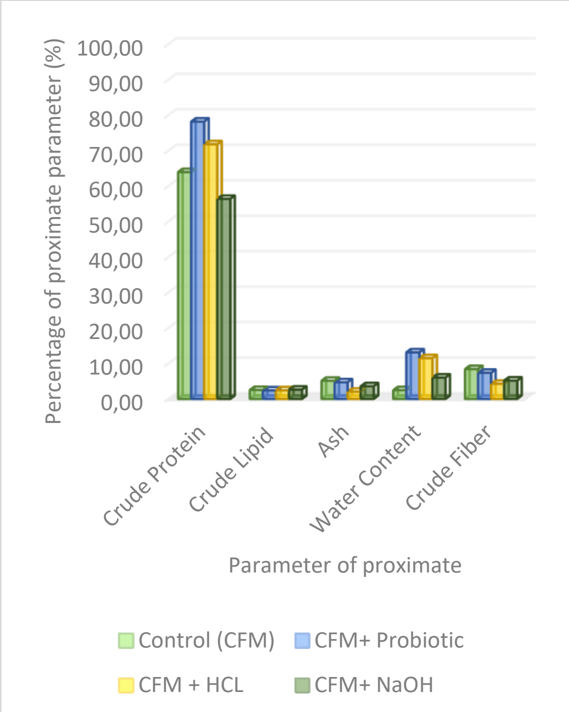

Figure 1 Proximate analysis of fermented and hydrolyzed chicken feather meal compared with control.

The control had an ash content of 5.12%, the highest compared to other treatments. Chicken feather hydrolysated using HCl, NaOH, and IW probiotic had an ash content of 2.05%, 3.55%, and 4.6%. The results showed that the control, fermentation, and hydrolysis treatments had no significant effect on the fat content of chicken feather meal. Controls and treatments had crude fat content of less than 5%. Fermentation and hydrolysis treatments increased the water content of chicken feather meal. The fermentation and hydrolysis treatment had a significant effect on the water content. Control chicken feathers had the lowest water content compared to the other treatments at 2.46%. Chicken feathers treated by hydrolysis of NaOH and HCl had water content of 6.07% and 11.50%. The highest water content was detected in the fermentation treatment with probiotics, which was 13.12%.

Treatment of probiotics, NaOH and HCl to chicken feather meal had a significant effect on crude fiber content. The crude fiber content of the control was the highest compared to the other treatments at 8.42%. The fermented and hydrolyzed chicken feather meal contained less crude fiber than the control, namely 7.38%, 4.27% and 5.16%.

Variations in amino acid concentrations from chicken feather meal using LCMS analysis. Based on the research results, it showed that chicken feather meal fermented using IW probiotics contained the highest amino acids arginine, phenylalanine and leucine compared to the control and hydrolysis treatment, at namely 33,321.63 µg/g, 22,815.65 µg/g and 44,511.41 µg/g. g. The amino acid lysine in the control chicken feather meal was the highest compared to the treatment, namely 30,875.1 µg/g (Figure 2).

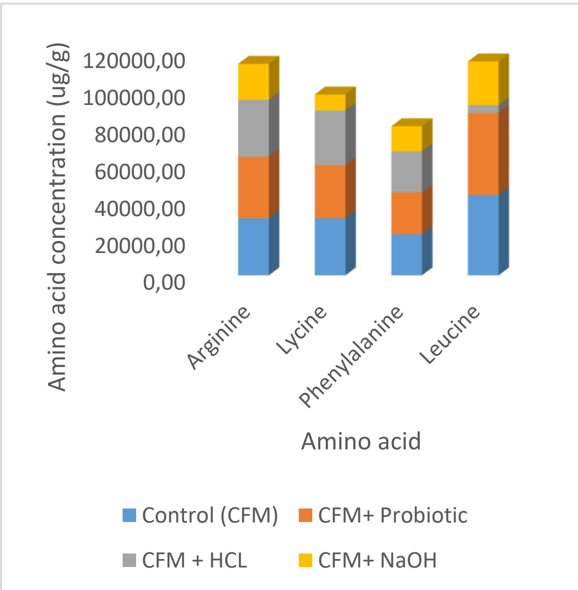

Figure 2. Concentrations of the amino acid arginine, lysine, phenylalanine, and leucine were contained in control and treatment chicken feather meal.

The treatment of probiotics and hydrolysis using strong basic (NaOH) and acids (HCl) had a significant effect on the variation of amino acids in chicken feather meal (Figure 3). The results showed that the levels of amino acids isoleucine and histidine in fermented chicken feather meal were the highest compared to the control and hydrolysis treatment, namely 14,763.26 µg/g and 12,043.825 µg/g. There was 4,509.66 µg/g of the highest valine for the control.

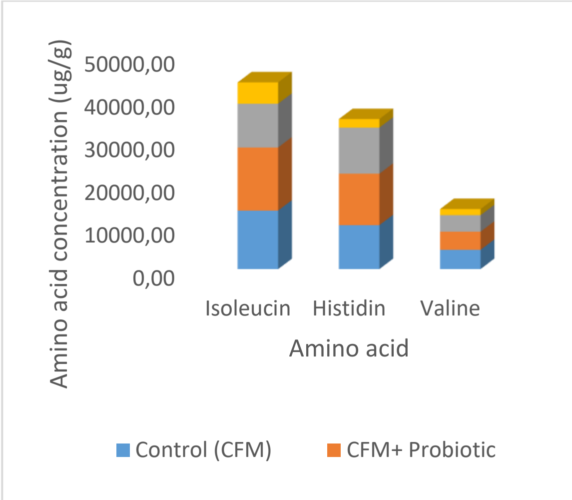

Figure 3. Concentrations of the amino acid isoleucine, histidine, and valine were contained in control and treatment chicken feather meal.

The content of methionine and glycine in probiotic-fermented chicken feathers was the highest compared to the control and hydrolysis treatment, namely 138.17 µg/g and 326.51 µg/g. The highest amino acid proline was detected in control chicken feathers, namely 30.60 µg/g (Figure 4).

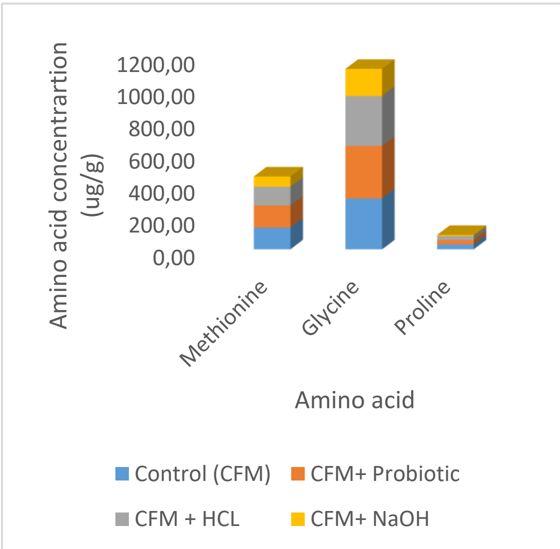

Figure 4. Concentrations of the amino acid methionine, glycine, and proline were contained in control and treatment chicken feather meal.

3.2. Discussion

There is still lack of research on the usage of chicken feathers as a fish feed ingredient. Although there is a vast potential market for chicken feathers, humans have only ever utilized it for cleaning, wall decorating, and sports equipment. Fish meal and soybeans are more expensive than chicken feathers, which can be used as an alternate source of protein for fish feed ingredients. For ruminants, pigs, and poultry, it has been utilized as a feed concentrate that can promote growth. The rising expense of buying feed can also be avoided by employing chicken feathers. While it comes from clean, fresh, and non-rotting feed materials, processing chicken feathers into meal for fish feed can be done in huge amounts.

Because chicken feathers contain a lot of keratins, a kind of protein, its digestion and utilization are low. Keratin contains high cross-linking disulfide bonds (Reddy et al., 2017). Due to the disulfide bonds that form between the amino acid cystine, keratin is difficult for chickens to digest by both proteolytic enzymes in the post-rumen digestive tract and rumen microbes. Through a variety of suitable processing techniques, the biological value of chicken feather meal can be improved. Hydrolysis and probiotics are two techniques that can be used to process chicken feathers for use as a raw material in fish feed (Yin et al., 2013; Sari et al. 2015; Chilakamarry et al., 2021). In this work, we employed feathers as the bacteria' feed source during solid state fermentation. Since using just chicken feathers as a substrate was inappropriate, we added molasse as the microorganism's carbon source during the fermentation process. The fermenting process was the reason for the rise in crude protein content in chicken feather meal. It was thought that the bacteria under test, specifically Bacillus and Lactococcus sp., produced keratinase activity. According to Saarela et al. (2017), bacterial fermentation improves the digestibility of feather keratin and causes certain amino acids to be produced by microorganisms during the degradation process. Mulia (2016) reported that Bacillus may produce huge amounts of keratinase enzymes. One protease enzyme that may degrade keratin substrates is called keratinase. Following fermentation, lactic acid bacteria's (LAB) keratinase activity led to a rise in the crude protein content of chicken feather meal. This enzyme may hydrolyze a wide range of soluble and insoluble proteins, including keratin. Because amino acids were broken during the hydrolysis process, the 10% NaOH treatment's crude protein concentration dropped.

Compared to other treatments, the control had the highest ash content. Ash is an organic material that is produced when organic things burn. To find the ash content, all organic materials are oxidized for six hours at a high temperature of 500–600 °C. Fermented and hydrolyzed chicken feathers had less ash because all the constituent parts that make up organic compounds burn and convert to gas. The water content of probiotic treatment also increased compared with control. The metabolism of microorganisms is generally followed by the release of water, which results in an increase in the water content of sample. The heat produced by the metabolic process causes a large amount of water vapor to be trapped in the fermenter. Another result also showed that the fermented chicken feather meal has the lower content of crude fiber. The decrease in crude fiber that occurred in fermented chicken feather meal was caused by a decrease in fiber in the keratin protein. Too high crude fiber levels result in longer digestion of nutrients and lower productive value. The larger amount of crude fiber in feed the lower the digestibility of the feed (Wulandari et al., 2013).

Numerous amino acids can be found in hydrolyzed chicken feather meals. According to the other findings (Cedrola et al. 2012; Grazziotin et al. 2006), the fermented feather concentrate has a high concentration of oligopeptide compounds and a few essential amino acids, including histidine (His) and lysine (Lys). There is a similarity in the amino acid of the feather meal and the fish meal. Fishmeal's amino acid composition includes the following: histidine (1.84 mg/100 g), isoleucine (4.21 mg/100 g), methionine (1.39 mg/100 g), phenylalanine (4.79 mg/100 g), valine (5.24 mg/100 g), arginine (4.56 mg/100 g), and histidine (8.83 mg/100 g) (Prado et al. 2016).

Fish require the important amino acids leucine, phenylalanine, and arginine to grow more quickly and efficiently. Because mature fish have low activity of arginine biosynthesis enzymes such as pyrroline-5-carboxylate (P5C) synthase, ornithine transcarboxylase (OTC), and carbamoyl phosphate synthase (CPS) III, Fish feed containing arginine is highly recommended to be given to fish (Andersen et al., 2016). Another amino acid which is necessary for fish is phenylalanine, an aromatic amino acid. Tyrosine, which is utilized as a precursor to epinephrine and norepinephrine, thyroid hormone, triiodothyronine, and thyroxine, is produced by the liver and kidneys from this amino acid. These substances are involved in the chemistry of the functioning brain (Ahmed 2022). Tyrosine is sometimes referred to as a semi-essential amino acid since, in situations when tyrosine levels are insufficient, fish production needs an extra supplement of phenylalanine to suit its growth and metabolic needs. Aromatic amino acids are essential for protein synthesis, growth, and metabolism. Fish lacking in phenylalanine will develop more slowly, feed less effectively, and antioxidants activity (Zehra and Khan 2014). According to Zhao et al. (2023), leucine can enhance the function of fish intestines by boosting tight junctions, humoral immunity, and antioxidant capacity.

Fish require isoleucine, also known as ille, as an important amino acid to support their immune system, lipid and synthetic protein metabolisms, and other physiological functions. Low and inadequate feeding efficiency is caused by isoleucine insufficiency as reported in Cyprinus carpio, Ctenopharyngodon idella, Ictalurus punctatus, Paralichthys olivaceus, Labeo rohita and Catla catla (Zhao et al. 2020). Histidine is a necessary amino acid that is involved in the synthesis of proteins, the development and repair of tissues, the preservation of osmoregulation, and the maintenance of the myelin sheath. In fish, histidine functions as an antioxidant and engages the immune system. A histidine deficit impairs feed consumption and growth performance. If taken in excess, it will upset the equilibrium of the amino acids in food, which can lead to toxicity or the death of welds in epithelial cells. Furthermore, histidine serves as a significant antioxidant and buffer in a variety of fish species' organs. Numerous studies have been carried out in aquaculture to maximize the amount of histidine present in the commercial diets of various fish species. For fish to grow, adequate amounts of amino acids must be provided (Mukhtar 2018).

Methionine is the major methyl donor in organisms via S-adenosylmethionine via the transmethylation pathway, which is important for several important metabolites, such as phosphatidylcholine and creatine. Methionine also plays a role in the synthesis of cysteine and glutathione via the transsulfuration pathway, which, along with methionine, are necessary for protein synthesis and the antioxidant system (Elango, 2020). Methionine functions not only as a substructure for protein synthesis, but also as a signaling molecule to regulate immunity, energy metabolism, and reproduction (Wang et al., 2021). Glycine is a non-essential amino acid that is very important for fish because it stimulates the immune system. According to Li and Wu (2018), 33% of collagen is composed of the amino acid glycine, which is involved in the synthesis of collagen. Furthermore, glycine contributes to the formation of glutathione, a potent and significant antioxidant molecule, working with glutamate and cysteine (Perez-Tores et al. 2017). Glycine thereby modifies the antioxidant system.

4. Conclusion

There are a few ways to turn chicken feathers into fish feed, including acid-based hydrolysis and microbial fermentation. The study's findings demonstrated that probiotic-fermented chicken feather meal had a higher protein and essential amino acid content than hydrolysis and control groups. Probiotic-fermented chicken feather meal can be fed to fish, and its digestibility value and effects on fish development, feed efficiency, and health state can be studied further.

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References

Adelina, A., Feliatra, F., Siregar, Y. I., Suharman, I., 2020. Utilization of feather meal fermented Bacillus subtilis to replace fish meal in the diet of silver pompano, Trachinotus blochii (Lacepede, 1801). AACL Bioflux. 13: 100-108.

Alamsyah, A.A.D., Christyawan, J., Tiarasukma, A. P., Paramita, V., 2013. Pembuatan pangan ternak lele organik berbahan baku protein dari bulu ayam dengan metode fermentasi bio. Prosiding SNST ke-4. 22-27. ISBN 978-602-99334-2-0.

Andersen, S.M., Waagbø, R., Espe M., 2016. Functional amino acids in fish health and welfare. Front Biosci. 8:143e69.

A.O.A.C., 2012. Official Method of Analysis: Association of Analytical Chemists. 19th Edition, Washington DC, 121-130.

Chilakamarry, C.R., Mahmood, S., Saffe, S.N.B.M., Arifin, M.A.B., Gupta, A., Sikkandar, M.Y., Begum, S.S., Narasaiah, B., 2021. Extraction and application of keratin from natural resources: A review. Biotech. 11: 220. https://doi.org/10.1007/s13205-021-02734-7

Chor, W.K., Lim, L.S. and Shapawi, R., 2013. Evaluation of feather meal as a dietary protein source for African catfish fry, Clarias gariepinus. Journal of Fisheries and Aquatic Science. 8(6): 697-705.

Dalle, N.S., Sembiring, S., Lazarus, E.J.L., 2022. The effect of including fermented feather meal as substitution of concentrate in the basal diet with different levels on the performance of landrace crossbred pigs. Indonesian Animal Science Journal. 17: 44-50. https://doi.org/10.31186/jspi.id.17.1.44-50

Directorate General of Animal Husbandry and Animal Health, Ministry of Agriculture-BPS, 2023. Broiler Meat Production by Province (Tons), 2020-2022. https://www.bps.go.id/indicator/24/488/1/produk-daging-ayam-ras-pedaging-menrut-provinsi.html

Elango, R., 2020. Methionine nutrition and metabolism: insights from animal studies to inform human nutrition. Journal of Nutrition. 150: 2518S–2523S

Fitriyanto, N.A., Ramadhanti, Y., Rismiyati, Rusyadi, I., Pertiwiningrum, A., Prasetyo, R.A. and Erwanto, Y., 2022. Production of poultry feather hydrolysate using HCl and NaOH as a growth medium substrate for indigenous strains. IOP conference series: Earth and environmental science 951.

Haryanto, A., Purwaningrum, M., Andityas, M., Wijayanti, N., 2017. Effect of chicken feather meal on the feed conversion ratio and blood lipid profile of broiler chickens. Asian J. Poult. Sci. 11: 64-69. DOI: 10.3923/ajpsaj.2017.64.69

Huang, H.J., Weng, B.C., Lee, Y.S., Lin, C.Y., Hsuuw, Y.D. and Chen, K.L., 2022. The Effects of Two-Stage Fermented Feather Meal-Soybean Meal Product on Growth Performance, Blood Biochemistry, and Immunity of Nursery Pigs. Fermentation. 8: 634. https://doi.org/10.3390/fermentation8110634

Li, P., Wu, G., 2018. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino Acids. 50: 29–38.

Masood, S., Hussaina, A., Javida, A. , Bukahria, S. M., Alia, W., Alib, S., Ghaffara, I., Imtiaza, A., Aminc, H. M. A., Salahuddin, H., Inayate, M., Razzaqa, S., Kafayatd, F., Rafiqa, H., Yasmeena, M., Muneeba, M. and Sattara. S., 2023. Fungal decomposition of chicken-feather waste in submerged and solid-state fermentation. Brazilian Journal of Biology. 83, e246389. https://doi.org/10.1590/1519-6984.246389

Mondal, K., 2014. Nutritional evaluation of fermented poultry feather meal in the formulated diets of fingerlings of Catla catla (Hamilton). Electronic Journal of Biology. 10:118-124.

Mukhtar A. Khan., 2018. Histidine Requirement of Cultivatable Fish Species A Review. Oceanography & Fisheries Open Access Journal, Juniper Publishers Inc., vol. 8(5).

Mulia, D.S., Yuliningshih, R.T., Maryanto, H., Purbomartono, C., 2016. Utilization of chicken feather waste into fish feed using Bacillus subtilis fermentation. J. man and environment, 23: 49-57.

Nugraha, T. A., Isnansetyo, A., Triyanto, Djalil, M., 2022. Fermented earthworms as a feed additive enhances non-specific immune response in catfish (Clarias gariepinus). Aquaculture International. 30: 211–226. https://doi.org/10.1007/s10499-021-00794-3

Oliviera C.C., Souza, A.K.S., Castro, A.K.S., 2019. Bioconversion of Chicken Feather Meal by Aspergillus niger: Simultaneous Enzymes Production Using a Cost-Effective Feedstock Under Solid State Fermentation. Indian Journal Microbiol. 59:209–216. https://doi.org/10.1007/s12088-019-00792-3

Pérez-Torres, I., María Zuniga-Munoz, A., Guarner-Lans, V., 2017. Beneficial effects of the amino acid glycine. Mini. Rev. Med. Chem. 17: 15–32.

Reddy, M.R., Reddy, K.S., Chouhan, Y.R., Bee. H., Reddy, G., 2017. Effective feather degradation and keratinase production by Bacillus pumilus GRK for its application as bio-detergent additive. BioresourTechnolol 243:254–263. https://doi.org/10.1016/j.biortech.2017.06.067

Saarela, M., Berlin M., Nygren, H., Lahtinen, P., Honkapaa, K., Lantto, R. and Maukonen, J., 2017. Characterization of featherdegrading bacterial populations from birds' nests potential strains for biomass production for animal feed. Int. Biodeter. Biodegrad. 123:262–268.

Sukendro, P.B., Indrawati, T., Rahmat, D., 2020. Optimasi proses hidrolisis protein dari limbah bulu ayam. Farmasains. 8: 7-14. Doi:10.22236/farmasains.v8i1.5171

Yin, X.C., Li, F.Y., He, Y.F., Wang, Y.R.M, . 2013. Study on effective extraction of chicken feather keratins and their films for controlling drug release. Biomaterials science. DOI:10.1039/c3bm00158j

Wang, W., Yang, P., He, C., Chi, S., Li, S., Mai, K., Song, F., 2021. Effects of dietary methionine on growth performance and metabolism through modulating nutrient related pathways in largemouth bass (Micropterus salmoides). Aquaculture Reports. 20:100642.

Wulandari, W., Hadi, W. and Rahayu, S., 2013. Digestibility of fat and energy monogastrick concentrate based hidrolyzate feather meal in vitro. Jurnal Ilmiah Peternakan, 1: 430-436.

Zehra, S. & Khan, M., 2014. Dietary phenylalanine requirement and tyrosine replacement value for phenylalanine for fingerling Catla catla (Hamilton). Aquaculture. 433:256–65. doi:10.1016/j.aquaculture.2014.06.023

Zhao, Y., Li, J., Jiang, Q., Zhou, X., Feng, L., Liu, Y., et al., 2020. Leucine improved growth performance, muscle growth, and muscle protein deposition through AKT/TOR and AKT/FOXO3a signaling pathways in hybrid catfish Pelteobagrus vachelli × Leiocassis longirostris, Cells 9. e327. https://doi.org/10.3390/cells9020327

Zhao, J., Zhao, Y., Liu, H., Cao, Q., Feng, L., Zhang, Z ., Jiang, W., Wu, P., Liu Y., , Luo, W., Huang. X . and Jiang, J ., 2023. Dietary Leucine Improves Fish Intestinal Barrier Function by Increasing Humoral Immunity, Antioxidant Capacity, and Tight Junction. Int. J. Mol. Sci. 24, 4716. https://doi.org/10.3390/ijms24054716