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Profile of Amino Acids and Proximate on the Extraction of Red Ginger Protease Enzymes in Commercial Feed for Aquaculture

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Abstract: Quality feed in aquaculture is very important to increase the growth and health of fish. Low feed efficiency can be overcome by feed supplementation using catalytic enzymes. One of the enzymes that can stimulate digestion is protease in red ginger plants. The protease enzyme in red ginger plants plays a role in breaking peptide bonds in place of carboxyl groups so that peptides are formed from complex compounds into simpler compounds. This study aims to determine the profile of amino acids and proximate in feed given red ginger enzymes. This research uses descriptive method. The feed sample used was commercial feed with 4 treatments namely Control (without red ginger enzyme), treatment A (1%), treatment B (3%), and treatment C (5%). The analyzes carried out included red ginger enzyme extraction, protease enzyme purification, proximate and amino acid analysis of each type of feed. The results showed that the results of the SDS-Page analysis on the red ginger protease sample contained at 67.1 kDa. The results of the proximate analysis showed the highest value in feed containing 3% red ginger protease (B) with a percentage of 42.69%. The resulting amino acid content consists of 8 non-essential amino acids namely, L-serine, L-glutamic acid, L-alanine, L-arginine, glycine, L-aspartic acid, L-tyrosine and L-proline. While the essential amino acids consist of 7 types of amino acids namely, L-Phenylalani, L-Isoleucine, L-Valine, L-Lysine, L-Leucine, L-Threonine and L-Histidine. The best type of feed is feed B, which is feed with the addition of 3% red ginger protease.

Keywords: Amino Acid; Feed; Protease Enzyme; Proximate; Red Ginger

Introduction

Fish farming requires high quality and nutritious feed to improve the growth and health of fish. Commercial feed accounts for significantly more than half of production costs. So, it is very important to improve feed efficiency and growth performance in cultured fish. Low feed efficiency can be overcome by feed supplementation using catalytic enzymes. This can solve digestive problems for fish, because many fish cannot digest fiber, while the feed contains high fiber, which reduces the utilization of nutrients by up to 10% (Purba, 2022). The quality of protein is related to the amino acid profile it contains. Classification of amino acids based on the body's ability to synthesize and metabolic needs. This classification is known as essential and non-essential amino acids.

Enzymes are proteins that have catalytic activity to lower the activation energy of a reaction so that the conversion of substrates into products can take place more quickly. One of the enzymes that has an important role in life is protease, which is a proteolytic enzyme that works to break down proteins into amino acids (Subandiyono et al., 2017). Proteases can be found from various sources, including plants, animals and microorganisms. One of the plants that contain protease enzymes is ginger. Zingiberene is the main compound of ginger which can increase physiological responses and

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fish welfare so that it can improve fish growth performance, zingiberene promotes fish growth. In addition, the bioactive components can change the morphology of the gut, increase the consumption and absorption of nutrients thereby increasing the growth of fish. Zingiberene is also reported to stimulate digestive enzyme activity which can increase feed efficiency and growth performance (Aqmasjed et al., 2023). According to Purwoko (2018), the protease enzyme in the ginger plant plays a role in breaking peptide bonds in place of the carboxyl group so that smaller peptides and free amino acids are formed. The use of enzymes in fish feed has been widely carried out, one of which is in the research of Faizal et al. (2017), adding a combination of crude enzyme extracts papain and bromelin to artificial feeds can produce the growth of tilapia (oreochromis niloticus) seeds, according to research by Marwan et al. (2022) papain enzyme in feed can increase the digestive enzyme activity of milkfish chanos chanos. However, there has been no further research on red ginger enzymes in feed, so the purpose of this study was to determine the profile of amino acids and proximate in feeds given red ginger enzymes.

Method

This research uses descriptive method. In this study the analysis of red ginger enzyme content, amino acid and proximate components of each feed with treatment. The feed used was commercial feed with 4 treatments namely Control (without red ginger enzyme), treatment A (1%), treatment B (3%), and treatment C (5%). The test feed will be tested for each treatment according to the proximate results on and amino acid profiles.

Red Ginger Enzyme Extraction

Enzyme extraction was carried out with reference to the research by Nafi et al. (2013), in the first step, 100g of red ginger was weighed and homogenized using a blender, 200 mL of 100 mM potassium phosphate buffer pH 7.0 was added, containing 10 mL of ascorbic acid stabilizer + EDTA. Each homogenate was filtered through a piece of cheesecloth and the filtrate was centrifuged (10000 rpm, 4oC, 30 minutes). Then proceed with partial purification of the protease enzyme from Moringa leaves using ammonium sulfate salt with a saturation level of 60%. After the protein deposition was obtained, it was centrifuged (7000 rpm, 4oC, 10 minutes). The precipitate obtained is dialyzed using a dialysis bag. Then tested for protein and protease activity.

Protease Purification

Purification with ammonium sulfate is carried out in order to separate other components from protein. The red ginger protease fractionation process refers to Wardani and Nindita (2012) which has been modified in partial purification of red ginger proteases using ammonium sulfate to separate enzymes from suspended matter. Addition of 60% ammonium sulfate in 200 ml of crude red ginger protease extract. The addition of ammonium sulfate is accompanied by stirring with a magnetic stirrer (4°C) with the addition of ice around the container so that the enzyme is prevented from damage. Then the extract is left overnight in the refrigerator. The extract precipitate was centrifuged at 7,000 rpm (4°C, 10 minutes). The addition of 16 ml of pH 7 phosphate buffer on the enzyme precipitate.

Proximate Analysis

Before the proximate analysis was carried out on the feed, the commercial feed was added to the protease extract from red ginger with each concentration of the extract according to research by Mohammad et al. (2015) which has been modified for each feed given a ginger protease dose of A: 10 ml/kg, B: 30 ml/kg, C: 50 ml/kg, Control: 0 ml/kg. Feed that has been sprayed is air-dried until completely dry. Proximate analysis was performed on treatments A, B, C, and controls. The proximate analysis performed included protein content, fat content, nitrogen-free extract material (BETN), moisture content, and ash content (AOAC, 1995).

Amino Acid Analysis

Analysis of feed amino acids using the HPLC method. The principle of amino acid analysis is that amino acids from proteins are released through a hydrolysis process with 6N HCl. Hydrolyzate is dissolved with sodium citrate buffer and each amino acid will be separated using HPLC. Prior to the hydrolysis process, protein was extracted using the Kjeldahl method (AOAC, 2005).

Result and Discussion

Purification of red ginger

The results of red ginger protease purification with the addition of a stabilizer combination of ascorbic acid + EDTA and followed by precipitation with 60% ammonium sulfate, then the dialysis process is carried out as shown in table 1. According to Babalola et al. (2023), that ammonium sulfate can purify 3-fold protease. Compared to the crude extract the results of dialysis have a higher specific activity of 1,130 units/mg to 2,580 units/mg, Research by Nafi et al. (2013), that the addition of ascorbic acid in combination (Ascorbic acid+EDTA) in extraction is more effective in increasing the activity of the protease enzyme and maintaining its stability, whereas according to Purwanto, (2016) the aim of precipitating protein with ammonium sulfate is to reduce the water content so as to obtain the enzyme with better activity because it can reduce contaminants. Precipitation with ammonium sulfate has the principle of salting out (separation of protein by salt). Where the addition of a certain salt concentration to a certain limit can result in decreased protein solubilitythis shows that with the dialysis process, some non-protease target proteins and non-protein molecules have been adsorbed into the dialysis bag because the crude extract still contains a lot of contaminant compounds such as non-protein and other proteins that are not proteases which can interfere enzyme activity. according to Hapsari et al. (2021), that the higher the specific activity value indicates the enzyme is pure and the enzyme works more efficiently because the amount of protein (mg) is smaller. However, it shows that the reaction rate remains the same or increases, this is due to reduced interference from the enzyme inhibitor. Meanwhile, according to Seidman and Mowery (2006) states that specific activity is a unit of protease activity in each milligram of protein contained in the enzyme, which can also indicate the level of purity of an enzyme. If the specific activity is lower than expected, it is caused by the denaturation of the enzyme protein or the much higher non-enzyme protein content.

Table 1. Results of red ginger protease purification

Stage	Total Enzyme Vol(ml)	Total Enzyme Activity (U)	Total Protein Content (mg)	Specific Activity (U/mg)	Yields	purification factor	Yield %
Crude Extract	200	3.270	2.94	1.130	100	1.00	99.97
Dialysis	23	2.063	0.8	2.580	63.09	2.32	25.55

Based on the results of SDS-Page analysis, the red ginger protease sample contained 1 band at 67.1 kDa as shown in Figure 1.

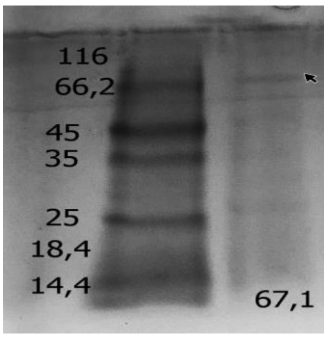

Figure 1. results of SDS-Page analysis on red ginger protease samples in band 67.1

This is in accordance with the opinion of Hou-Pin et al. (2009) the results of SDS-Page from ginger with the addition of ascorbic acid stabilizer has a high protiolytic activity of 82 kDa and 62 kDa. The result of SDS-Page is the success of the crude red ginger protease extract, the band resulting from the study shows a very thin band, according to Rachmania et al. (2017), that the SDS-PAGE band is thin due to low protein concentration so that the concentration affects the the thickness of the SDS-PAGE protein bands. This is because the thick and thin bands of the SDS-PAGE protein indicate proteins that have the same molecular weight and band position.

Proximate Composition

In this study, there were three experimental feeds with the addition of red ginger protease and control feed. Analysis of the proximate composition of fish feed has been analyzed with the results presented in table 2. The function of the proximate fish feed is to provide information about the nutritional composition of fish feed, such as protein, fat, fiber, ash, and carbohydrates content. This information is important in determining the right type and amount of feed for certain fish so that they can grow optimally and healthily. Based on the research results, it was found that the nutritional content in the feed was very different. However, the highest protein content was found in feed treated with red ginger protease of 3% (B) which was 42.69%. Furthermore, each protein content of the control treatment (K) was 41.3%,

Based on the protein content of the four feeds, it is very good for fish growth, because protein plays an important role in fish growth because it contains many amino acids, both essential and non-essential amino acids. Quality artificial feed must meet several criteria, namely the nutritional content of the feed, especially protein that is able to meet the needs of fish, the nutritional content of the feed is easily absorbed by the body, low ash content, and high efficiency (Defrizal, 2015; Gunawan, 2015). Protein is needed by fish, especially as a source of energy. Because the energy production value generated by protein is higher in fish compared to other animals, where the increase in heat due to consumption of protein is lower in fish. Besides that, most of the energy that can be digested in protein (digestible energy) can also be metabolized better in the fish's body. Fish protein is not only a source of energy, but also functions as a repair agent for damaged tissue and supports fish growth. The fish body needs this protein constantly, because the amino acids they contain are always needed, especially to replace damaged proteins during the growing season and to make new proteins during the growth and reproduction phase (Manik & Arleston, 2021).

Enzymes in the feed can reduce the crude fiber and ash content in fish feed, high crude fiber in the feed can reduce the digestibility of the feed. Crude fiber is part of carbohydrates that cannot be digested, high use of crude fiber in feed can reduce growth due to the time required for intestinal emptying and reduced digestibility (Nurfitasari et al., 2017). According to Rina and Subhan (2017), the optimal crude fiber content to support the growth of tilapia is around 4 – 8%. Crude fiber helps accelerate the excretion of leftover feed through the digestive tract, the presence of crude fiber in the feed is insufficient in the digestibility of the feed. One of the factors that can affect the digestibility of feed is the specific differences in the digestive system in fish which can cause differences in the ability of fish to digest feed (Megawati et al., 2012)

The nutritional content of feed for fish can affect fish survival. protein content of feed can influence fish growth. Protein functions to form new tissue for the growth and maintenance of the fish's body. Protein content that is too high will be absorbed and used to form damaged body cells, while the rest will be used as energy (Azzahra et al., 2023). Carnivorous fish require feed protein ranging from 40 - 55%, while the average fat content in feed ranges from 4-8% (Gunawan and Khalil, 2015). Meanwhile, Mudjiman (2004), the fat requirement for freshwater fish ranges from 4%–18%, while the minimum standard for fish feed is 3%. according to Huda and Gusmarwani, (2020) a good ash content in feed should be less than 12% because ash in feed affects fish digestibility.

Table 2. The proximate composition of the feed given

Treatment	Mark (%)				
	Water content	Ash	Proteins	Crude Fat	Coarse Fiber
K	7.99	9.05	41.30	4.69	2.25
A	24.91	9.00	42.56	4.84	1.82
B	36.39	7.96	42.69	6.17	1.69
C	30.95	8.40	39.15	5.20	1.76

Description: (K) control; (A) Feed + red ginger protease 1%; (b) Feed + red ginger protease 3%; (C) Feed + red ginger protease 5%

The proximate results of the nutritional content of the feed with the addition of enzymes are still optimal for fish, feeds added with enzymes show higher protein content compared to feeds without enzymes. according to Qiao et al., (2009) the protease content in ginger plants can improve the quality of feed processing, proteases in ginger can accelerate protein absorption because they can decompose proteins into amino acids. The addition of enzymes to fish feed can increase the utilization of feed components. Enzymes have been confirmed to increase the nutritional value of feed (Hassaan et al., 2018. Baehak et al. (2015), stated that the greater the dose of enzyme added to the feed, the greater the value of the degree of hydrolysis of the feed. Hasnaliza et al. (2010) increasing enzyme concentrations will lead to an increase in dissolved nitrogen content.

Protease in feed not only improves feed quality, however, several studies on protease added to feed have shown to improve the immune system in shrimp. Protease supplementation also increases free radical scavenging in white shrimp, Litopenaeus vannamei, and tilapia, Oreo chromis niloticus × O. aureus, the digestibility of feed protein in carp and tilapia feed increased with the addition of protease, which shows that optimally digestible feed protein can protect fish from intestinal damage (Wiszniewski et al., 2022). Protease enzymes in feed not only improve performance growth but also improves the water quality in the tank cultivation (Patil, et al., 2019). adding protease in the feed also provides an optimum limit for each fish for its digestibility and growth. research by Farrag et al. (2013), showed the growth optimal and protein efficiency in Labeo rohita seeds is achieved at a dose of 10 g of papain enzyme per kg of feed, while research Sinaga et al. (2020) added 3% enzyme to feed could increase the growth and survival of channa striata.

Amino Acid Content

The amino acid composition of each feed treatment contains essential and non-essential amino acids. Non-essential amino acids consist of 8 types of amino acids namely, L-Serin, L-Glutamate Acid, L-Alanine, L-Arginine, Glycine, L-Aspartic Acid, L-Tyrosine and L-Proline. While the essential amino acids consist of 7 types of amino acids namely, L-Phenylalani, L-Isoleucine, L-Valine, L-Lysine, L-Leucine, L-Threonine and L-Histidine. The results of the amino acid composition of each feed treatment given to snakehead fish can be seen in Table 3.

The content of non-essential amino acids with the highest average was glutamic acid (4.82% -6.03%). Glutamic acid is a feed additive that functions as a growth booster and can increase the body's protein synthesis. Glutamic acid supports the body's metabolism and acts as a substrate and mediator for the synthesis of amino acids and protein building blocks (Walker and Donk, 2016). Furthermore, the highest essential amino acid content is leucine (2.37% -2.65%). Leucine is an essential amino acid that plays an important role in the growth and development of fish. leucine supplementation in fish rations can improve growth performance and survival rates. Optimal dietary leucine levels can vary depending on the age and size of the fish. Leucine is a member of the branched chain amino acid family, has an important anabolic function that stimulates protein synthesis in fish through targeting the rapamycin signaling pathway (Lansard et al., 2010; Wei et al., 2020).

The amino acid profile in the feed given other red ginger proteases is high, namely Arginine, while the higher Lysine content occurs the higher the addition of protease to the fish feed. Lysine is one of ten essential amino acids that can be used as an amino acid reference. According to Miles & Chapman (2007), There are several reasons for choosing lysine as a reference amino acid. First, the major function of lysine in the animal body is to deposit protein tissue because its needs are not influenced by other metabolic roles. Second, depending on the fish species and type of raw material, lysine usually plays a major role in limiting amino acids because it is known that the need for lysine is much greater than for other amino acids. Mulia et al., (2021) stated that the requirement for feed amino acid content for arginine is around 1.2-2.04% and leucine 3.3-5.3%. according to the National Research Council (2011) and National Research Council (1993), fish amino acid requirements range from 1.2-2.04% arginine, leucine ranges from 0.95-1.4% and lysine ranges from 1.43-1.80%. according to Nunes et al. (2014) Lysine content requirements for fish feed range from 1.2% to 3.3% and 1.6% to 2.1% of feed for cultivated shrimp.

According to Mile et al. (2023), High protein is protein that contains all types of essential amino acids in the right proportions for growth. In this case, treatment B (3%) has optimal amino acids for fish supported by high protein in Table 2. Results of feed proximate analysis. The concentration of essential amino acids is very important in evaluating the possibility of substitution in fish feeds, because essential amino acids cannot be made by the body and must be obtained from the diet. Besides leucine, there is the amino acid lysine which plays a role in the metabolism of fish growth. Lysine is an amino acid whose bioavailability can affect fish growth and feed efficiency (Abimorad et al., 2014). In the study by Wei et al. (2020), showed that lysine and leucine in the Lys-Leu form were used more efficiently for feed utilization compared to the free amino acid form.

The balance of amino acid components in feed is the main factor influencing fish growth and health (Pratama et al., 2019). fish or shrimp that are fed feed that lacks essential amino acids show decreased growth and higher mortality rates (Rachmawati et al., 2020). Unbalanced amino acid availability and lower amino acid availability in feed can affect the digestion, absorption and, metabolism of nutrients (Alam et al., 2012).

Table 3. Results of analysis of amino acid content in the feed given

Parameter	Mark %			
	K	A	B	C
L-Serine	1.86	1.78	1.63	1.95
L-Glutamic Acid	5,19	4.82	5.05	6.03
L-Phenylalanine	1.88	1,8	1.44	2,29
L-Isoleucine	1.35	1.29	1.28	1.42
L-Valin	1,6	1.52	1.52	1.69
L-Alanine	1.52	1.48	1.48	1.71
L-Arginine	2.44	2,32	1.97	2,7
Glycine	1.56	1.48	1.39	1.6
L-Lysine	1.66	1.62	1.76	1.86
L-Aspartic Acid	2.6	2.4	2.54	3.12
L-Leucine	2.55	2.43	2.37	2.65
L-Tyrosine	0.96	0.95	0.76	1.18
L-Proline	1.63	1.57	1.57	1.74
L-Threonine	1.68	1.62	1.45	1.76
L-Histidine	1.33	1.3	1.03	1.5

Conclusion

In this study, the results of the SDS-Page analysis showed that the red ginger protease sample contained 1 band at 67.1 kDa. The results of the proximate analysis showed the highest value in feed containing 3% red ginger protease (B) with a percentage of 42.69%. The resulting amino acid content consists of 8 non-essential amino acids namely, L-serine, L-glutamic acid, L-alanine, L-arginine, glycine, L-aspartic acid, L-tyrosine and L-proline. While the essential amino acids consist of 7 types of amino acids namely, L-Phenylalani, L-

Isoleucine, L-Valine, L-Lysine, L-Leucine, L-Threonine and L-Histidine. The content of non-essential amino acids with the highest average is glutamic acid (4.82% - 6.03%) as a growth booster and able to increase the body's protein synthesis. Meanwhile, the highest essential amino acid content was leucine (2.37% -2, 65%) which plays an important role in the growth and development of fish. Based on the proximate test, the best type of feed is feed B, which is feed with the addition of 3% red ginger protease.

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