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Optimization of Glutamate Production from Lactobacillus plantarum Originating from Minangkabau Fermented Food as a Feed Supplement for Broiler

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Abstract Background and Objective: Glutamate is a non-essential amino acid and it improves the perception of the taste umami and serves as a building block of protein and physiological functions of the body. Increased use of glutamate in animal feed causes glutamate to rise globally. The aim of this study was to obtain the optimal conditions for glutamate production by Lactobacillus plantarum VM. Materials and Methods: Lactobacillus plantarum VM (L. plantarum VM) is a lactic acid bacteria originating from Minangkabau fermented foods and produces glutamate. The increased production of glutamate from Lactobacillus plantarum VM can be achieved by improving the nutrition and the growth environment of the bacteria. This study was designed in the form of a laboratory experiment protocol and was repeated 3 times. The variables measured in this study were the medium pH, temperature, incubation time, carbon source and nitrogen source. Results: The results of this study showed an optimum 5.5 pH (161.519 mg LG1), incubation time (36 h), temperature (36EC) (350.001 mg LG1), 11% glucose (566,535 mg LG1) and 0.5% peptone (680.525 mg LG1). Conclusion: Optimization of the initial pH of the media, incubation time, temperature, source C and source N can increase glutamate production. Key words: Glutamate, umami, building block, Lactobacillus plantarum, Minangkabau, fermentation food Received: March 06, 2018 Accepted: May 14, 2018 Published: June 15, 2018 Citation: Vabera Maslami, Yetti Marlida, Mirnawati, Jamsari and Yuliaty Shafan Nur, 2018. Optimization of glutamate production from Lactobacillus plantarum originating from minangkabau fermented food as a feed supplement for broiler. Pak. J. Nutr., 17: 336-343. Corresponding Author: Yetti Marlida, Department of Animal Science, Andalas University, West Sumatra, Indonesia Competing Interest: The authors have declared that no competing interest exists. Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION The use of amino acids as a supplement in animal feed is practiced extensively. One of the amino acids that is added to animal feed is glutamate. Glutamate is a non-essential amino acid, it enhances the perception of the taste umami and it is a building block for proteins1,2. Apart from its role as a flavor enhancer and a building block for proteins, glutamate acts as a neurotransmitter in the brain and has a number of physiological functions3. Glutamate as a dietary supplement has been widely used in broiler diet. The addition of glutamate in broiler chicken rations can increase body weight and decrease mortality, crude protein ration and fossil ammonia4-6. In addition to improve performance, glutamate can also improve carcass quality. According to Berres et al.7 and Fujimura et al.8, adding glutamate can lower abdominal fat, reduce bruising in carcasses and increase the perception of the taste umami in meat. Increased use of glutamate as a feed supplement for livestock causes an increased glutamate demand globally. According to or Sano9, the total world glutamate production with fermentation is estimated at 2 million tons/year. Glutamate is an amino acid that has dominated demand and was valued at over 8 billion USD in 201410. Therefore, it is necessary to innovate to increase glutamate production. Innovations to increase glutamate production can be done with new organisms, increasing the nutrient content of media and altering the growth environment11,12. According to Niaz et al.13 and Zareian et al.14, temperature, pH and incubation time can all affect glutamate production. Glutamate is excreted by many bacteria in response to different nutritional conditions and production

of glutamate can then be influenced by the concentration of C and N in the culture media. Nadeem et al.15 suggests that the optimization of C and N sources can increase glutamate fermentation using various sources of carbon and nitrogen in the production media. One of the bacteria that can produce glutamate is Lactobacillus plantarum VM. Lactobacillus plantarum

VM is a lactic-acid bacteria (LAB) isolated from Minangkabau fermented food. To increase the production of glutamate, it is necessary to optimize the production of Lactobacillus plantarum VM. The purpose of this study was to obtain optimal glutamate production by improving the nutrient content of the medium and growth environment of Lactobacillus plantarum VM. MATERIALS AND METHODS Time and place of study: The study was conducted from 1st August

to 1st November, 2017. The study was implemented at the Industry Feed Technology Laboratory, Faculty of

Animal Husbandry, University Andalas. Experimental design: The research was designed in the form of a laboratory experiment descriptive protocol and repeated 3 times. Microbes and media content: This study was conducted using Lactobacillus plantarum VM bacteria isolated from Minangkabau fermented foods. The Lactobacillus plantarum VM sample was a collection of bacteria from previous studies. Lactobacillus plantarum VM was cultured on MRS broth media. Optimizing glutamate production: The glutamate production with minerals media contained (g L G1): 1 g KH2PO4, 0.4

g MgSO4A7H2O, 0.01 g FeSO4A7H2O and 0.01 g MnSO4A4-5H2O16. The first optimization of glutamate production was testing the pH of the media. To decrease the pH, 2 M NaOH was used and it was raised using 2 M HCl to pH values of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7. Second, the optimum temperature was determined (30, 33, 36, 39, 42 and 45EC). Third, the total incubation time was optimized (12, 24, 36, 48, 60, 72, 96 and 108 h). Fourth, the optimal carbon source and dose were determined. Carbon sources used were glucose, sucrose, maltose and lactose. After the best carbon source was determined, the optimum concentration of that carbon source was determined (1, 3, 5, 7, 9, 11, 13 and 15%). Fifth, the optimal sources and concentration of nitrogen were determined (peptone, yeast extract, skim milk, NH4NO3 and KNO2). After obtaining the best nitrogen source, a determination of the optimum dosage of nitrogen source (0.1, 0.3, 0.5, 0.7, 0.9, 1 1.1, 1.3 and 1.5%) was performed. Determination of glutamate content: Quantitative measurements of

glutamate were performed using HPLC analysis using the method described by Yang et al.17. The mobile

phase comprised a mixture of 60% solution A (aqueous solution of 10.254 g of sodium acetate, 0.5 mL tri-

ethylamine and 0.7 mL of acetic acid in 1000 mL, with a final pH of 5.8), 12% solution B (acetonitrile)

and 28% solution C (aquabides). A gradient HPLC separation was performed on a Shimadzu (Kyoto, Japan)

LC 20AT apparatus. A Prevail C18 column (250× 4.6 mm I.D., particle size 5 µm LG1, Alltech,

IL, USA) was used during the analysis. The mobile phase from the gradient elution was pumped

at a 0.6 Ml minG1 flow rate at 27EC and glutamic acid detection was performed at 254 nm. 180 160 140 Glutamate (mg LG1) 120 100 80 60 40 20 0 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 pH medium Fig. 1: Effect of pH medium on glutamate production 300 250 Glutamate (mg LG1) 200 150 100 50 0 12 24 36 42 60 72 84 Incubation time

(h) Fig. 2: Effect of incubation time on glutamate production RESULTS AND DISCUSSION The medium pH is an important factor for Lactobacillus growth in biological processes and glutamate production20,21. Optimum pH medium: Optimization of the production of glutamate from growing Lactobacillus plantarum VM isolates at different pH values (pH 2-7) can be seen in Fig. 1. The results show that at pH values from 2-7, the isolates could still grow, as is indicated by the turbidity formed in the media and glutamate production. The optimum pH for growth of Lactobacillus plantarum VM in this study was acidic (pH 5). According to Zareian et al.14, an acidic pH can trigger the gdh gene of LAB to produce higher glutamate. The pH of the growth media of Lactobacillus plantarum VM, however is different compared to other studies. As Zareian et al.18 reported the optimum pH for the growth of LAB for glutamate production is pH 4.5. Furthermore, pH 6 has been reported to be the optimum condition of Lactobacillus plantarum to produce glutamate19. Decreases in the initial pH of the media may inhibit the growth of Lactobacillus, thereby causing a redirection of 2-Oxoglutarate efflux to glutamate production that also increases glutamate excretion19,22. In addition to growth factors,

glutamate production increases due to Lactobacillus plantarum production of ammonia in an acidic environment, as

this contributes to the pH of homeostasis to allow the survival of microorganisms through

neutralizing pH23. Thus, the ammonia produced can be used to form glutamate. Incubation time: The incubation time for Lactobacillus plantarum VM can be seen in Fig. 2. The best incubation time was 36 h, with a production 260,551 mg LG1. The optimal incubation time in this study differs from several other studies. Lawal et al.24 reported the optimum incubation time using 400 350 300 Glutamate (mg LG1) 250 200 150 100 50 0 30 33 36 39 42 45 Temperature (EC) Fig. 3: Effect of temperature on glutamate production Bacillus spp. as 96 h. According to Zaraien et al.18, the best incubation time of Lactobacillus plantarum was 96 h for glutamate production at 1.032 mmol. Furthermore, Ahmad et al.25 reported 100 h as the best incubation time for producing glutamate. The optimum time difference of bacterial growth in producing glutamate is influenced by the growth rate of Lactobacillus cells. According to Nampoothiri et al.26, the growth of

Lactobacillus cells increases exponentially between 18 and 72 h of fermentation in MRS broth media. This stage is

recognized as the log phase or exponential phase. The exponential growth phase has a certain time limit. This is because the nutrients in the media will decrease and the nutrients used for bacterial growth will affect the production of glutamate produced18. In addition, differences in bacterial strains and media nutrition are another factor responsible for differences in outcomes14. Optimum temperature: To determine the optimum temperature of Lactobacillus plantarum VM in producing glutamate, incubation at a range of temperatures (30, 33, 36, 39, 42 and 43EC) was performed. The effect of

temperature on glutamate production can be seen in Fig. 3. The results showed that the optimum temperature of Lactobacillus plantarum VM in producing glutamate was 36EC (366.76 mg LG1). There was a difference in the production of glutamate with temperature shifts that can be seen in Fig. 2. The optimum temperature obtained in this study is different from that reported by other studies. According to Ahmet et al.26, 31EC is the optimum temperature of Corynebacterium glutamicum for producing glutamate. Furthermore, Zareian et at.14 and Lawal et al.24 stated that the optimum temperature of Lactobacillus plantarum in producing glutamate is 37 and 32EC is the optimum temperature for Bacillus spp. The occurrence of differences in glutamate production at each temperature change caused every microbe to have an optimum, maximum and minimum temperature for growth. If the environmental temperature is less than the minimum temperature or greater than the maximum temperature for growth, then enzyme activity can stop and at too high temperature, enzyme denaturation will occur27. According to Lehniger et al.28, lower temperatures decrease the fluidity of cell membranes by

increasing the fraction of fatty acids containing shorter carbon chains and cis-double bonds. This

change in lipid composition increases the fluidity of the lipid double layer in the cell membrane and

will increase the transport of metabolites and nutrients to maintain the same level as normal growth temperatures. Therefore, as an increase in membrane fluidity, is achieved by increasing the temperature of cultivation, the production of glutamate will increase11. This is the same as is conveyed by Uy et al.29 increasing the temperature of cultivation can inhibit the dehydrogenase a-ketoglutarate complex. Thus, it can cause the transfer of 2-Oxoglutarate

flux to glutamate production and thus increase the excretion of glutamate22. Carbon source optimization: Determination of the best carbon source was then performed, comparing different carbon sources to produce

glutamate. The carbon sources used in this study were glucose, lactose, sucrose and maltose. Once the highest carbon source to produce glutamate was selected, then a determination of the optimum concentration was performed (1, 3, 5, 7, 9, 11, 13 and 15%). The effect of carbon source and optimum concentration can be seen in Fig. 4-5. The results showed that the best carbon source for glutamate production was glucose at 351.14 mg LG1. However, this is not much different when compared with sucrose, which produced 150 mg LG1 of glutamate. The difference in 360 350 Glutamate (mg LG1) 340 330 320 310 300 Glucose Lactose Sucrose Maltose Carbon sucrose Fig. 4: Effect of carbon sources on glutamate production 700 600 Glutamate (mg LG1) 500 400 300 200 100 0 1 3 5 7 9 11 13 15 Carbon concentration (%) Fig. 5: Effect of carbon concentration on glutamate production glutamate production results from the ability of different microbes to deregulate different carbon sources. According to Nadeem et al.16, each microbe has a different metabolism in the degradation of carbon sources that affects the formation of biomass and the production of primary or secondary metabolites. Research on the production of glutamate from carbon sources has been reported, suggesting that

glucose is the best source of carbon for producing glutamate14.24. High glutamate production is obtained because the bacterium has changed glucose through the use of Krebs cycle intermediates30. Glucose can

be changed to pyruvic acid through glycolysis, the TCA cycle and the electron transport chain31. However, different results were reported by Kiefer et al.32, where fructose and sucrose were the best carbon

sources for producing glutamate. Thus, the production of glutamate from microbes can be influenced by available carbon sources. Glucose is the best source of carbon in producing glutamate and author then sought to determine

the optimal glucose concentration. The effect of glucose concentration on glutamate production can be seen in Fig. 7. The results showed that the optimum glucose concentration was 11%, which was higher than in other studies. The best glucose concentrations for glutamate production obtained in this study were lower than that of Zareian et al.18, who reported a concentration of 12% of a carbon source. The high production of glutamate at 11% concentration is due to microbial growth itself. This was also stated by Nampoothiri and Pandey26 that the utilization of sugars in the media by bacteria will continue in accordance with the growth pattern of the bacteria. Optimization of nitrogen source: Determination of the best nitrogen source was made by comparing the sources of nitrogen (peptone, yeast extract, skim milk, NH4NO3 and KNO3) for glutamate production. After obtaining the best nitrogen source, it was then determined the optimum concentration of nitrogen. The nitrogen source and the optimum concentration of nitrogen can be seen in Fig. 6-7. The results showed that the best source of nitrogen for producing glutamate was peptone, with a production of 500 450 400 Glutamate (mg LG1) 350 300 250 200 150 100 50 0 P e pton extract NO3 NO3 k i m m ilk NH4 K Y e ast S Nitrogen source Fig. 6: Effect of nitrogen source on glutamate production 800 700 600 Glutamate (mg LG1) 500 400 300 200 100 0 0.1 0.3 0.5 0.7 0.9 1.1 1.3 Nitrogen concentration (%) Fig. 7: Effect of nitrogen concentration on glutamate production 415.23 mg

LG1. In this study, the best results obtained differed from those reported by Li et al. 33 and Nadeem

et al. 15, where ammonium sulfate was the best source of nitrogen to produce glutamate. According to Savijoki et al. 34, the determination of suitable nitrogen sources is essential for the needs of amino acids and microbial peptides in producing glutamate. The ability of microbes in synthesizing nitrogen sources has an effect on the production of glutamate expression34. Furthermore, each LAB strain has different needs on each nitrogen source and nitrogen is an

essential growth factor or stimulating factor35,36. After obtaining results indicating peptone as the best source of nitrogen, then the optimum concentration of peptone was determined, which can be seen in Fig. 7. The results showed that the best peptone concentration was 0.9%. Nitrogen plays an important role in the fermentation of glutamate -producing bacteria. Nitrogen is taken up by bacterial cells and then assimilated to achieve metabolism37. The uptake of nitrogen sources into bacterial cells occurs through passive diffusion (ammonium) or active transport38. The ammonia concentration is low so that as diffusion into the cells becomes limited, the ammonium transporter (AmtB) activates to overcome this nitrogen deficiency and the nitrogen is assimilated with glutamine synthetase39. Conversely, in high nitrogen concentrations, nitrogen diffusion (NH3) occurs across cytoplasmic membranes. This encourages the growth of bacterial cells and the nitrogen is assimilated by glutamate dehydrogenase to form glutamate. De Angelis et al.40 also showed that most of the nitrogen is assimilated by glutamate dehydrogenase to form glutamate, which has been shown to exhibit high activity in Lactobacillus plantarum. The implications of this study are that glutamate production from Lactobacillus plantarum originating from Minangkabau fermented food can be increased. Optimizing glutamate production by determining the optimum pH, temperature, incubation time and carbon and nitrogen sources can all increase glutamate production. It is recommended to use more efficient carbon and nitrogen sources to further increase glutamate production and reduce production costs. CONCLUSION An increase in glutamate production was obtained using the optimized Lactobacillus plantarum VM. Optimization as well as the adjustment of nutrients and environmental conditions affects glutamate fermentation, resulting in the increased production of glutamate. The media pH, incubation time, temperature, C source and N source all impacted glutamate production. SIGNIFICANCE STATEMENT This study determined optimal conditions for glutamate production from Lactobacillus plantarum originating from Minangkabau fermented food. This study helps researchers to increase the production of glutamate by defining the optimal media, temperature, incubation time, carbon source and nitrogen source, which has not been explored by many other researchers. Thus, a new theory and new lactic-acid bacteria isolated from Minangkabau fermented foods can be used as a feed supplement for poultry, such as broiler chickens, layer or any monogastric animal, leading to increases in the quality immune systems of carcasses. ACKNOWLEDGMENT This study was funded by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through PMDSU No: 1387/E4./2015. We are very grateful to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia and the Rector of Universitas Andalas for their support.

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