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Research Article

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Improved Quality of Lamtoro Leaf Meal Fermented *Bacillus laterosporus* with the Addition of Supplement Methionin-lysine Synthetic

Nita Yessirita[1], Tinda Afriani[2], Sunadi[3]

[1]Faculty of Agriculture Ekasakti University, Veteran Dalam Street No 26 B Padang 25116, Indonesia
[2]Faculty of Animal Husbandry, Andalas University, Campus Unand Limau Manis Padang 25163, Indonesia
[3]Faculty of Agriculture, Tamansiswa University, Tamansiswa Street No. 9 Padang 25138, Indonesia

Abstract This study aimed to determine the level of provision of optimal amino acid methionine-lysine in fermentation leucaena leaf meal (LLM) with *Bacillus laterosporus* for the preparation of ration ducks. This study used a completely randomized design with three replications. Each treatment consists of A = LLM fermentation with *Bacillus laterosporus*+0% supplement amino acid methionine-lysine (control), B = A+0.25% methionine+0.50% lysine, C = A+0.30% methionine+0.75% lysine, D = A+0.35% methionine+1.00% lysine and E = A+0.40% methionine+1.50% lysine. Parameters measured were amino acids methionine and lysine after methionine-lysine supplementation. The result indicated that the content of the amino acid methionine for the addition of amino acid supplements methionine-lysine was not significant (P>0.5) but significant (P<0.05) the content of the amino acid lysine. It can be concluded that supplementation of amino acid methionine 0.40%+1.50% lysine to LLM fermentation with *Bacillus laterosporus* gives the best results.

Keywords supplementation, amino acid, fermentation, *Bacillus laterosporus*

Introduction

The content of nutrients produced in the fermentation process, especially protein increases, it is caused by bacteria / microorganism during fermentation containing single cell protein and also produce enzymes that count as protein. On the other hand the product of fermentation produces the nucleic acid that is non protein nitrogen (NPN) which is not a protein so that the use in poultry should be tested to determine whether the amino acids of proteins containing amino acids are good [1]. By products of fermentation is a nucleic acid which is a protein that contains NPN where proteins are not utilized optimally in poultry because it does not have the enzyme ribonuclease and will be discarded along with the feces, so that the resulting protein is not a protein-containing amino acids are complete. Then added [2] that Leucaena deficiency in amino acids methionine and lysine, to be used optimally need additional methionine amino acid lysine synthesis.

A protein quality feed ingredient, among others, determined by the completeness and balance of essential amino acids contained therein. High-quality proteins usually contain essential amino acids complete, the numbers are sufficient and balanced. The preparation of poultry rations is now the focus of attention is no longer on the amount of protein that must be provided, but more attention to the balance between the energy of the essential amino acids, because the essential amino acids can not be synthesized in the body [3], so it needs to be supplied in the ration is consumed by the addition of amino acid synthesis.

Amino acids are the fundamental building blocks of protein. About 22 different amino acids contained in the proteins of the body. All that is not to be available in poultry rations and can be synthesized in the body. but the amino acids are the following: lysine, arginine, histidine, leucine, isoleucine, valine, methionine, threonine, phenylalanine tryptophan and its presence in the diet is absolutely necessary because the duck can not synthesize in the body, it is classified as ten amino acid essential [4].

Methionine is one of the essential amino acids, therefore must be provided in the diet in sufficient quantities, in addition to the amino acid methionine is a major barrier in chicken rations [5]. Furthermore, [6] suggests that the methionine is a substance that is essential for poultry, which is in line with the statement [7] and [8], that the establishment of the breast meat in broilers is very sensitive influenced by methionine in the rations.

Lysine which has many uses in the body is an amino acid that can not be synthesized by the body of the chicken, so classified in essential amino acids are critical for very low levels in the feed. Due to the lack of essential amino acids in feed ingredients, then chicken rations need to be supplemented with synthetic lysine amino acid in accordance with the needs of the poultry [9].

Furthermore, in preparing the feed of poultry is highly considered amino acids methionine-lysine, because amino acids are essential amino acids and are also called amino acids barrier that must be brought in from outside because the poultry can not produce it themselves, an amino acid that is not derived from plant materials but from the animal. Methionine is an amino acid deficiency-lysine of vegetable can also be met by the addition of methionine-lysine synthesis. Thus will lysine methionine amino acid supplementation on quality of leucaena leaf meal fermented with *Bacillus laterosporus* needs to be done. It is expected to help farmers reduce the use of fish meal and soybean meal in poultry rations.

Material and Methods

The study was conducted at the Integreted Laboratory of Kopertis Region X Padang and the Integrated Laboratory of IPB Bogor and the Center For Post Harvest Development in Bogor, Year of 2016. The studies include the enrichment of the bacteria to gain rejuvenation and manufacture of inoculum fermentation bacteria (*Bacillus laterosporus*) as well as the manufacture of products leucaena leaf meal (LLM) fermented with *Bacillus laterosporus* without the addition of amino acid supplements-lysine and methionine plus supplemental amino acids methionine-lysine by using a Complete Randomized Design (CRD), 5 treatments with 3 replications. Parameters measured were amino acids methionine, lysine without and after supplementation of amino acids lysine and methionine and also *betacarotene* content.

The composition of the treatment is:

A = LLM Ferm *Bac. laterosporus* without supplement met-lysine (as control)
B = LLM Ferm *Bac. laterosporus* + 0,25% and 0,50% supplement met-lysine
C = LLM Ferm *Bac. laterosporus* + 0,30% and 0,75% supplement met-lysine
D = LLM Ferm *Bac. laterosporus* + 0,35% and 1,00% supplement met-lysine
E = LLM Ferm *Bac. laterosporus* + 0,40% and 1,25% supplement met-lysine

Results of analysis of variance included in the table to determine the effect of treatment. Test of DMNRT at the level of 5% was used to compare between treatments. Data was analyzed according to the procedures of [10].

Leucaena leaf meal fermentation process with *Bacillus laterosporus,* according to the following procedure:

1. Substrate preparation leucaena leaf Leucaena. Leucaena used in this study, is a local leucaena that have a high 2-5 m, located was obtained around the City of Padang, West Sumatera, the leaves are taken, then the oven temperature of 60^0C for 24 hours, then milled to be used as LLM.
2. Preparation of inoculums. Making inoculum using substrates bran 100 g plus 60 ml of distilled water in the autoclave for 30 minutes at a temperature of 120^oC, 1 atm and then cooled to a temperature of about 37 °C. Taken tube containing *isolate*, add 20 ml of distilled water, then crushed loopful. Furthermore then in vortex so that a homogeneous solution, mix into a plastic isolate containing bran, stirring until evenly distributed and closed then a hole to keep the aeration. Incubated for 24 hours at a temperature of 37^0C.
3. Procedure of fermentation. Dry substrate LLM weighed with a weight of 1 kg. Added 800 ml of distilled water. Then autoclaved for 30 minutes at a temperature of 121^0C, 1 atm. After that inoculated with *Bacillus laterosporus* as much as 6% of the amount of substrate, then incubated for 24 hours [11], so the fermented product is dried at 60^0C for 24 hours. The dried product ready for use. The process of making meal products leucaena leaf meal are fermented *Bacillus laterosporus* for amino acids and *betacarotene* analysis, the best results are used for further research.

The process of making leucaena leaf meal of product fermented with *Bacillus laterosporus* which can be seen in Figure 1.

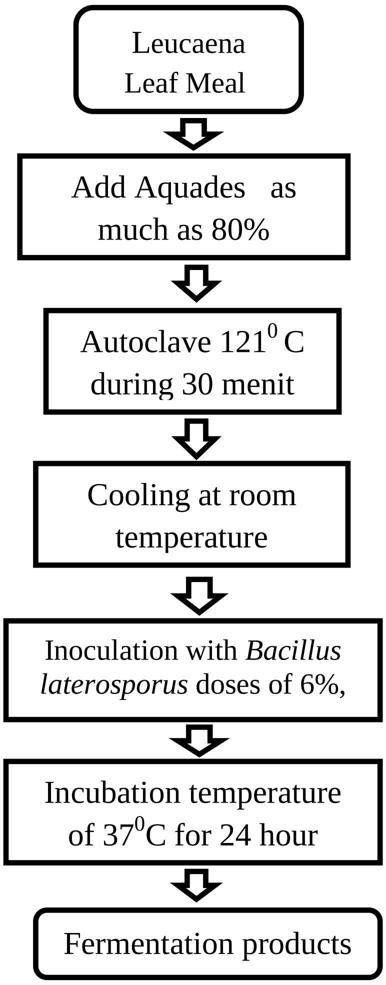

Figure 1: Making procedures LLM fermentation with Bacillus laterosporus (modified from [12]).

Targeted Results

1. Getting the best level LLM fermented with *Bacillus laterosporus* with a dose of 6% and fermentation time 24 hours, the best before and after supplementation be given lysine amino acid methionine.
2. Getting the analysis of amino acids methionine and lysine fermentation leucaena leaf meal best products before and after the supplements of amino acids methionine-lysine.

Results and Discussion

Effect of Amino Acid Supplementation on Methionine-lysine content of Methionine Amino Acid Fermentation Products

Methionine amino acid content of LLM fermentation products methionine-lysine supplements given during the study are:

Table 1: Mean content of amino acids methionine LLM fermentation given supplements methionine-lysine for research.

Treatment	The Content of Metionin (%)
A = LLM ferm without treatment	0,183^{a}
B = LLM ferm *Bac. laterosporus* + 0.25% methionine + 0.50% lysine	0,200^{a}
C = LLM ferm *Bac. laterosporus* + 0.30% methionine + 0.75% lysine	0,207^{a}
D = LLM ferm *Bac. laterosporus* + 0.35% methionine + 1.00% lysine	0,208^{a}
E = LLM ferm *Bac. laterosporus* + 0.40% methionine + 1.25% lysine	0,300^{a}

The numbers followed by the same letter are not significantly different according to the test of DNMRT on a real level 5%

Results of variance showed that the level of supplementation of amino acids Methionine-lysine to methionine amino acid content of LLM fermentation products provide no significant effect (P> 0.05).

The results in Table 1 showed that the level of supplementation with the amino acid methionine-lysine to the amino acids methionine fermented leucaena leaf meal (LLM) showed that the treatment E (Extra supplement amino acids 0.40% methionine + 1.25% lysine) increased content the amino acid methionine highest compared with other treatments is 0.30% and the value is better than the results of the control that is 0.183%.

Described by the [13-15] that methionine is an amino acid superior to other amino acids in the increased weight of the eggs, as an amino acid synthetic in the form of a mixture of DL-methionine acts as a donor methyl, utilization in the form of isomer 100%, so it plays a role in helping other metabolism in the body such as metabolism choline, protein and carbohydrates. Synthetic amino acids should be used to meet the needs of limiting amino acids useful for the reduction of amino acids as part of the feed protein [16]. Added [17] required the addition of amino acids methionine 0.1%-0.2% in the ration to increase egg weight and high usage efficiency ration. According to [18] Supplementation of L-methionine and L-Linin amino acids have an effect on the decrease of abdominal fat deposits in broilers, so that the duration of spawning time becomes longer.

Effect of amino acid supplementation on methionine-lysine content of lysine amino acid fermentation products.

Lysine amino acid content of leucaena leaf meal fermentation products methionine-lysine supplements given during the study are:

Table 2: Mean content of amino acids lysine LLM fermentation given supplements methionine-lysine for research

Treatment	**The Content of Lysine (%)**
A = LLM ferm without treatment	0.873^{a}
B = LLM ferm *Bac. laterosporus* + 0.25% methionine + 0.50% lysine	0.993^{ab}
C = LLM ferm *Bac. laterosporus* + 0.30% methionine + 0.75% lysine	1.193^{ab}
D = LLM ferm *Bac. laterosporus* + 0.35% methionine + 1.00% lysine	1.063^{ab}
E = LLM ferm *Bac. laterosporus* + 0.40% methionine + 1.25% lysine	1.353^{b}

The numbers followed by the same letter are not significantly different according to the test of DNMRT on a real level 5%

Results of variance showed that the level of supplementation of amino acids methionine-lysine to the amino acids lysine of LLM fermentation products provide a significantly different effect (P <0.05).

The results in Table 2 showed that the level of supplementation with the amino acid methionine-lysine to the amino acids lysine fermented of LLM showed that the treatment E (Extra supplement amino acids 0.40% methionine + 1.25% lysine) increased content the amino acid lysine highest compared with other treatments is 1.353% and the value is better than the results of the control that is 0.873%.

Described by [19] and [9] that lysine which have many uses in the body is an amino acid that can not be synthesized by the body of the chicken, so classified in essential amino acids essential for very low levels in the feed. Lysine produce energy inhibits the formation of fat. Due to the lack of essential amino acids in feed ingredients, then chicken rations need to be supplemented with synthetic lysine amino acid in accordance with the needs of livestock. Furthermore, [20] states that the supplementation of the amino acid methionine (0.47%) and lysine (1.1%) with a protein content of 15% can increase the performance of crossbred Mojosari-Alabio ducks.

Effect of amino acid supplementation on methionine-lysine content of betacarotene fermentation products.

Betacarotene content of leucaena leaf meal fermentation products with the Methionine-lysine can be seen in bar chart on Figure 2.

Betacarotene is a carotenoid group unstable and easily oxygenated become *xanthophyl* and *xanthophyl* must come from outside because the poultry are not able to synthesize [21-22]. Obtained from the treatment accorded treatment supplementation of amino acids methionine 0.40% + 1.25% lysine gives the best results *betacarotene* namely 68, 49%.

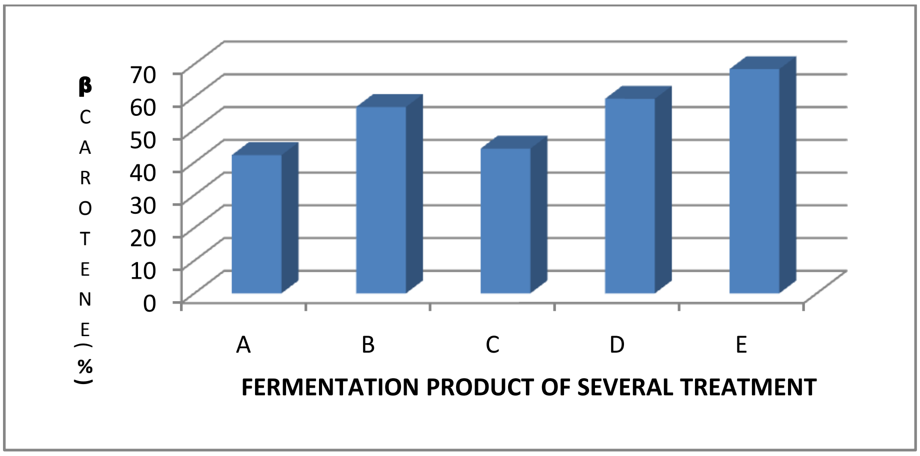

Figure 2: Betacarotene content of fermentation product in several treatments

Conclusion

The research results can be concluded that treatment of fermentation obtained leucaena leaf meal (LLM) with supplementation of amino acids methionine and lysine to the level of 0.40% methionine and 1.25% lysine are best able to improve the content of 24.22% methionine and 21.56% lysine compared with no treatment (control).

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