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Research Article

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Improvement Crude Fiber Digestibility, N Retention and Energy Metabolism of Broiler through Fermentation LLM and Methionine-Lysine Supplementation

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Abstract *Leucaena* is a tropical legume plants that can be used as animal feed ingredients because it has good nutritional value but a deficiency of the amino acid lysine methionine, for that it must be imported from outside because poultry cannot produce it. This study was conducted to determine the crude fiber digestibility, Nitrogen retention and energy metabolism of broiler that were given *Leucaena* leaf meal (LLM) ration with some treatments. Research used 30 of broiler chicken 6-8 weeks of age. The experiment was designed by a completely randomized design, which consist of 3 treatments and 8 replications. The treatment were: R0= based ration + 7.5% LLM unfermented, R1= based ration + 7.5% LLM fermented by *Bacillus laterosporus* and R^2= based ration + 7.5% LLM fermented by *Bacillus laterosporus* with suplementation 0.40% methyonine 1.25% lysine. Data were analyzed by analysis of variance and continued by Duncan New Multiple Range Test. Parameters observed were crude fiber digestibility, Nitrogen retention and energy metabolism. The results showed that treatment highly significant ($P < 0.01$) on crude fiber digestibility, Nitrogen retention and energy metabolism of broiler chicken. Based on this study it can be concluded that LLM fermented by *Bacillus laterosporus* with suplementation 0.40% methyonine 1.25% lysine in ration can be used as one of alternative feed ingredients in the ration of broiler chickens seen from the increasing value crude fiber digestibility (57.66%), Nitrogen retention (68.35%) and while viewed from energy metabolism (301.49 kcal/kg) of broiler chickens.

Keywords legume, amino acid, treatment, fermented

Introduction

Poultry such as broilers are the right choice in meeting the needs for quick protein supplements. In maintaining poultry, it is important to consider the nutritional needs related to feed costs which play an important role because they affect 70% of the production costs. To reduce the cost of the feed, alternatives are sought by utilizing local materials such as waste and forage, which are cheap and widely available that if properly processed can be used as the high quality broilers feed e.g. LLM.

Leucaena leaf meal according to [1] is a proximate composition of 88.2% of dry LLM, 21.8% crude protein, 15.1% crude fiber, 3.1% ash, 8.6% fat, and 50.7% BETN. [2] reported the results of the research from several researchers stated that *Leucaena* is an important source for feed ingredients because it contains lots of protein, essential amino acids, minerals, carotenoids and vitamins. Even though it also contains mimosine and is toxic that is often mentioned as the barrier in its intensive use.

The fermentation of *Leucaene* leaf meal with *Bacillus laterosporus* is done by using bacteria derived from the digestive tract of Pitalah ducks as a fermentation inoculum can detoxify *Leucane* leaf mimosine and reduce

Mimosine for 64.89% (from 2.62% down to 0.92%) and to increase Beta-carotene for 96.91% (from 972.75 ppm to 1915.48 ppm) [3].

The problem is that the fermentation by products contain nucleic acids, where proteins are not utilized maximally in poultry because they do not have the Ribonuclease enzyme and will be wasted with feces which means that the resulting protein is not a protein that contains complete amino acids [4]. Furthermore [5] added that *Leucaena* deficiency of the amino acids methionine and lysine that must be added manually because the poultry cannot produce it themselves. It means that it is necessary to supplement the synthetic amino acid of methionine lysine to overcome the problem [6]. The amino acids lysine and methionine, are amino acids that need to be considered in the preparation of rations because lysine is the main limiting amino acid of poultry followed by methionine as the second limiting amino acid, which is an essential amino acid in poultry feed [7]. The fermentation of LLM supplementation of 0.40% methionine and 1.25% lysine can increase the crude protein by 6.88%, decrease crude fiber by 39.30%, and the quality of beta-carotene is 68.49% better than the fermentation products without methionine-lysine supplementation [8].

Nitrogen retention and energy metabolim is one method for assessing protein quality and the energy of the rations. According [9] nitrogen retention for each type of livestock, age, and genetic factors is different. The amount of nitrogen retained in the animal's body will cause the excreta to contain less nitrogen and energy compared to cattle that do not retain nitrogen. The level of nitrogen retention depends on the consumption of nitrogen and the energy metabolism of the ration, but an increase in the metabolic energy of the ration is not always followed by an increase in nitrogen retention [10].

The measurement of digestibility or digestibility of a feed ingredient is an attempt to determine the amount of nutrients from a feed material that is degraded and absorbed in the digestive tract. Digestion is also a presentation of nutrients absorbed in the digestive tract whose results can be known by looking at the difference between the amount of nutrients eaten and the amount of nutrients released through feces. Nutrients that are not present in feces are assumed to be digested and absorbed values [11].

Energy metabolism according to [12] is the difference between the gross energy content of feed ingredients or rations and the gross energy released through excreta. In preparing rations for poultry in addition to nutrient content such as carbohydrates, fats, proteins, vitamins and minerals. In addition, the energy content also needs to be considered given the level of ration energy determines the amount of feed consumed [10]. Energy needs are standard in the preparation of livestock rations, so knowledge of quantitative energy content is very important [13]. [14] states that energy sources of nutrition are carbohydrates, fats, and proteins, To find out the quality of the ration given to broilers, it can be seen its nitorgen retention, digestibility of crude fiber and its energy metabolism in the chicken.

Materials and Methods

This research was conducted in an experimental cage in Lolong village, Padang city. The methods of determining nutritional quality, treatment of metabolic energy, and nitrogen retention used in this research is the method of [15] modified by [16]. Analysis of N retention and energy metabolism was conducted in the non-Ruminant Nutrition Laboratory and technology and feed industry Laboratory of the Faculty of Animal Husbandry, Andalas University, Padang. This research was conducted on 30 Cobb strain broilers, 6-8 weeks old, body weight ± 1600 grams. The cages used are 30 units of battery cages size 20x30x30 cm, equipped with food and beverage containers. Equipment used are digital O'Haus scales (for weighing broilers, rations and excreta), excreta storage containers, spray tubes, syringes to bend treatment rations to broilers, aluminum foil, stirrers, and ovens to dry the excreta. The chemicals used are 1 liter aquades and H_2SO_4 0.3 N. feed used is, grits, bran, soybean meal, fish meal, premix A, LLM: unfermented, fermented with *Bacillus laterosporus* and, fermented with *Bacillus laterosporus* plus 0.40% methionine with 1.25% lysine supplementation. The composition of food substances and the metabolic energy of food ingredients making up the ration can be seen in Table 1. This study is an experimental study using a completely randomized design (CRD) [17] consisting of 3 treatments and 8 replications, with the following arrangement:

R0 = basal ration + LLM is unfermented

R1 = basal ration + LLM fermented with *Bacillus laterosporus*

R2 = basal ration + LLM fermented with *Bacillus laterosporus* supplemented with 0.40% methionine and 1.25% lysine

In this study, broilers were placed in battery cages individually and given treatment feed each 10% of body weight (BW). Excreta shelter is done after broiler chicken is kept for 24 hours. Every excreta is sprayed with H_2SO_4 every 3 hours, so that the nitrogen does not evaporate. Chickens are water ad libitum. Then the excreta is dried and ground and weighed for analysis. The parameters measured are a) its digestibility of crude fiber, b) Nitrogen retention and b) energy metabolism.

The composition of foodstuffs and substances and energy metabolism ration experiments can be seen in Table 1.

Table 1: The Composition of Foodstuffs and Substances and Energy Metabolism of Experimental Rations

Feed ingredients	**Treatment Ration (%)**		
	R0	**R1**	**R2**
Milled corn	48.50	49.50	48.50
Rice bran	21.00	21.00	21.00
Soybean Meal	10.00	10.00	10.00
Fish Meal	11.00	11.00	11.00
LLMF + methionine + lysine	0.00	0.00	7.50
LLMunfermentation	7.50	0.00	0.00
LLMF	0.00	7.50	0.00
Premix A	1.50	1.50	1.50
Palm Oil	0.50	0.50	0.50
Total	100	100	100
Nutrient Content			
Crude protein (%)	20.02	20.21	21.19
Fat (%)	6.45	6.34	6.52
Crude Fiber (%)	5.43	5.23	5.14
Ca (%)	0.89	0.76	0.54
P (%)	0.69	0.53	0.67
Methionine (%)	0.4539	0.4531	0.4576
Lysine (%)	0.8165	0.8770	0.9637
EM (kcal/kg)	2987.45	3056.13	3115.23

Index : a = Laboratory Analysis. Non Ruminant Nutrition and Laboratory Analysis Technology and Feed Industry (2019)

b = Integrated Laboratory analysis IPB (1997) and Wahju[10]

R0 = Basal ration + LLMunfermentation.

R1 = Basal Ration + LLM Fermentation

R2 = Basal Ration + LLM Fermentation + 0.40% Methionine + 1.25% Lysine

Research Implementation

LLM Fermentation Process with *Bacillus laterosporus*

1. Preparation for the LLM substrate

   Leucaena used in this study, is a local *leucaena* (River Tamarind) which has a height of 2-5 m, leaves are peeled off, then roasted at 60^{o}C for 24 hours, then milled to grains (for ration R0).

2. Preparation for Inoculum

   The preparation of inoculum is using 100g bran substrate plus 60 ml distilled water in the autoclave for 30 minutes at a temperature of 120^{o}C, 1 atm, and then cooled to a temperature of of 37^{o}C. Take a test tube containing the isolate, add 20 ml of distilled water, then crushed with an ose needle. Next vortex them to get homogeneous solution, mix the isolate into plastic containing bran, stir until evenly distributed and covered then make a hole to maintain the aeration. Incubate them for 24 hours at 37^{o}C.

3. Fermentation Procedure

   1 Kg of dried substrate of LLMis added with 800 ml of distilled water. Then it is autoclaved for 30 minutes at 121^{o}C, 1 atm. Then, it is inoculated with 6% *Bacillus laterosporus* of the amount of the substrate (for ration R1). For another treatment, the fermented LLM is supplemented with 0.40%

methionine and 1.25% lysine (for ration R2). It then incubated for 24 hours so that the fermentation product is dried at 60^{o}C for 24 hours. The dried product is ready to use, for animal feed.

The calculation of crude fiber digestibility and energy metabolismis calculated based on the following formula Table 1:

A. Crude Fiber Digestibility

$$\text{Digestion of Crude Fiber (\%)} = \frac{\text{CF Consumption} - \text{CF Excreta}}{\text{CF Consumption}} \times 100\%$$

Index:

CF = crude fiber

CF Consumption = the level of crude fiber ration multiplied by the amount of consumption

CF Excreta = the number of excreta multiplied by the crude fiber excreta

B. Nitrogen Retention

1. Nitrogen Consumption (g)

   This value is obtained by multiplying the amount of dry matter feed consumption with the nitrogen content in the treatment feed. Nitrogen Consumption (g) = Consumption of Feed (DM) x Feed N Content

2. Nitrogen Retention/RN (g)

   Nitrogen retention is the difference between the value of nitrogen consumption and the value of nitrogen which is excreted with excreta after being corrected with endogenous nitrogen excretion values.

C. Energy Metabolism

For the measurement of Nitrogen Corrected Pseudo Metabolic Energy (EMSn) and Nitrogen Corrected Pure Metabolic Energy (EMMn), two approaches are used:

Based on the method of [12]

Pseudo Metabolic Energy Corrected Nitrogen (EMSn) (Cal/kg) EMSn =

$$\frac{(EB \times K) - \{(EBe \times Y) + (8{,}22 \times RN)\}}{K} \times 1000$$

Index:

EB = gross energy of feed ingredients (Cal/kg)

EBe = gross energy of excreta (Cal/kg)

EBk = gross energy of fasted chicken (endogenous) (Cal/kg)

K = consumption of dry matter ration (g)

Y = dry weight of chicken excreta fed with treatment feed (g)

Z = endogenous) (g) dry weight of chicken excreta

RN = nitrogen retention (g)

8.22 = corrected value as gout (Cal/kg) in [12]

Results and Discussion

Average value of crude fiber digestibility, N retention and the average energy metabolism of Broiler using LLM with several treatments are presented in Table 2 and average of crude fiber digestibility, N retention and energy metaboilism of Broiler, can be seen in Figure 1 below:

Table 2: Average Value of Protein Consumption, Crude Fiber Digestibility, Nitrogen Retention, and Energy Metabolism of Broiler That were Given LLM Rations with Some Treatments

Parameter	Treatments		
	R0	R1	R2
Consumption of Protein (g)	34,04	45,05	48,24
Crude Fiber Digestibility (%)	42,21^a	52,52^b	57,66^c
Nitrogen Retention (g)	1,52	2,43	2,69
Nitrogen Retention (%)	57,54^a	62,90^b	68,35^c
Energy Metabolism (Kcal / kg)	2288,15^a	2706,80^b	3013,49^c

Note: Different superscripts in the same column show very significant differences (P <0.01)

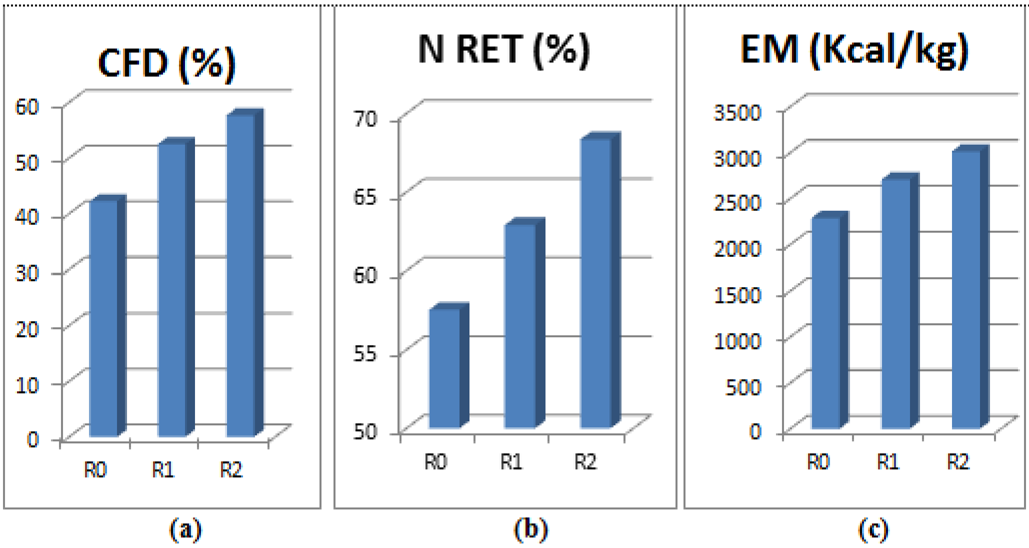

Figure 1: Average Value of Crude Fiber Digestibility, N Retention and Energy Metaboilism of Broiler That were Given LLM Ration with Some Treatments

A. The Effect of the Treatment on Crude Fiber Digestibility Values

The average crude fiber digestibility value of broiler chickens with LLM from some treatments in this study ranged from 42.21-57.66%. The results of the diversity analysis showed that the treatment of LLM from several treatments showed a very significantly different (P <0.01) to the value of crude fiber digestibility of the broilers. From Figure 1, it can be seen that the digestibility value of crude fiber, N retention and energy metabolism of the highest broiler chickens was found in the R2 treatment containing LLM fermentation plus supplementation of methionine-lysine.

The digestibility of crude fiber increased significantly with different treatments, i.e. R0 (42.21%), R1 (52.52%) and R2 (57.66%), respectively. The digestibility of crude fiber in the ration of the treatment was different because the crude fiber content of each treatment was also different. The digestibility of crude fiber treatment ration has a very significant effect because of the crude fiber content and consumption of crude fiber treatment rations that are different. The content of crude fiber in the ration, the composition of the composition of crude fiber and the activity of microorganisms affect the digestibility of crude fiber. According to [18] the digestibility of crude fiber is influenced by several factors including feed consumption, fiber content in feed, composition of crude fiber composition and microorganism activity. According to [19] states that the higher content of crude fiber in the ration leads to lower digestibility of crude fiber and vice versa.

B. The Effect of the Treatment on Nitrogen Retention Values

The average Nitrogen retention value of broiler chickens with LLM from several treatments in this study ranged from 1.52 to 2.69 g and the percentage of Nitrogen retention ranged from 57.54 to 66.84%. The results of the diversity analysis showed that the treatment of LLM from several treatments showed a very significantly different (P <0.01) to the value of Nitrogen retention of the broilers.

The high number of Nitrogen retention is influenced by the increase in the digestibility of nitrogen because of the fermentation process [20]. While the low number of nitrogen retention in the R0 diet (basal rasum added with unfermented LLM) is predicted because the crude fiber content in this ration is high compared to other rations and its protein consumption is also low in compared to the other rations. In accordance [21] and [22] who state that the high concentrate of the coarseness can increase the digesta rate so that the digestibility value of food substances including energy becomes low due to a decrease in the digestibility of the material resulting in a decrease in food substances and the energy.[10] added that Nitrogen retention is influenced by protein consumption, protein digestibility and the balance of food substances in the ration, besides that the protein content in the ration also determines how much nitrogen is capable of retention. If the quality of the protein produces is low, one of the amino acids produced will be reduced. By then, nitogen retention is also low [23]. The high and low levels of Nitrogen in feces affect the nitrogen retention. The more nitrogen left in the body, the nitrogen that is wasted with feces is also decreasing [24]. The differences are caused partly by the difference in formula ration and the metabolic energy measurement method used.

C. The Effect of the Treatment on the Energy Metaboilsm Value

The results of the statistical analysis presented that the results of the diversity analysis of the treatment of LLMfrom several treatments showed a very significant difference (P<0.01) to the metabolic energy value of broilers. The energy metabolism in this experiment is around 288.15-3013.49kcal/kg. The high metabolic energy in the R2 treatment is related to the condition of the crude fiber and the increase in the digestibility of the crude fiber so that the energy used by the broilers are also increased. In accordance,[25] stated that the digestibility of a feed ingredient is affected by a decrease in crude fiber, the balance of food substances and broilers factors and subsequently affects the value of metabolic Energy. [22] added that the less energy expended, the higher the ration energy absorbed or digested by the body, so that the efficiency of ration energy use is high. On the contrary, according to [10] high-fiber food has a low metabolic energy due to high crude fiber content, so it cannot be digested and can carry other food substances that have been digested out with feces, so that livestock metabolic energy becomes low .

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Conclusion

Based on the results of the study, it can be concluded that *Leucaena* leaf meal fermented with *Bacillus laterosporus* with supplementation 0.40% methyonine and 1.25% lysine can be used as an alternative feed ingredient for broilers rations seen from increasing the digestibility value of crude fiber (57.66%), N retention (68.35%) and energy metabolism (3013.49 kcal/kg) of broiler chicken.

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