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Bio-emulsifier supplementation from *Pseudomonas putida* that cultivated on medium containing waste palm oil as feed additive on poultry

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Abstract. The purpose of this study to determine effect of bio-emulsifier from *Pseudomonas putida* as feed supplement on the performance of broiler chickens. The experiment was divided in two phases. In phase 1: test of oil bubbles formation in water using bio-emulsifier, and phase 2 : *in vivo* test bio-emulsifier in animal. 200 one-day-old male and female broiler strain Cobb were used. On d 14, chicks were separated by sex and placed in 40 pens, sex separate (100 males or 100 females) and randomly assigned 4 treatments and 5 replicates of 5 birds each for 14 d (21d-35 d). The diets were based of corn, concentrate and rice bran meal and supplemented with: 0, 0.5,1 and 1.5 g/kg bio-emulsifier. The observed parameters were feed intake (FI), average daily gain (ADG), and feed conversion ratio (FCR). Result of experiment 1: as expected, supplementation of bio-emulsifier was increased small oil bubbles formation in medium oil in water, whereas in experiment 2 showed FI, ADG and FCR unaffected by dietary supplementation with bio-emulsifier on broiler at finisher phase (week 4 and 5) (P>0.05) and did not decrease on broiler performance. The results give new information that bio-emulsifier from *Pseudomonas putida* could potentially as a new alternative feed additive in the future on broiler performance.

1. Introduction

Energy in animal feed is the main nutrient in addition to protein, fat and minerals that are needed by livestock. The cause of in-efficient of nutrient metabolism was the lack energy availability in the feed. Lack of energy consumption caused disturbances in poultry production. Therefore, energy consumption must be able to meet the standard needs of broiler chickens 2800-3200 Kcal/kg [1-3] . Corn has been recognized as the main source of energy in poultry, the use of this material ranges from 50-60 percent, but that amount is still needed for other energy sources to meet energy requirement. Among the ingredients in feed, oil contains energy and essential fatty acids [4]. Oil contains higher calory than carbohydrates which is responsible for providing increased energy density [5].

The addition of oil in the digestive tract of poultry is difficult to digest because it cannot mix with water. The low fat absorption capacity, especially in young cattle, due to incomplete physiological functions and low lipase enzyme production [6]. Fat to be absorbed is hydrolyzed by lipase enzymes and bile acid contribute to helping the formation of micelles before they can be absorbed in the cell membrane [7]. To increase the ability of micelle formation, it is necessary to add an emulsifying agent, namely an emulsifier. The addition of an emulsifier can increase feed digestibility and body weight of starter phase chickens [7]. The ability to break down fat in the digestive tract will stimulate easier absorption of fat in cell membranes [8]. Micelles are small globules that contain polar compounds on their surface, so that they will easily penetrate the un stirrer water part of the hydrophilic cell membrane, thus facilitating the process of simple fat absorption [9].

Emulsifiers that function as surfactants are mostly made from petroleum materials which are increasing competitive with fuel requirements, for this reason, surfactants from organic materials that do not cause contamination, are easily degraded and are effective in a certain temperature and pH *Pseudomonas putida* is one type of microbe that can produce biosurfactants for emulsifiers [10]. The

reaction between glucose and fatty acid or triglycerides as a substrates of *Pseudomonas putida* will produce a type of glycolipid surfactant [11]. This reaction can be produced when the bacterial growth medium contains fatty acids and glucose. Fatty acids can come from waste palm oil and glucose from molasses materials. The use of this bio-emulsifier is expected to increase the formation of micelles so as to facilitate the absorption of fat in the cell membrane so that it can increase the energy produced so as to improve performance in poultry. The objective of this present study at evaluating the effect of addition a bio-emulsifier as feed supplement on the performance of broiler chickens.

2. Materials and Methods

2.1. Production of bio-Emulsifier

The wild type of *Pseudomonas putida* was originated from Feed technology laboratory, Politeknik Negeri Jember. *Pseudomonas putida* was grown in the cultivated in the medium that containing: molasse 360 g/L, peptone 50 g/L, Potato Dextrose Broth (PDB 50 g/L mixed to 1 g/L, 1.5% waste palm oil, ammonium sulfate (Sigma Aldrich, Germany) and distillated water. The media was sterilized using an autoclave at temperature of 121 ^{o}C, 15 min and cooled for 2 hours. The media contained in the test tube was then planted with *Pseudomonas putida* bacteria and incubated at 37 ^{o}C ,200 x g for 24 h in a shaker incubator. After 24 hours, medium were transferred to volume 1000 ml Erlenmeyer and shake with the same procedure as before. Cell biomass was centrifuged at 4 ^{o}C, 9000 x g for 10 min. The harvested then dried in the oven during 48 h 40 ^{o}C and used for *in vivo* trial.

2.2. Bio-emulsifier test oil in water

The test was divided into 4 treatments, P0, control P1, 200 μl P2 300 μl, and P3 400 μl. The precipitate bio-emulsifier that obtained previously was mixed with 100 ml of distilled water and, shake for 1 min at 27 ^{o}C, then put in sterilized the petri dish (90 mm). Introduced 10 ml palm oil and observed sum of small bubbles formation during 15 minutes in petri dish.

Table 1. Treatment ration formulation

	(%)			
Parameter	P0	P1	P2	P3
Feed ingredients				
Corn	58	58	58	58
Concentrate	30	30	30	30
Rice Bran	10	10	10	10
Palm oil	1	1	1	1
Premix[1]	1	1	1	1
Bio emulsifier) g/kg feed*	0	0.5	1	1.5
Total %	100	100	100	100
Chemical composition				
Protein (%)	19.8	19.8	19.8	19.8
Fat (%)	5.45	5.45	5.45	5.45
Crude Fiber (%)	4.21	4.21	4.21	4.21
ME (Metabolism energy) (Kcal/kg)	2.929	2.929	2.929	2.929
Ca (Calcium) (%)	1.35	1.35	1.35	1.35
P (Phospor) (%)	0.66	0.66	0.66	0.66

[1]Premik each kg contains: vitamin A, 8000 IU; vitamin D, 2100 IU; vitamin E, 12 IU; vitamin B1 , 0.5 mg; vitamin B6, niacin 1 mg, 15 mg, pantothenic acid, 12.5 mg.

2.3 Animals and diets

200 one-day-old male and female broiler strain Cobb were used. On d 14, chicks were separated by sex and placed in 40 pens, sex separate (100 males or 100 females). There were consisted 2 dietaries: starter phase (1 to 21 d), finisher (21 to 35 d). Chickens individual body weight and feed intake were measured at 21-28 d and 28-35 d to evaluate bird performance. The diets were based of corn, concentrate and rice bran meal and supplemented with: P0, 0 (control), P1: 0.5, P2: 1, and P3 : 1.5 g/kg bio emulsifier from *Pseudomonas putida*. Feed nutrient was referred in the basal diet met that suggested by NRC (1994). The composition of the feed that will be used for the trial is shown in **Table 1.**

2.4. Data collection

Data collection starts from the beginning to the end of maintenance. The data taken included feed intake (FI), average daily gain (ADG) and feed conversion ratio (FCR) was taken once a week.

2.5.Statistical Analysis

Data were submitted to analysis of variance using the ANOVA of Minitab software. Means were compared by the Tukey test

3. Results and Discussion

3.1 Bio-emulsifier oil in water test

The result In the bubble test formation from experiment 1 is presented in **Table 2** and showed that P3 treatment with the addition of a bio emulsifier caused highest number of bubbles compared to P2, P1. formation of the number of bubbles indicates the occurrence of a fraction of oil in water (O in W). The oil fraction shows or indicates the occurrence of an emulsification process. *Pseudomonas sp*. produced biosurfactants that are surface active, characterized by a decrease in the surface tension of the liquid medium [12]. The biosurfactant produced by *Pseudomonas spp*. is a low molecular weight biosurfactant was identified as the amphiphilic surface-active glycolipids (rhamnolipids) that consisting of hydrophobic and hydrophilic molecules [13].

Table 2. The number of small bubbles after introduction bio-emulsifier (unit) in medium oil in water

Treatment	Small bubbles formation
P0	475±65.05[a]
P1	602±9.90[b]
P2	595±130.00[b]
P3	690±67.00[c]

According Gumel [14] *Pseudomonas putida* can growth in medium fatty acid C8:0 and C18: 1 as carbon and ammonium as nitrogen source that found in waste palm oil effluent.

3.2. Bio-emulsifier test in Broiler performance

During the observation period in the *in vivo* study, the chickens were in good condition and did not appear to have health problems and showed normal livestock behavior. Chickens did not display a number clinical symptom. Analysis of variances was showed no significant effect on feed consumption among treatments ($P>0.05$). The coefficient variation for feed intake were slightly higher for the chickens reared with sexes separated with males have larger variation than female. The absence of differences between treatments is thought to be due to the relatively the same energy content. According Massuquetto [15] the energy content in feed will affect the amount of feed consumed. Chickens will stop eating when the energy consumed is sufficient for chicken activities. The addition of bio emulsifier is expected to reduce feed consumption. This

is in accordance with the opinion Guerreiro [16] which states that the addition of an emulsifier does not cause a change in performance.

Table 3. Broiler performance was fed treated by glycolipid as bio emulsifier produced from *Pseudomonas putida*

Parameter	P0	P1	P2	P3	SEM	*P value*
Cummulative Week 4-5						
Male						
Feed intake	1635,50	1567,50	1483,69	1456,26	390	0.754
ADG	873,89	808,92	797,49	833,12	90.74	0.711
FCR	1,87	1,94	1,86	1,75	0.18	0.289
Female						
Feed intake	1491,95	1382,85	1308,96	1345,18	131	0.187
ADG	770,55	709,51	728,59	714,76	153.1	0.959
FCR	1,94	1,95	1,80	1,88	0.22	0.337

SEM = Standard Error Mean

Data from ADG and FCR showed that the addition of bio-emulsifier had no significant effect (P>0.05) for both males and females. The increase in body weight gain is expected to be achieved by the addition of a bio-emulsifier. Bio-emulsifier agent is expected to increase fat absorption, because it can increase the hydrolyze of fat globules. There was no significant effect, this was thought to be caused by the product application carried out finisher period at week 4 (21-28 d). According Polin and Hussein [17] to in this period chickens produced lipase at a high concentration compared to chickens aged 42 days. The ability of fat absorption is sensitive in young broilers, where lipase production is not balanced with fat absorption capacity. [18] . The addition of an emulsifier in 7-day-old chicks can reduce oil use by 20%, when compared to 14 d and 21 d. the bio-emulsifier ability was not affected due to lipase activity at concentrations that were still able to increase fat absorption, so that the bio-emulsifier effect was not effectively effective. significantly in suppressing FCR which is related to the efficiency of production performance on broiler.

4. Conclusion

The results give new information that bio-emulsifier from *Pseudomonas putida* could potentially as a new alternative feed additive in the future for broiler performance.

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