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Nutrition Evaluation of Organic Protein Flour as Feed Ingredients on AME, AMEn, Nitrogen Retention, and Protein Digestibility in Layers

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Abstract**—** *This research aims to evaluate nutrition of the use Organic Protein (OP) flour as a feed ingredient for digestibility in laying hens. In this research, 20 laying hens of the Lohman Strain layer phase aged 33 weeks were used. The Singel Cell Protein (SCP) used liquid Organic Protein from waste Monosodium Glutamate (MSG) is atrademark produced by PT. Daesang Ingredients Indonesia which is processed into flour. The research method used was a field experiment with a completely randomized design (CRD). This research consisted of 5 groups, namely basal feed without treatment and four types of feed which were differentiated based on the percentage of use of Organic Protein flour, respectively 1%, 2%, 3%, and 4%. Each treatment consisted of 4 replications and each replication consisted of 1 laying hens. The observed variables consisted of Apparent Metabolizable Energy (AME), Apparent Metabolizable Energy N Corrected (AMEn), Nitrogen Retention (RN) and Protein Digestibility. The data obtained during the study were tabulated and statistically analyzed using analysis of variance (ANOVA) with a completely randomized design (CRD). If the results are significantly different (P < 0.05) or very significant (P < 0.01), then proceed with Duncan's multiple distance test. The results of the analysis of diversity (ANOVA) showed that the use of organic protein flour gave a significant difference (P<0.05) on the AME, AMen, nitrogen retention, protein digestibility digested by laying hens. The results showed that AME, AMen, nitrogen retention and protein digestibility were highest in laying hens that received T0 treatment. Further research is needed so that organic protein flour is able to maximize digestibility in laying hens.*

Keywords**—** *Organic Protein, AME, AMEn, Nitrogen Retention, Protein Digestibility.*

I. INTRODUCTION

Feed is one of the impoortant factors in supporting the success of a laying hen business. Businesses that produce eggs as a selling commodity allocate at least 60-70% of their production costs for feed. The method used by the majority of laying hen breeders to reduce feed costs is by applying the self-mixing method. Selfmixing is a method of formulating feed in which breeders mix fees ingridients themselves into feed. In laying hen farms with large populations, this method is considered to be able to reduce the price of feed calculated in kilogram conversion to be more economical so as to significantly increase the efficiency of feed costs. However, the dynamics of feed ingredient costs can also cause high feed cost calculations. Nasir, et al. (2018) reported that the costs of laying hen feed had increased sharply due to the fact that many of the main feed ingredient components were still imported, such as corn, soybean meal, fish meal, and MBM.

The limited availability of quality feed ingredients causes laying hen breeders to depend on conventional feed ingredients which are relatively expensive, especially protein source feed ingredients (Kim, et al., 2019). One way that needs to be done is to look for alternative ways that can substitute conventional protein sources from biotechnology development products called Single Cell Protein (SCP). SCP is a biomass product that has a high protein content produced from microbes. SCP-producing microbes generally grow in industrial waste which contains carbon and nitrogen elements. The main components of single cell proteins are amino acids and minerals. In addition, single cell protein can also produce mycoprotein which has a high nutritional content (Nigam, 1998). The use of single-cell protein as a substitute for protein-sourced feedstuffs has begun to be pioneered in Indonesia, with the hope that it will reduce dependence on the supply of imported commodity protein-sourced feedstuffs (Attia et al., 2014). Giola and Biavati, 2018) and (Nunes and Kumar, 2018) the result of the research that has been done show that the use of yeast culture/SCP in feed show the result has increased production, egg quality, hatchability and chick survival.

The organic protein used is production waste from the manufacture of Monosodium Glutamate (MSG) produced by PT Daesang Ingredients Indonesia. however the use of Organic Protein from PT Daesang Ingredients Indonesia has not been optimally utilized. Therefore there is a need for further research regarding the use of Organic Protein in poultry so that it can be an alternative feed ingredient for laying hens. This research is expected to be able to show the value of protein quality and feed energy content contained in organic protein flour. Based on this description, the authors conduct a research on the effect of using Organic Protein Flour on the value of nitrogen retention and metabolic energy of laying hen rations.

II. MATERIALS AND METHODS

Research Materials

In this research, 20 laying hens of the Lohman Strain Layer phaseaged 33 weeks were used. The SCP used is liquid organic protein from PT Daesang Ingredients Indonesia's Monosodium Glutamate (MSG) waste which is floured with

nutrient content as shown in Table 1. The basal feed used is self-mix feed with nutrient content as shown in Table 1.

Research Methods

The research method used was a field experiment with a completely randomized design (CRD). This study consisted of 5 groups, namely basal feed without treatment and four types of feed which were differentiated based on the percentage of use of Organic Protein (OP) flour, each of 1%, 2%, 3%, and 4%. Each treatment consisted of 4 replicates and each repetition consisted of 1 laying hen. The feed treatments were:

P0: 100% basal feed (no treatment)
P1: 99% basal feed + 1% OP flour
P2: 98% basal feed + 2% OP flour
P3: 97% basal feed + 3% OP flour
P4: 96% basal feed + 4% OP flour

Observational Variables

The variables observed as indicators to determine the effect of feed fed with OP flour on laying hens included

1. Apparent Metabolizable Energy (AME)

The calculation of Apparent Metabolizable Energy (AME) or Apparent Metabolic Energy (EMS) is determined using the equation from Farrell (1978) as follows:

$$AME=\frac{\text{GE feed-GE excreta}}{\text{GE feed}}$$

2. Apparent Metabolizable Energy N Corrected (AMEn)

Apparent Metabolizable Energy N Corrected (AMEn) or Corrected Apparent Metabolizable Energy N (EMSn) is calculated based on the equation from Farrell (1978) as below:

$$AMEn=\frac{\text{GE feed}-\text{GE excreta}}{\text{GE feed}}-(8{,}73\times \text{retention N})$$

3. Nitrogen Retention

Nitrogen retention is known using the Black and Griffiths equation (1975) as below:

Retention N = N feed – N excreta

4. Protein Digestibility

Digestibility of crude protein is calculated by the formula (Wahju, 1997) as below:

$$PD\ (\%)=\frac{\text{Excreta Protein}-\text{PK Consumption}}{\text{Protein Consumption}}\times 100\%$$

TABLE 1. Substances contained in organic protein flour and basal feed

	BK (%)	Abu (%)	PK (%)	LK (%)	SK (%)	Ca (%)	P (%)	GE (Kal/g)
[1)] Flour OP	92,84[1)]	8,59[1)]	37,99[1)]	10,11[1)]	4,56[1)]	0,53[1)]	0,91[1)]	4051[1)]
[2)] Basal Feed	89,32[2)]	-	19,38[2)]	-	-	-	-	3790[2)]

Information:
1) laboratory proximate test results of the Livestock and Fisheries Service of Blitar Regency
2) laboratory proximate test results of the Pontianak Plantation and Livestock Service Office

TABLE 2. Treatment feed content

Nutrien	P0	P1	P2	P3	P4
Dry Weight (%)	89,32	89,35	89,38	89,42	89,46
Crude Protein (%)	19,38	19,57	19,76	19,96	20,15
Gross Energy (Cal/g)	3790	3792	3795	3797	3800

Note: calculation based on data calculation.

III. Results and Discussion

Effect of Treatment on Variables

This research was conducted as a experiment to determine the effect of using Organic Protein (OP) treatment on the predetermined variables, including Apparent Metabolizable Energy (AME), Apparent Metabolizable

Energy N Corrected (AMEn), and Nitrogen Retention. Metabolic energy and nitrogen retention is a method to measure the quality of energy and protein rations (Scott et al., 1998). Data from research on the effect of treatment on Apparent Metabolizable Energy (AME), Apparent Metabolizable Energy N Corrected (AMEn), Nitrogen Retention, and Protein Digestibility are presented in Table 3.

TABLE 3. Data on the average effect of treatment on the digestibility of laying hens

which variable Observed	Treatment				
	P0	P1	P2	P3	P4
Apparent Metabolizable Energy (AME) (kkal/kg)	3095,1±35,8[b]	2933,3±143,3[a]	2962,4±77,8[a]	2939,1±61,6[a]	2860,2±120,9[a]
Apparent Metabolizable Energy N Corrected (AMEn) (kkal/kg)	3079,7±35,6[b]	2920,2±141,1[a]	2948,1±76,4[a]	2924,1±60,4[a]	2845,6±120,2[a]
Retention Nitrogen (g)	1,77±0,21[b]	1,50±0,28[a]	1,64±0,21[a]	1,71±0,15[a]	1,67±0,09[a]
Protein Digestibility (%)	62,12±3,03[b]	50,63±7,4[a]	57,03±5,3[a]	55,06±3,9[a]	52,44±3,6[a]

Note: a-b different Super scripts on the same line indicate a significant difference in each treatment (P<0.05)

Effect of Treatment on Apparent Metabolizable Energy (AME)

Apparent Metabolizable Energy (AME) is a method used to measure the digested metabolic energy value of poultry. Apparent Metabolizable Energy (AME) is the difference between the gross energy consumed from feed and the energy expended through excreta (Barzegar, et al., 2020). The effect of using OP flour in laying hens feed on Apparent Metabolizable Energy (AME) during the study can be seen in Table 3.

The results of the analysis of variance (ANOVA) showed that the use of OP flour had a significant (P<0.05) effect on the Apparent Metabolizable Energy (AME) digested by laying hens. The results of Duncan's multiple range test showed that P0 was significantly different from P1, P2, P3, and P4. Meanwhile, between P1, P2, P3, and P4 there was no significant difference. Based on Table 3, it can be seen that the highest AME digestibility was at P0 of 3095.1 ± 35.8 kcal/kg, while the lowest was at P4 of 2860.2 ± 120.9 kcal/kg. The results showed that laying hens that received feed treatment with OP flour did not give better results on AME digestibility compared to basal feed without treatment.

The use of OP flour with a higher percentage is directly proportional to the increase in the gross energy content of the feed, but not directly proportional to the digested AME. The amount of AME digested by laying hens is not onlybe affected by the gross energy content of the feed, but also depends on the digestibility of the feed. It is appropriate with the steament of Bahri and Rusdi (2008) that the level of metabolic energy is closely related to the digestion and absorption of nutrients. Mcdonald et al. (2002) added that the low digestibility of a feed ingredient will result in a lot of energy being lost in the form of excreta, so that the metabolicenergy values becomes low.

Effect of Treatment on Apparent Metabolizable Energy N Corrected (AMEn)

Apparent Metabolizable Energy N Corrected (AMEn) is the AME value corrected with nitrogen, so the value is lower than AME (Lase, et al. 2014). The AMEn value is obtained from the apparent metabolic energy (AME) value which is then corrected/reduced by the multiplication beetwen retention value of N with a coefficient of 8.73 which is the calorific value of 1 gram of nitrogen. Natsir (2007) stated that the results of calculating the metabolic energy of feed ingredients without N correction are considered to be less estimated the energy value of a feed ingredient because nitrogen is stored in body tissues (Retained Nitrogen/RN). If a process of catabolism occurs, it will become energy that is lost as urine. Therefore, with the calculation of Apparent Metabolizable N Corrected (AMEn), it shows the value of metabolic energy that is no longer affected by Nitrogen. The effect of using OP flour in laying hens feed on Apparent Metabolizable N Corrected (AMEn) during the study can be seen in Table 3.

The results of the analysis of variance (ANOVA) showed that the use of OP flour had a significant difference ($P<0.05$) on Apparent Metabolizable Energy N Corrected (AMEn) in laying hens. The results of Duncan's multiple range test showed that P0 was significantly different from P1, P2, P3, and P4. Meanwhile, between P1, P2, P3, and P4 there was no significant difference. Based on Table 3, it can be seen that the highest AMEn digestibility was P0 of 3095.1 ± 35.8 kcal/g, while the lowest was at P4 of 2860.2 ± 120.9 kcal/g. The results showed that the use of OP flour was not able to increase the AMEn digested by laying hens.

The effect of research showing a significant difference by feed. The digestibility value of a protein shows the percentage of food that can be digested. The protein digestibility value of the feed is related to the size of the feed content. The content of nucleic acids in feed can interfere with protein digestion and absorption of essential amino acids. This is apropiate with the steatment of Giec and Skupin (1988) where 10-15% of SCP is nucleic acid. Nucleic acids are macromolecules that undergo different metabolic processes from proteins and have low digestibility. Another thing that affected the administration of 1 – 4% Organic Protein flour had no effect on AMEn because the protein content in the treated feed did not differ significantly causing no significant difference. This is accordance with the results of Mario et al. (2014) which concluded that the protein content or protein iso of the feed was almost the same in each treatment so that the Apparent Metabolizable Energy corrected Nitrogen was not significantly different.

Effect of Treatment on Nitrogen Retention

Nitrogen retention is nitrogen in protein feed that enters the body and is then absorbed and left in the body and then used by livestock. Nitrogen retention is a method of assessing feed quality by measuring the difference between nitrogen consumption and nitrogen excreted in excreta (Sutrisno, et al., 2013). This retained nitrogen illustrates the efficient use of protein in poultry (Siabandi, et al., 2018). The effect of using OP flour in laying hen feed on nitrogen retention during the study can be seen in Table 3.

The results of the analysis of variance (ANOVA) showed that the use of OP flour had a significant ($P<0.05$) effect on nitrogen retention in laying hens. The results of Duncan's multiple range test showed that P0 was significantly different from P1, P2, P3, and P4. Meanwhile, between P1, P2, P3, and P4 there was no significant difference. Based on Table 3 it can be seen that the highest nitrogen retention was P0 of 1.77 ± 0.21 g, while the lowest was at P1 of 1.50 ± 0.28 g. The results showed that the use of OP flour in laying hen feed was not able to increase nitrogen retention when compared to basal feed without treatment as a control.

The use of Organic Protein flour with a higher percentage is directly proportional to the increase in crude protein content in the feed, but not directly proportional to the nitrogen that can be retained. This is thought to be caused by the nucleic acid content in OP flour which has a low digestibility value. The low digestibility of nucleic acids was reported by Samadi, et al. (2012) who suggested that single cell proteins have a fairly high nucleic acid content. In another study, Giec and Skupin (1988) reported that 10-15% of a single cell protein is nucleic acid. Nucleic acids are macromolecules that have low digestibility when compared to pure proteins.

The level of nitrogen that is able to be retained by laying hens is strongly be affected by the digestibility of the feed consumed. According to Wahju (2004) he argues that nitrogen retention be affected by several factors including protein digestibility, protein quality, and the balance of nutrients in the ration. If the quality of the protein is low, or one of the amino acids is lacking, nitrogen retention will be low. Indrasari, et al. (2014) argues that maximum protein digestibility can increase N retention. The results of Sutrisno et al.'s research. (2013) reported that the higher the digestibility of crude protein, the higher the nitrogen retained in the chicken's body. Mateos, et al., (1982) states that more nitrogen is retained, partly due to the better digestion and absorption of food substances, which speeds up the rate of passage.

Effect of Treatment on Protein Digestibility

Protein digestibility is the amount of protein that can be digested by livestock and not excreted with excreta (Nuraini, et al., 2016). Protein digestibility is calculated based on the percentage of digested protein to protein from the feed consumed by laying hens. Meanwhile, digested protein is known from the difference between the protein from the feed consumed and the protein in the excreta of laying hens. According to Tillman, et al., (1991) argues that protein

digestibility is determined by two important things, including the amount of protein contained in the feed and the amount of digestible protein which can be known if there has been a process of digestion in the feed. The effect of using organic protein flour in laying hens feed on protein digestibility during this research can be seen in Table 3.

The results of the analysis of variance (ANOVA) showed that the use of organic protein flour had a significant ($P<0.05$) effect on protein digestibility in laying hens. Based on Table 3, it can be seen that the highest percentage of protein digestibility was P0 of 62.12 ± 3.03 %, while the lowest was in P1 of 50.63 ± 7.4 %. Duncan's multiple range test results show that P0 is significantly different from P1, P2, P3, and P4. Meanwhile, between P1, P2, P3, and P4 there was no significant difference.

The use of organic protein flour as a substitute for basal feed with a proportion of 1 – 4% can increase the crude protein content in the feed linearly as shown in Table 2. However, the feed using organic protein flour has a low protein digestibility value when compared to the control feed as shown in Table 2, shown in Table 3. This is thought to be caused by the nucleic acid content in organic protein flour which has a low digestibility value, so that a lot of crude protein is wasted through excreta. The results in this research are in accordance with the results of the research by Nasseri, et al. (2011) who suggested that even though single cell protein has high nutritional value because it contains high protein, vitamins, essential amino acids, and fat. However, there are doubts to replace conventional protein sources because of the high nucleic acid content and slow digestibility. Besides that livestock are also be able to respond that single cell proteins as a foreign material in the body which can cause allergic reactions.

IV. CONCLUSION

The use of organic protein flour can increase the crude protein content and gross energy in feed, but has not been able to increase the digestibility of Apparent Metabolizable Energy (AME), Apparent Metabolizable Energy N Corrected (AMEn), nitrogen retention, and protein digestibility in laying hens

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